J. Membrane Biol. 191, 133–139 (2003)
DOI: 10.1007/s00232-002-1049-x

The Journal of
Membrane
Biology

Basolateral Cl^- Transport Is Stimulated by Terbutaline in Adult Rat Alveolar Epithelial Cells

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Received: 17 June 2002/Revised: 12 September 2002

Abstract. Stimulation of adult rat alveolar epithelial cells with terbutaline was previously shown to activate Cl^- channels in the apical membrane. In this study, we show that terbutaline stimulates net transepithelial (apical-to-basolateral) Cl^- absorption from 0.19 ± 0.13 to 1.43 ± 0.31 $\mu mol \times cm^{-2} \times hr^{-1}$. Terbutaline also increases net Cl^- efflux across the basolateral membrane under conditions where an outward $[K^+]$ gradient exists and the membrane voltage is clamped at zero mV. When the $[K^+]$ gradient is eliminated, the effect of terbutaline on net Cl^- efflux is inhibited to the extent that no significant Cl^- efflux can be detected across the basolateral membrane. RT-PCR experiments detected mRNA for three KCl cotransport isoforms (KCC1, KCC3 and KCC4) in monolayer cultures of alveolar epithelial cells. Western blot analysis using antibodies to the four cloned isoforms of KCl cotransporters revealed the presence of KCC1 and KCC4 isoforms in monolayer cultures of these cells. These results provide evidence suggesting a role for KCl cotransport in terbutaline-stimulated transepithelial Cl^- absorption.

Key words: KCC — KCl cotransport — CFTR — Cystic fibrosis — Alveolar fluid clearance — Cl^- channels

Introduction

The adult alveolar epithelium is responsible for regulating the ionic composition and volume of alveolar fluid along the apical surface of the alveolus through active absorption of electrolytes and solutes from the alveolar space (Cheek, Kim & Crandall, 1989, Kim, Cheek & Crandall, 1991, Matalon & O'Brodovich, 1996, Lazrak, Nielsen & Matalon, 2000). Alveolar fluid and electrolyte absorption is stimulated by treatment with β-adrenergic receptor agonists such as terbutaline and isoproterenol (Crandall et al., 1986, Saumon et al., 1987, Cheek et al., 1989, O'Brodovich, 1996, Jiang, Ingbar & Grady, 1998, Saldias et al., 1998, Lazrak et al., 2000, O'Grady Jiang & Ingbar, 2000, Widdcombe, 2000). Previous isotopic flux measurements showed that alveolar epithelial cell monolayers actively absorb Na^+ and Cl^- in response to β-adrenergic stimulation (Kim et al., 1991). Addition of terbutaline to monolayer cultures of adult rat alveolar epithelial cells produced an initial rapid decrease in short circuit current (I_{sc}), consistent with anion absorption. This decrease in current was followed by a slow increase in I_{sc} that reached a new steady-state plateau that was sometimes greater than the basal current (Jiang et al., 1998). This time-dependent increase in current was inhibited by apical addition of amiloride, suggesting that terbutaline increases transepithelial Na^+ absorption and that amiloride-sensitive Na^+ channels are involved in the process (O'Grady et al., 2000).

In previous studies, terbutaline activated apical membrane Cl^- channels (Jiang et al., 1998, Lazrak et al., 2000, Jiang et al., 2001). Our data and previously published flux experiments suggested that absorption of Cl^- by the epithelium involved uptake of Cl^- from the luminal fluid through terbutaline-activated Cl^- channels. At this time, the mechanism of Cl^- efflux across the basolateral membrane is unknown. One likely possibility is that KCl cotransport is involved. Presently, four distinct isoforms of the KCl cotransporter have been cloned and characterized in heterologous expression systems (Lauf & Adragna,

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2000). In rat, the KCC1 isoform is widely expressed in most tissues and is thought to play a role in cell volume regulation and salt transport in epithelial cells (Gillen et al., 1996, Mercado et al., 2000). The KCC3 and KCC4 isoforms are also widely expressed, with the KCC3 isoform being most abundant in heart, kidney and brain, and the KCC4 isoform expressed in muscle, brain, lung, heart and kidney (Race et. al., 1999, Mount et al., 1999). In contrast to these other isoforms, KCC2 is present only in neurons (Payne, Stevenson & Donaldson, 1996).

