Chloride and potassium channel function in alveolar epithelial cells

Scott M. O’Grady and So Yeong Lee

Department of Physiology, University of Minnesota, St. Paul, Minnesota 55108

O’Grady, Scott M. and So Yeong Lee. Chloride and potassium channel function in alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 284: L689–L700, 2003; 10.1152/ajplung.00256.2002.—Electrolyte transport across the adult alveolar epithelium plays an important role in maintaining a thin fluid layer along the apical surface of the alveolus that facilitates gas exchange across the epithelium. Most of the work published on the transport properties of alveolar epithelial cells has focused on the mechanisms and regulation of Na+ transport and, in particular, the role of amiloride-sensitive Na+ channels in the apical membrane and the Na+-K+-ATPase located in the basolateral membrane. Less is known about the identity and role of Cl− and K+ channels in alveolar epithelial cells, but studies are revealing important functions for these channels in regulation of alveolar fluid volume and ionic composition. The purpose of this review is to examine previous work published on Cl− and K+ channels in alveolar epithelial cells and to discuss the conclusions and speculations regarding their role in alveolar cell transport function.

alveolar fluid clearance; Cl− absorption; K+ secretion; epithelial ion transport

THE ALVEOLAR EPITHELIUM is composed of two major epithelial cell types referred to as type 1 (AI) and type II (AII) cells (5, 10, 30, 46). The transport and water permeability characteristics of these cells play a critical role in regulating the volume and electrolyte composition of alveolar lining fluid. In the fetal state, alveolar epithelial cells are involved in the secretion of fluid into the developing alveoli and airways (1, 19, 30, 52, 55, 67, 68, 87). Shortly before birth, fluid secretion is inhibited and a net increase in lung liquid absorption develops in preparation for breathing air (1, 19, 46, 67, 68, 70). This change in fluid transport occurs as a consequence of a shift in electrolyte transport properties of the epithelium from anion secretion to electrogenic Na+ absorption. Moreover, electrogenic Na+ absorption persists as the basal transport process associated with adult alveolar epithelial cells (10, 28, 45, 46, 48, 49). Inhibition of Na+ transport across the apical membrane by amiloride produces a decrease in fluid absorption from the alveolar space (47). Most of the in vitro work on Na+ transport across the alveolar epithelium has focused on AII cells, which cover 2–5% of the lung surface area and are known to produce and secrete surfactant (5, 30). The cellular and molecular mechanism of Na+ transport and its regulation by corticosteroids and catecholamines have been the subject of several recent review articles and special topic papers (9, 10, 37, 45–49). These articles have focused on the importance of apically localized, amiloride-sensitive Na+ channels as the principle pathways for Na+ uptake from the alveolar fluid. Two Na+ channel phenotypes have been identified with differing Na+ selectivities and amiloride sensitivities. In cultures of adult rat AII cells grown in the absence of corticosteroids or reduced oxygen delivery, the predominant Na+ entry pathway is a 21-pS nonselective cation (NSC) channel with an Na+/K+ permeability ratio (PNa/K) of 1 (26). In late-gestation fetal distal lung epithelial cells, a similar NSC channel was previously identified with single-channel conductance estimates of 23 and 27 pS (42, 62, 63) and a PNa/K ratio of 0.9 (63). When adult rat AII cells were grown in the presence of glucocorticoids and an apical air interface, the predominant apical conductance was a 6-pS, highly selective Na+ channel (HSC; PNa/K ratio = 80:1) that was blocked by amiloride ([half-maximal inhibition (K0.5) = 37 nM; (7, 26)]. Similarly, in experiments with A549 human alveolar epithelial cells, it was shown that treatment with dexamethasone for 24–48 h produced a twofold increase in amiloride-sensitive whole cell current and decreased the K0.5 for amiloride from 833 to 22 nM (38). In addition, dexamethasone decreased single-channel conductance from 8.6 to 4.4 pS and increased Na+ selectivity, as reflected by a shift in reversal potential from 47 to 66 mV. These changes in biophysical and pharmacological properties of A549 cell Na+ channels were associated with increased expression of mRNA...
and protein for both β- and γ-epithelial Na⁺ channel (ENaC) subunits, but no significant change in α-ENaC expression (38). It has been previously proposed that different combinations of ENaC subunits can form Na⁺ channels with differing biophysical and pharmacological properties (25). Expression of the α-subunit alone produces NSC channels, whereas channel proteins consisting of α-, β-, and γ-subunits exhibit biophysical and pharmacological properties consistent with HSC (26). Thus steroid induction of β- and γ-subunit mRNA and protein expression is most likely responsible for the change in Na⁺ channel characteristics in A549 cells. However, in primary rat AlI cells, all three ENaC subunits could be detected in the absence of glucocorticoid treatment. Thus the presence of α-, β-, and γ-subunit proteins within the same cell does not necessarily result in expression of HSC. This observation would suggest that steroid-mediated increases in Na⁺ selectivity and low single-channel conductance also depend on proper assembly and insertion of channel subunits into the apical membrane and not solely on the level of expression of each subunit (26).