Previous studies of KCl cotransport function in epithelia showed that basolateral KCl cotransport activity played an important role in transepithelial Cl^- absorption in *Necturus* gallbladder and in renal tubule epithelial cells (Reuss, 1983, Warnock & Eveloff, 1989). A more recent study in rat colonic epithelia showed that the KCC1 isoform mediated K^+ absorption and that its expression was enhanced under K^+ depletion conditions (Sangan et al., 2000). To date, specific KCl cotransport isoforms have not been identified in alveolar epithelial cells. The goal of the present study was to investigate the mechanism and potential regulation by terbutaline of Cl^- transport across the basolateral membrane and to determine whether known KCl cotransport isoforms are expressed in monolayers of adult rat alveolar epithelial cells.

Materials and Methods

Materials

Male pathogen-free Sprague-Dawley rats weighing 150–174 g were purchased from Harlan (Indianapolis, IN). Elastase was purchased from Worthington Biochemical (Freehold, NJ). Rat immunoglobulin G (IgG), deoxyribonuclease I, non-essential amino acids, bovine serum albumin (BSA), L-glutamine, HEPES, trypsin inhibitor, terbutaline and glibenclamide were obtained from Sigma (St. Louis, MO). Dulbecco's modified Eagle's medium, Ham's F-12 nutrient mixture in a 1:1 ratio (DMEM/F12), penicillin-streptomycin and phosphate buffered saline (PBS) were purchased from GIBCO/BRL (Grand Island, NY). 120- and 40-μm Nitex mesh was purchased from Tetko (Elmsford, NY). Tissue-culture-treated Transwell polycarbonate filters were purchased from Corning Costar (Cambridge, MA). ^{36}Cl was purchased from ICN (Aurora, OH).

Cell Preparation and Culture

Alveolar epithelial cells were isolated from adult rat lungs using a modification of the protocol previously described by Borok et al. (1996) and Jiang et al. (1998). Lungs were perfused with (in mM): 140 NaCl, 5 KCl, 2.5 NaH_2PO_4, 1.3 $MgSO_4$, 2.0 $CaCl_2$, 6 glucose, 10 HEPES. Following removal, the lungs were repeatedly lavaged with (in mM): 140 NaCl, 5 KCl, 2.5 NaH_2PO_4, 6 glucose, 10 HEPES) to eliminate macrophages. The lungs were then filled with elastase-containing solution (2.7 IU/ml) and were incubated at 37°C for 30 min in a shaker bath. Finely minced tissues were filtered through 120- and 40-μm Nitex mesh. Cells were further purified by panning on IgG-coated culture dishes to remove remnant macrophages and suspended directly in serum-free DMEM/F12 medium supplemented with 1.25 mg/ml BSA, 0.1% nonessential amino acids, 2.0 mM glutamine, 100 U/ml sodium penicillin G, and 100 μg/ml streptomycin. The cells were seeded onto Transwell membrane filters (4.52 cm^2, 0.4 μm pore size) at a density of 1.5×10^6 cells/cm^2 to prepare confluent monolayers. The medium was changed every other day. The resistance of the monolayers was measured using an epithelial voltohmmeter (World Precision Instruments, New Haven, CT). Transport studies were performed on day 7 following isolation.

Chloride Flux Experiments

High-resistance monolayers were mounted in Ussing chambers and bathed with identical DMEM/F12 media at 37°C. The bathing solutions were bubbled with 95% O_2-5% CO_2 on each side to maintain a pH = 7.4. Transepithelial flux experiments were performed under voltage-clamp conditions where the transepithelial potential was held at zero mV. $^{36}Cl^-$ (4 μCi) was added to either the apical or basolateral solutions of paired monolayers with transepithelial resistances within 10% of each other. The monolayers were incubated with isotope for 30 min prior to the beginning of the first flux period. Two 20-minute flux measurements were conducted. The first flux period was used to determine the basal apical-to-basolateral and basolateral-to-apical unidirectional fluxes and the second-period flux was performed in the presence of 2 μM terbutaline (basolateral addition). To measure Cl^- fluxes across the basolateral membrane, the apical membrane was first permeabilized with amphotericin B (10 μM) and the apical surface bathed with intracellular solution (in mM: 120 $KMeSO_4$, 30 mannitol, 3 Ca-gluconate, 0.7 $MgSO_4$, 20 $KHCO_3$, 0.3 KH_2PO_4 and 10 NaCl). The basolateral surface was bathed with the same solution except that 120 $KMeSO_4$ was replaced with 120 $NaMeSO_4$. Under these conditions, [CI] (10 mM) was equal on both sides and the basolateral membrane was voltage-clamped at zero mV. ^{36}Cl (4 μCi) was subsequently added to either the apical or basolateral solutions of paired monolayers with resistances within 10% of each other. Isotope was allowed 30 minutes to equilibrate with the intracellular compartment. Again, two 20-minute flux measurements were made to initially determine the basal unidirectional Cl fluxes across the basolateral membrane followed by a second 20-minute flux in the presence of 2 μM terbutaline.