It is worth noting that the effects of corticosteroids on alveolar Na⁺ transport function are not limited to the apical membrane. Glucocorticoids have been shown to increase Na⁺-K⁺-ATPase subunit expression and activity in fetal and adult AlI cells (3, 6, 11, 17, 67, 74). In alveolar cells, the predominant Na⁺-K⁺-ATPase catalytic subunit is α₁, which contains binding sites for Na⁺, K⁺, and ATP. The β₁-subunit has also been identified in AlI cells and is presumably involved in pump insertion into the basolateral membrane. In the fetal lung epithelial cell line FD18, dexamethasone increased steady-state levels of α₁- and β₁-subunit mRNA by 3.8- and 2.8-fold, respectively (6). This increase in mRNA was shown to be the result of increased promoter activity with no change in RNA stability. In adult rat AlI cells, dexamethasone was shown to increase β₁- but not α₁-mRNA transcript levels. Both α₁- and β₁-protein expression were increased along with stimulation of Na⁺-K⁺-ATPase activity (3). Thus glucocorticoid receptor stimulation produces transcriptional and translational regulation of Na⁺-K⁺-ATPase function in AlI cells. Moreover, coordinate regulation of apical and basolateral Na⁺ transport pathways following glucocorticoid stimulation enhances the rate of transepithelial Na⁺ absorption (11).

Until recently, the standard model for electrolyte and fluid transport across the alveolar epithelium proposed that AlI cells were the principle cell type involved in NaCl transport and that AlI cells were primarily involved in fluid transport and gas exchange (10, 46–48). Recent studies of AlI cells in culture, however, have demonstrated amiloride-sensitive Na⁺ uptake that exceeds rates of Na⁺ influx measured in parallel cultures of AlI cells by 2.5-fold (30). In addition, immunocytochemistry experiments with cultured AlI cells revealed the presence of all three ENaC subunits as well as the α₁- and β₁-subunits of the Na⁺-K⁺-ATPase. Western blot analysis of α-ENaC expression indicates that the amount of α-subunit/μg protein in AlI cells exceeds that observed in AlI cells by threefold. These results were further supported by Borok et al. (5), who used antibodies to specific AlI and AlI cell marker proteins in situ and cultured AlI cells to distinguish sites of expression for ENaC and Na⁺-K⁺-ATPase subunits. The results showed immunolocalization of α₁- and β₁-Na⁺-K⁺-ATPase subunits in AlI cells in situ and localization of the α-ENaC subunit in cultured AlI cells. In addition, α₁- and β₁-Na⁺-K⁺-ATPase subunits, as well as the α-ENaC subunit, were detected by RT-PCR in mRNA samples obtained from highly purified populations of freshly isolated AlI cells. The results of these recent studies provide evidence in support of a significant role for AlI cells in electrogenic Na⁺ absorption across the alveolar epithelium. Moreover, the observation that AlI cells comprise >95% of the internal surface area of the lung suggests that most of the Na⁺ absorption is mediated primarily by AlI cells.

Although considerable interest exists in understanding mechanisms and regulation of transepithelial Na⁺ transport and its role in fluid absorption across the alveolar epithelium, less is known about Cl⁻ and K⁺ transport pathways and their physiological significance in alveolar cells. Chloride secretion by fetal alveolar epithelial cells is well accepted as important for establishing the osmotic driving force necessary for fluid secretion into the lumen of the developing lung (19, 35, 52, 53, 55). The role of Cl⁻ channels and transcellular Cl⁻ absorption in alveolar fluid clearance in the adult lung has been controversial, but recent studies in mouse and human lungs have provided compelling evidence showing that activation of the cystic fibrosis transmembrane conductance regulator Cl⁻ channel (CFTR) is essential for increased fluid clearance following β-adrenergic stimulation (18, 36). The role of specific K⁺ channels known to exist in alveolar epithelial cells is even less well understood. Patch-clamp studies have shown that alveolar epithelial cells express an interesting array of K⁺ channel families, including voltage-gated (Kv-type) channels, inwardly rectifying K⁺ (Kir) channels, and Ca²⁺-activated K⁺ channels (12, 24, 33, 65, 72, 81). The purpose of this review is to discuss what is known about Cl⁻ and K⁺ channels present in both fetal and adult alveolar epithelial cells and to offer some speculation on the physiological role of these channels in alveolar epithelial electrolyte transport.

**CHLORIDE CHANNELS IN ALVEOLAR EPITHELIAL CELLS**

Cl⁻ channels were first identified in cultured alveolar epithelial cells by single-channel patch-clamp recording techniques in the mid-1980s. The first Cl⁻ channel identified in adult rat AlI cells was a high-conductance (350–400 pS), voltage-sensitive channel that was located in the apical membrane (76). The channel was found to be selective for anions relative to Na⁺ (PNa/PCl = 0.015) and to have an anion selectivity of I⁻ > Br⁻ > Cl⁻ > NO₃⁻. At that time, the mechanism
of transepithelial Na\(^+\) transport across cultured rat AII cells was not well understood, but the authors did speculate that this high-conductance Cl\(^-\) channel might be involved in transcellular Cl\(^-\) transport across the alveolar epithelium. Several years later, a high-conductance (375 pS), stilbene-sensitive, G protein-regulated Cl\(^-\) channel was identified in cell-free patches from freshly isolated fetal guinea pig AII cells (31). Smaller-conductance Cl\(^-\) channels (25 pS) were also identified in cell-attached patches. The authors speculated that these large-conductance, G protein-regulated Cl\(^-\) channels may play a role in mediating Cl\(^-\) efflux from cells that are involved in active Cl\(^-\) secretion.

**Chloride secretion and Cl\(^-\) channel function in fetal alveolar epithelial cells.** Early studies of fetal alveolar epithelial cell transport function were carried out on rat alveolar buds that aggregate to form cysts in submersion culture (34, 53, 54). These cysts accumulate fluid and exhibited lumen negative transepithelial potentials. Treatment with the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransport inhibitor bumetanide produced a 70% decrease in transepithelial potential (34) and reduced the size and number of cysts, consistent with inhibition of fluid transport into the lumen (54). Stimulation with a membrane-permeant analog of cAMP in combination with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) increased the size of the cysts and the lumen negative transepithelial potential difference from 4.6 to 7.3 mV (53, 54). These effects were also blocked by bumetanide. Stimulation with the β-adrenergic receptor agonist isoproterenol or epinephrine also increased the lumen negative potential difference, consistent with an increase in Cl\(^-\) secretion in alveolar cells from rat and late-gestation fetal sheep (53, 83). In more recent studies of rat fetal distal lung epithelial (FDLE) cells, β-adrenergic receptor stimulation with isoproterenol was shown to increase a glibenclamide-sensitive Cl\(^-\) conductance of the apical membrane (9). Similarly, forskolin treatment of FDLE cells was shown to stimulate both Cl\(^-\) and HCO\(_3\)\(^-\) secretion. The effects of forskolin were blocked by apical addition of glibenclamide [an inhibitor of CFTR Cl\(^-\) channels and ATP-sensitive potassium (K\(_{ATP}\)) channels] and basolateral treatment with bumetanide (39). The results of these studies suggest a model (see Fig. 1A) for alveolar anion and fluid secretion similar to that proposed for a variety of secretory epithelia (4, 20). The essential features of this model include Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporters, Na\(^+\)-K\(^+\)-ATPase enzymes, and K\(^+\) channels located in the basolateral membrane and apical Cl\(^-\) channels that are regulated by cAMP. Cl\(^-\) uptake across the basolateral membrane is mediated by electroneutral Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransport. Recycling of Na\(^+\)<ref>

**Fig. 1.** Na\(^+\) and Cl\(^-\) transport models for alveolar epithelial cells. A: anion secretion in early-gestation fetal alveolar epithelial cells. NSC, nonselective cation channel; CFTR, cystic fibrosis transmembrane conductance regulator; AC, adenylyl cyclase. B: transcellular NaCl absorption in adult rat alveolar epithelial type II (AII) cells. HSC, highly selective Na\(^+\) channel. (See text for discussion.)

AJP-Lung Cell Mol Physiol • VOL 284 • MAY 2003 • www.ajplung.org
and K⁺ out of the cell depends on Na⁺-K⁺-ATPase activity and K⁺ channels, respectively. Electrogenic K⁺ efflux sustains the electrical driving force for Cl⁻ efflux across the apical membrane when Cl⁻ channels are activated by increases in cytosolic CAMP. Although a number of details remain to be elucidated, this general model of alveolar cell anion secretion appears to account for the presently available data.

The observations that Cl⁻ and fluid secretion could be stimulated in fetal alveolar epithelial cells by agents that increased cAMP suggested that CFTR may be involved in mediating Cl⁻ exit across the apical membrane. This hypothesis was initially tested by in situ hybridization studies with radioactive labeled anti-sense CFTR probes to localize CFTR mRNA in human fetal lung tissue and cultured lung explants. CFTR mRNA was detected in first- and second-trimester lung tissue. All cells in cultured explants were also found to contain CFTR mRNA, suggesting a potential role in alveolar anion and fluid secretion (55). In a subsequent study, a monoclonal CFTR antibody was shown to label the apical membrane of epithelial cells in cultured human fetal lung explants. These cells were shown to exhibit isoproterenol- and forskolin/IBMX-stimulated Cl⁻ secretion that was blocked by apical addition of the Cl⁻/H⁺ channel inhibitor diphenylamine-2-carboxylate (DPC) and by basolateral addition of bumetanide (52). In rat FDLE cells, CFTR protein was identified by immunocytochemistry, again consistent with a role in Cl⁻ and HCO₃⁻ secretion described above (39). Although a direct link between Cl⁻ channel function and CFTR expression was not absolutely established by these studies, the in situ hybridization and immunolocalization results provide compelling evidence for CFTR expression in fetal alveolar epithelial cells.

CFTR-independent anion secretion has also been shown in fetal mouse lung following stimulation with keratinocyte growth factor (KGF). Treatment with KGF for several hours produced increases in lumen volume of alveolar buds and disrupted branching morphogenesis (87). This effect was observed in both wild-type and CFTR knockout mice and was blocked by treatment with bumetanide. In addition, KGF also inhibited expression of the α-subunit of ENaC, suggesting a reduction in transepithelial Na⁺ absorption. The persistence of anion secretion in the absence of CFTR expression indicates activation of a second anion conductance in the apical membrane by KGF. The molecular identity of this anion conductance is presently unknown.

Cl⁻ channel identification and function in cultured adult alveolar epithelial cells. After the original report of high-conductance, voltage-dependent Cl⁻ channels in adult rat AII cells (31, 76), there have been differing results and conclusions regarding the expression of cAMP-activated Cl⁻ channels in these cells. In a previous study (88), patch-clamp recording techniques were used to measure single channel and whole cell Cl⁻ currents from AII cells stimulated with 500 μM 8-(4-chlorophenylthio)-adenosine 3',5'-cyclic monophosphate (8-cpt-cAMP), a cell-permeant form of cAMP. Only one of 33 cell-attached patches exhibited Cl⁻ channel activation following 8-cpt-cAMP stimulation. Moreover, whole cell current measurements also failed to show increases in Cl⁻ current after stimulation with either 8-cpt-cAMP or the calcium ionophore ionomycin. These results lead to the conclusion that only a very small fraction of adult AII cells expresses cAMP-activated Cl⁻ channels and suggested that Cl⁻ transport occurs passively across the alveolar epithelium through tight junctions between the cells. In contrast to the findings of that study, Jiang et al. (27) demonstrated that stimulation of adult rat alveolar epithelial cell monolayers with the selective β₂-adrenoceptor agonist terbutaline resulted in activation of Cl⁻ channels located in the apical membrane. Similarly, forskolin-activated Cl⁻ channels were also identified in the apical membrane of cultured adult rabbit alveolar epithelial cells (75). Characterization of terbutaline-activated Cl⁻ channels in adult rat alveolar epithelial cells was initially conducted using permeabilized monolayers, where the basolateral membrane was perforated with the pore-forming antibiotic amphotericin B, thus allowing apical-membrane voltage-clamp experiments to be performed (27). The terbutaline- or 8-cpt-cAMP activated Cl⁻ channels possessed a near-linear current-voltage relationship (Fig. 2B) and a reversal potential that became progressively more positive as the Cl⁻ concentration gradient was decreased across the apical membrane. The anion selectivity sequence was found to be SCN⁻ > Br⁻ > Cl⁻ > I⁻, and the channel was blocked by known Cl⁻ channel inhibitors in a concentration-dependent manner (Fig. 3). In a subsequent study, whole cell perforated patch-clamp experiments revealed that both freshly isolated alveolar epithelial cells and alveolar cells maintained in monolayer culture under air-interface conditions expressed terbutaline-activated Cl⁻ currents that were blocked by 5-nitro-2-(3-phenylpropylamino)benzoate or glibenclamide (29). In addition, CFTR protein expression was established by immunocytochemistry, and identification of CFTR mRNA in monolayer cultures of adult rat alveolar epithelial cells was recently demonstrated by RT-PCR (40, 61).

In monolayer cultures of adult rat alveolar cells it has been shown that Cl⁻ channel activation produces stimulation of transepithelial Cl⁻ absorption (32, 40, 61). This flux data support the interpretation that the immediate decrease in short-circuit current (Isc) produced by terbutaline stimulation results from Cl⁻ uptake across the apical membrane through CFTR-like Cl⁻ channels. Although other Cl⁻ channels have been observed in patch-clamp studies of cultured alveolar epithelial cells (31, 76), CFTR is the only cAMP-activated Cl⁻ channel known to be present. Replacement of extracellular Cl⁻ with a nontransported anion or treatment with Cl⁻ channel blockers known to inhibit CFTR abolishes this current response (27, 29). Thus CFTR appears to serve as the major influx pathway in support of transcellular Cl⁻ absorption in adult rat alveolar epithelial cells following stimulation by β-adrenergic receptor agonists. The transporter responsible...
for Cl⁻ exit across the basolateral membrane is not certain; however, a likely mechanism would involve electroneutral cotransport with K⁺/H⁺. Such a mechanism would take advantage of the outwardly directed chemical potential for K⁺/H⁺, and Cl⁻ efflux would not be limited by a relatively depolarized basolateral membrane. In support of this idea, recent studies of Cl⁻/H⁻ transport across the basolateral membrane indicate that terbutaline stimulates an electroneutral Cl⁻/H⁻ efflux mechanism that is dependent on an outwardly directed K⁺/H⁺ concentration gradient (40, 61). Moreover, RT-PCR experiments revealed that monolayers of adult rat alveolar epithelial cells contain mRNA for K⁺/Cl⁻ cotransporter (KCC) 1, KCC3, and KCC4 isoforms of the KCl cotransporters and that expression of protein for the KCC1 and KCC4 isoforms was detected by Western blots. Thus these findings support a model for terbutaline regulation of transcellular Cl⁻ absorption involving Cl⁻ uptake by CFTR Cl⁻ channels located in the apical membrane and Cl⁻ exit across the basolateral membrane mediated by KCl cotransport (Fig. 1B). In contrast to results obtained with adult rat alveolar cells, agents that increase cytosolic cAMP in alveolar cells from adult rabbits produce an increase in bumetanide-sensitive Iₛₑ, consistent with Cl⁻ secretion (58).

At this time, the distribution of CFTR into AI and AII cells within the alveolus is not clearly defined. Unlike recent studies describing the identification and functional characterization of Na⁺ transport proteins within AI cells (5, 30), there are no data confirming the presence of CFTR or any other Cl⁻ channel proteins in AI cells in the intact lung. As mentioned above, cultured alveolar epithelial cells have been shown to express CFTR mRNA and protein, but these cultures most likely contain both AI and AII cell populations (9, 29, 40, 61). Therefore, further studies will be needed to establish the localization of Cl⁻ channel proteins in AI cells, and this could have implications on what additional signaling pathways maybe involved in regulation of Cl⁻ transport within the alveolus.
Role of CFTR in alveolar fluid clearance in mouse and human lung. A recent, seminal investigation utilizing in situ perfused lung experiments with wild-type and ΔF508 CFTR-expressing mice and ex vivo human lung perfusion studies demonstrated a direct role for CFTR Cl\(^-\) channels in alveolar fluid clearance (18). In experiments with wild-type mice, instillation of fluid containing Cl\(^-\) channel blockers (known to inhibit CFTR) into the airways and alveolar space completely blocked isoproterenol-stimulated fluid absorption (Fig. 4A). In addition, mice expressing the ΔF508 CFTR mutation failed to exhibit a significant increase in fluid clearance following isoproterenol stimulation (Fig. 5). Experiments with ex vivo human lungs also showed that infusion of the CFTR Cl\(^-\) channel inhibitor glibenclamide inhibits the effects of terbutaline on fluid clearance (Fig. 4B). These blocker experiments and the results obtained with ΔF508 CFTR mice indicate that CFTR activation is critical to stimulation of fluid absorption by adrenergic agonists. To establish that CFTR is involved in Cl\(^-\) uptake from the alveolar fluid, \(^{22}\text{Na}\) and \(^{36}\text{Cl}\) uptake measurements were performed in the mouse lung at 23°C, a temperature that inhibits alveolar fluid clearance. It was observed that \(^{22}\text{Na}\) uptake from the alveolar fluid was similar to that of \(^{36}\text{Cl}\) under basal conditions, but following isoproterenol stimulation, a significant increase in \(^{36}\text{Cl}\) uptake was observed with no change in \(^{22}\text{Na}\) uptake. Moreover, when these experiments were repeated with ΔF508 CFTR mice, no increase in \(^{36}\text{Cl}\) removal was detected. These results indicate that CFTR is involved in \(^{36}\text{Cl}\) uptake from the alveolar fluid and that isoproterenol stimulation increases the Cl\(^-\) conductance of the alveolar epithelium.

Interestingly, the complete inhibition of isoproterenol-stimulated fluid clearance by Cl\(^-\) channel blockers in wild-type mice and the lack of effect of adrenergic stimulation on fluid clearance in ΔF508 CFTR-expressing mice may indicate that adrenergic stimulation does not increase apical Na\(^+\) conductance in adult mouse alveolar cells. The same may also be true for perfused human lung, where blockers of CFTR completely abolished the terbutaline-stimulated increase in fluid absorption. Alternatively, Na\(^+\) and Cl\(^-\) uptake across the apical membrane may be tightly coupled so that inhibition of CFTR results in depolarization that limits Na\(^+\) uptake despite increases in apical membrane Na\(^+\) channel activity.

The issue of whether CFTR is involved in the resolution of alveolar edema was studied in a hydrostatic volume overload model using wild-type and ΔF508 CFTR-expressing mice (18). In this model, volume overload was established by saline infusion resulting in a \(\approx 30\%\) increase in wet-to-dry weight ratio of lungs from wild-type and heterozygous ΔF508 mice. In homozygous ΔF508 mice, the ratio was 64% with histological evidence for significant alveolar edema. Blockade of endogenous catecholamine effects on the lung epithelium using the \(\beta\)-adrenergic receptor antagonist propranolol produced an increase in the wet-to-dry weight ratio of lung tissue from wild-type and heterozygous ΔF508 CFTR mice to a level comparable with that of homozygous ΔF508 mice. These results indicate that CFTR activation is an important physiological response to fluid accumulation in the distal airways and alveoli and suggest that patients suffering from cystic fibrosis (CF) may have a greater susceptibility to pulmonary edema.

**Relationship between Cl\(^-\) channel activation and Na absorption in adult alveolar cells.** In our initial study of ion transport in monolayer cultures of adult rat alveo-

---

![Fig. 4. Effects of isoproterenol (100 \(\mu\)M) or terbutaline (100 \(\mu\)M) and glibenclamide (100 \(\mu\)M) on fluid clearance in mouse and human lung (reproduced from Ref. 18).](image-url)
Data reported as means ± SE; *P < 0.05 compared with control. [From Fang et al. (18).]

Fig. 5. Fluid clearance from the distal air spaces of wild-type (open bars) and ΔF508 CFTR (solid bars) mice (data reproduced from Ref. 18). Measurements were performed using in situ perfused lung at 37°C under basal conditions (n = 24 wild type, n = 7 ΔF508) and in the presence of 100 μM isoproterenol (n = 9 wild type, n = 6 ΔF508). Data reported as means ± SE, *P < 0.05 compared with control. [From Fang et al. (18).]

Lar epithelial cells (27), we suggested that Cl− channel activation by terbutaline plays a role in stimulating amiloride-sensitive Na+ transport by increasing the driving force for Na+ entry across the apical membrane. This explanation was based on the observation that no increase in amiloride-sensitive conductance could be detected following stimulation of monolayers with terbutaline and that Cl− replacement or voltage clamping the apical membrane to 0 mV abolished the time-dependent increase in Isc or apical membrane current. This interpretation has been challenged by results of other investigations that have reported increased apical membrane Na+ channel activity following adrenergic receptor stimulation (1, 7, 9, 37, 46). The basis for these differing results is most likely related to differences in culture conditions or perhaps the original source of alveolar cells, as may be the case for rat FDLE cells grown under high-O2 conditions as a method to produce the adult transport phenotype (1, 9, 70). In our experiments, monolayers were not treated with corticosteroids and were most likely expressing amiloride-sensitive NSC channels and HSC channels as suggested by the reversal potential (47 mV) of the amiloride-sensitive Na+ current. In addition, the cells were grown under serum-free conditions, and a relatively low concentration of terbutaline (2 μM) was used to stimulate the cells. One possible explanation for not observing an increase in Na+ conductance in response to terbutaline is that, in the absence of corticosteroids, the pool of Na+ channels available for insertion into the apical membrane may be relatively low compared with cells cultured in the presence of dexamethasone or aldosterone. Thus the magnitude of increased Na+ channel activity may have been too small to detect in voltage-clamp experiments using amphotericin B-permeabilized monolayers. In addition, previous studies (20) have reported using much higher concentrations of β-receptor agonists (20 μM or more), perhaps leading to greater increases in cytosolic cAMP and subse-

quent higher levels of Na+ channel expression and activity in the apical membrane. Another interesting point regarding the cells used in our studies is that terbutaline stimulation at either 2 or 20 μM does not lead to an increase in cytosolic calcium concentration (unpublished data). This result was similar to studies by Isohama et al. (23), where treatment with 10 μM terbutaline had no effect on intracellular Ca2+ concentration, but when added in combination with phorbol ester, a significant increase in cytosolic Ca2+ was observed. Thus adrenergic receptor stimulation in combination with protein kinase C activation was necessary to increase intracellular calcium. This was not the case in alveolar cells grown in the presence of corticosteroids, where terbutaline alone was sufficient to increase cytosolic Ca2+ concentration (7). This effect of terbutaline on intracellular Ca2+ has been shown to be necessary for NSC channel activation (7, 43, 44) and presumably explains why activation of these channels was not observed in our previous studies.

In a recent study (35), the direction of Cl− flux across rat FDLE cell monolayers (grown under conditions where they exhibited the adult alveolar cell transport phenotype) was shown to be differentially regulated by adrenergic and purinergic receptor agonists. These authors observed that β-adrenergic receptor stimulation with isoproterenol produced an apical-to-basolateral Cl− flux as measured using Cl−-selective microelectrodes. Cl− absorption (50–70%) was inhibited by Cl− channel blockers, indicating that Cl− channels present in the apical membrane were responsible for Cl− uptake. In contrast, stimulation with the P2Y receptor agonist UTP produced an increase in the basolateral-to-apical Cl− flux. Previous studies on Na+ transport in rat FDLE cells showed that UTP inhibited apical Na+ channels, whereas stimulation with isoproterenol increased apical membrane Na+ conductance (9, 22, 71). Although the mechanisms underlying the actions of isoproterenol and UTP are not completely understood, it is reasonable to suggest that the direction of Cl− movement across the apical membrane depends on the influence of Na+ channel activation or inhibition on membrane voltage relative to the Cl− channel reversal potential. Apical membrane depolarization (by isoproterenol) or hyperpolarization (following UTP) as a consequence of Na+ channel regulation would contribute to the driving force and influence the direction of Cl− movement across the membrane. The results of this study would indicate that Na+ and Cl− movement across the apical membrane are interdependent and influence each other through changes in apical membrane potential.

K+ CHANNELS IN ALVEOLAR EPITHELIAL CELLS

K+ channels in epithelial cells play an essential role in transepithelial electrolyte and fluid transport. In Cl−-secreting epithelia for example, basolateral K+ channels are typically activated in parallel with apical Cl− channels in response to secretagogue stimulation (2, 13, 14, 50, 51, 77, 78, 82). K+ efflux through these
channels is essential in preventing significant cell depolarization, thus helping to sustain the electrical driving force for electrogenic Cl\(^{-}\) exit across the apical membrane (2, 13, 50, 77, 82). Inhibition of these K\(^{+}\) channels effectively blocks transepithelial Cl\(^{-}\) secretion. K\(^{+}\) channels also play a similar role in Na\(^{+}\)-absorbing epithelia, where K\(^{+}\) exit helps to offset membrane depolarization produced by electrogenic Na\(^{+}\) influx (8, 85, 86). At this time, an extensive literature on the identity and function of K\(^{+}\) channels in alveolar epithelial cells is not available. However, studies so far in fetal and adult alveolar epithelial cells suggest the presence of multiple K\(^{+}\) channel families and accessory regulatory proteins. The following section describes what is known about the molecular identity, localization, and proposed function of specific K\(^{+}\) channel family members found in alveolar epithelial cells.

*Kv-type K\(^{+}\) channels.* Two distinct types of K\(^{+}\) channels similar to delayed rectifier potassium channels have been identified in isolated adult rat alveolar type II cells and human lung adenocarcinoma cell line A549 (12, 33, 65). DeCourcey et al. (12) identified the potassium channels in adult rat alveolar type II cells as either normal (n)- or large (l)-type channels, depending on their voltage dependency and tetraethylammonium (TEA) sensitivity. Most alveolar type II cells possessed the n-type channel that was activated at more negative potentials (−30 mV) compared with l-type channels, which were activated at −10 mV. Peers et al. (65) also showed two distinct potassium channel currents in adult AII cells that possessed different voltage dependency and blocker sensitivities. Low threshold currents were activated at more depolarized voltages than −40 mV, and these currents were blocked by 2 mM 4-aminopyridine (4-AP). In contrast, high threshold currents were activated at −20 mV and were 4-AP insensitive. In human A549 cells, a portion of the K\(^{+}\) current was inhibited by TEA, and the residual current was blocked by cesium (33). Recently, more rapidly inactivating potassium channel currents have been identified in adult rat alveolar epithelial cells (Fig. 6, A–C). The molecular identity of K\(v\) channels was determined using RT-PCR, Western blot, and immunocytochemistry. Eight distinct \(\alpha\)-subunits (Kv1.1, Kv1.3, Kv1.4, Kv2.2, Kv4.1, Kv4.2, Kv4.3, and Kv9.3), three \(\beta\)-subunits (Kv\(\beta\)1.1, Kv\(\beta\)2.1, and Kv\(\beta\)3.1), and two K\(^{+}\) channel-interacting proteins (KChIP: KChIP2 and KChIP3) were detected in monolayer cultures of these cells by RT-PCR. Among the \(\alpha\)-subunits, Kv1.1, Kv1.3, Kv1.4, Kv4.2, and Kv4.3 were identified at the protein level by Western blot analysis and were localized to the apical membrane of adult rat alveolar epithelial cells by immunocytochemistry (Table 1).

In considering the role of K\(v\) channels in alveolar cell electrolyte transport, we find it interesting to note that the K\(^{+}\) concentration in alveolar epithelial lining fluid is greater than plasma, suggesting K\(^{+}\) secretion (15, 16, 59, 75, 84). The finding that K\(v\) channels are present in the apical membrane of alveolar epithelial cells suggests the possibility that these channels are involved in K\(^{+}\) secretion into the alveolar space. The mean window current, derived from activation and steady-state inactivation curves obtained from whole cell patch-clamp experiments, lies between −40 and 10 mV (Fig. 6D). Thus K\(v\) channels possess significant
open probability at voltages within this range and could contribute to basal potassium secretion, provided that the apical membrane is sufficiently depolarized. Another possible function of Kv channels may be related to oxygen sensing. In cultured alveolar cell monolayers, an electrically silent K\(^+\) channel \(\alpha\)-subunit, Kv9.3 (79, 80), was detected by RT-PCR (Table 1). Previously, Kv2.1/Kv9.3 heteromeric channels have been shown to be significantly inhibited by hypoxia in several expression systems, including COS cells (64) and mouse L cells (21). In addition, chronic hypoxia downregulates mRNA expression of Kv1.1, Kv4.3, and Kv9.3 \(\alpha\)-subunits in pulmonary artery smooth muscle cells (69). Kv \(\beta\)-subunits are also known to confer O\(_2\) sensitivity to Kv4.2 in HEK293 cells (66). Therefore, Kv \(\alpha\)- and \(\beta\)-subunits expressed in rat alveolar epithelial cells may function as oxygen sensors, perhaps detecting differences in alveolar ventilation or changes in \(O_2\) diffusion across the apical membrane.

**Kir channels.** Alveolar type II cells isolated from fetal guinea pig exhibited Kir currents that were blocked by 1 mM Ba\(^{2+}\) and regulated by both protein kinase A and protein phosphatases (PP) including PP-1/2A and PP2C (56, 57). In this cell preparation, Kir2.1 was detected by RT-PCR, and we have subsequently identified Kir2.1 mRNA transcripts in adult rat alveolar cells (Table 1). The localization of Kir2.1 in either fetal guinea pig AII cells or adult rat alveolar epithelial cells has not been determined. In a previous study, a Kir channel (Kir1.1) was identified in the apical membrane of epithelial cells from the thick ascending limb of Henle’s loop (41). In these cells, K\(^+\) uptake into the cell occurs through an Na\(^+-K^+\)-2Cl\(^-\) cotransporter and is recycled back into the tubule lumen through Kir1.1. Thus Kir1.1 plays an important role in sustaining Na\(^+\) and Cl\(^-\) transport across epithelial cells from this nephron segment. However, the role of Kir channels in alveolar epithelial cells is unclear. One possibility could be that Kir2.1 is located in basolateral membrane, where it may play a role in K\(^+\) recycling necessary for maintaining Na\(^+-\)K\(^+\)-ATPase activity. This may be particularly important under conditions of stimulated Na\(^+\) absorption where increased Na\(^+-\)K\(^+\)-ATPase activity may depend on the rate of K\(^+\) recycling.

In an earlier study by Sakuma et al. (73), the presence of ATP-sensitive potassium (K\(_{\text{ATP}}\)) channels in human alveolar cells was suggested from experiments with 2-(3,4-dihydro-2,2-dimethyl-6-nitro-2H-1,4-benzoxazin-4-yl) pyridine N-oxide (YM934), which increased both potassium influx into the alveolar space and alveolar fluid clearance in human lung. Addition of glibenclamide, a K\(_{\text{ATP}}\) channel blocker, inhibited the YM934-stimulated increase in alveolar fluid clearance, providing additional evidence to suggest a role for K\(_{\text{ATP}}\) channels. Although K\(_{\text{ATP}}\) channels may be present in

### Table 1. Molecular evidence of K\(^+\) channels identified in alveolar epithelial cells

<table>
<thead>
<tr>
<th>Standard Names of K(^+) Channels</th>
<th>Accession No. (species)</th>
<th>mRNA</th>
<th>Protein</th>
<th>Cell System</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1.1</td>
<td>X12589 (rat)</td>
<td>+</td>
<td>+</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv1.2</td>
<td>M74449 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv1.3</td>
<td>X16001 (rat)</td>
<td>+</td>
<td>+</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv1.4</td>
<td>M32867 (rat)</td>
<td>+</td>
<td>+</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv1.5</td>
<td>M27158 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv1.6</td>
<td>X17621 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv2.1</td>
<td>X16476 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv2.2</td>
<td>M77482 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv3.1</td>
<td>M68880 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv3.2</td>
<td>M59211 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv3.3</td>
<td>M84210 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv3.4</td>
<td>X62841 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv4.1</td>
<td>M64226 (mouse)</td>
<td>+</td>
<td>+</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv4.2</td>
<td>S64320 (rat)</td>
<td>+</td>
<td>+</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv4.3</td>
<td>U42975 (rat)</td>
<td>+</td>
<td>+</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv9.3</td>
<td>AF029056 (rat)</td>
<td>+</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>AF043472 (human)</td>
<td>+</td>
<td>N/A</td>
<td>cell line A549</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Kv8.1</td>
<td>X70662 (rat)</td>
<td>+</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv8.2</td>
<td>X76724 (rat)</td>
<td>+</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kv8.3</td>
<td>X76723 (rat)</td>
<td>+</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>KChIP1B</td>
<td>AY142709 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>KChIP2</td>
<td>AP269283 (rat)</td>
<td>+</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>KChIP3</td>
<td>AB043892 (rat)</td>
<td>+</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td>Kir2.1</td>
<td>L48490 (rat)</td>
<td>+</td>
<td>N/A</td>
<td>adult rat</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Z48971 (guinea pig)</td>
<td>−</td>
<td>N/A</td>
<td>fetal guinea pig</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Kir6.1</td>
<td>D42145 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>unpublished data</td>
<td></td>
</tr>
<tr>
<td>Kir6.2</td>
<td>X97041 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>unpublished data</td>
<td></td>
</tr>
<tr>
<td>SUR1</td>
<td>L40624 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>unpublished data</td>
<td></td>
</tr>
<tr>
<td>SUR2</td>
<td>D85598 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>unpublished data</td>
<td></td>
</tr>
<tr>
<td>Slo1</td>
<td>U59995 (rat)</td>
<td>−</td>
<td>N/A</td>
<td>unpublished data</td>
<td></td>
</tr>
<tr>
<td>IK1</td>
<td>AF022797 (human)</td>
<td>+</td>
<td>N/A</td>
<td>cell line A549</td>
<td>81</td>
</tr>
</tbody>
</table>

Kv, voltage-gated K\(^+\) channel; KChIP, K\(^+\) channel-interacting protein; Kir, inwardly rectifying K\(^+\) channel. +, Detected; −, Not detected; N/A, data not available.
human alveolar epithelial cells, the components of these potassium channels [Kir6.1, Kir6.2, sulfonylurea receptor (SUR 1, and SUR2)] could not be detected in primary adult rat alveolar epithelial cells by RT-PCR (Table 1).

**Ca**<sup>2+</sup>–activated **K**<sup>+</sup> channels. Two-types of **Ca**<sup>2+</sup>–activated **K**<sup>+</sup> channels, large-conductance (BK) and intermediate-conductance (IK) **K**<sup>+</sup> channels, have been identified in A549 cells. Ridge et al. (72) observed an ~242-pS **K**<sup>+</sup> channel that was activated by increases in [**Ca**<sup>2+</sup>] and membrane depolarization. Blockers including Ba**<sup>2+</sup>** and TEA, and quinidine were shown to inhibit this large-conductance **K**<sup>+</sup> channel. BK channels may play a role in repolarizing cells following depolarization or calcium entry. However, the physiological role of BK channels in primary alveolar epithelial cells remains to be resolved. IK channels were activated by adenosine or nucleoside transport blockers such as nitrobenzylthioinosine in A549 cells (81). In addition, clotrimazole, a selective blocker of IK channels, was also shown to inhibit the channel. In RT-PCR experiments, mRNA transcripts for IK-1 were detected in these cells. However, we were unable to detect slo1, the principal subunit of BK channels and IK-1 in cultured adult rat alveolar epithelial cells by RT-PCR. Thus at this time there is no functional or molecular evidence for expression of these **Ca**<sup>2+</sup>–activated **K**<sup>+</sup> channels in primary rat alveolar epithelial cells (88).

**SUMMARY AND CONCLUSIONS**

**Cl**<sup>−</sup> and **K**<sup>+</sup> channels serve multiple functions in excitable and nonexcitable cells, including prominent roles in cell signaling, control of cell volume, and regulation of membrane potential. In alveolar epithelial cells, **Cl**<sup>−</sup> and **K**<sup>+</sup> channels are involved in regulating the volume and ionic composition of alveolar fluid. Results from previous investigations with cultured fetal alveolar epithelial cells and more recent work with adult cells and perfused lung studies has shown that CFTR plays a role in both anion and fluid secretion or NaCl and fluid absorption, depending on stage of development. In the fetal state, anion secretion establishes an osmotic driving force necessary for fluid secretion into the developing alveoli and airways. In the adult lung, CFTR activation following adrenergic receptor stimulation appears to be essential for increasing alveolar fluid clearance. This observation suggests that an increase in apical membrane anion permeability is necessary for increased fluid transport across the alveolar epithelium in response to adrenergic agonists. Whether CFTR modulates the expression or activity of ENaC in the apical membrane of alveolar epithelial cells is presently unknown. Evidence that CFTR is important for adrenergic receptor stimulation of alveolar fluid clearance in the adult human lung suggests the possibility that patients with CF may be less responsive to terbutaline treatment under conditions of hydrostatic or lung injury pulmonary edema.

Investigations over the past 15 yr have shown that alveolar epithelial cells express a variety of **K**<sup>+</sup> channels, including Kir channels, **Ca**<sup>2+</sup>–activated **K**<sup>+</sup> channels, and Kv channels. The functional roles of these channels in alveolar epithelial cell transport function remain to be elucidated, but some ideas have been suggested. For example, the proposed localization of Kir2.1 channels in the basolateral membrane of fetal guinea pig AI cells suggests a possible role in K<sup>+</sup> recycling, in support of increases in Na<sup>+</sup>–**K**<sup>+</sup>–ATPase activity. In contrast, the apical localization of at least some of the conducting α-subunits of Kv channels suggests a role in K<sup>+</sup> secretion, or perhaps O<sub>2</sub> sensing, given that Kv β-subunits have been previously shown to confer oxygen sensitivity to certain Kv channels in smooth muscle cells. Clearly, more studies are needed to understand the physiological importance of these channels and to identify other **K**<sup>+</sup> channel types that are important in sustaining transepithelial Na<sup>+</sup> and Cl<sup>−</sup> transport across the alveolar epithelium.

**REFERENCES**