Identification of CFTR, KCC Isoforms and NKCC1 From Rat Alveolar Cells by RT-PCR

Reverse transcription-polymerase chain reaction (RT-PCR) was used to identify CFTR, NKCC1 and KCC isoforms in rat alveolar epithelial cells. Total RNA was extracted from rat alveolar cells grown in monolayer culture for 7 days and from rat brain using TRIzol reagent (Gibco, Grand Island, NY). Total RNA (2 μg) was reverse-transcribed using random hexamer primers (Life Technologies, Rockville, MD) and the Superscript II reverse transcription kit (Gibco, Grand Island NY). Primers used in this study are shown in Table 1. The initial denaturation condition was 94°C for 4 min, followed by 94°C for 45 sec, annealing temperature 54–65°C (*see* Table 1) for 45 sec, and 72°C for 1 min for 30 cycles. All of the PCR products were gel-purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and purified products were sequenced using the gene-specific primers to confirm the amplified sequences.

Western Blot Analysis

Cells grown in monolayer culture for 7 days were disrupted with TRIzol reagent, homogenized, and resuspended in cold 1% SDS,

Table 1. Forward and reverse primers for PCR reaction

Standard Names (GenBenk Accession No.)	Fragment Size (bp)	Forward/Reverse Primers	Annealing Temperature (°C)
CFTR*	800	5′-CTGCTGACCACCTGTCTGAA-3′ 5′-GAAGAAGGCGGAGCTAGTGA-3′	54
KCC1 (U55815)	804	5′-GGACTTTCTTCTGCCACAGC-3′ 5′-AGTTGGGGGTCCTCAGAAGT-3′	54
KCC2 (U55816)	399	5′-CTCAACAACCTGACGGACTG-3′ 5′-GCAGAAGGACTCCATGATGCCTGCG-3′	65
KCC3 (AF211854)	799	5′-ACTCCATCACAGGGGAGCAC-3′ 5′-CCAGCATAGATAGCCAAGAT-3′	60
KCC4 (AF105365)	362	5′-CTGGCCAACTACACCAACCT-3′ 5′-GTGCCCAGGTAGAAGCAGAG-3′	60
NKCC1 (AF051561)	566	5′-GGTTCTCCAAACTCACGG-3′ 5′-GTCTTGCCATCCTCTTCCTC-3′	60

*Fiedler et al., 1992.

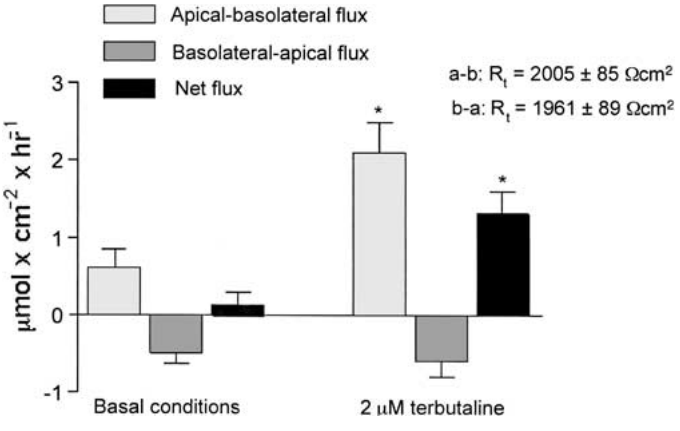

Fig. 1. Transepithelial Cl^- fluxes measured under continuous short-circuited conditions (voltage-clamped at zero mV). Monolayers were bathed on both sides with DMEM/F12 media as described in the Methods ($n = 6$ paired monolayers using cells pooled from 8 adult rats).

EDTA (1mM) plus protease inhibitors (in μg/ml: PMSF 50, Aprotinin 1, Pepstatin 1, Leupeptin 1, all from Sigma). A protein assay was performed using a BCA Protein Assay Kit (Pierce, Rockford, IL). Proteins were separated by standard polyacrylamide gel electrophoresis (8%). Electroblotting was done using Immobilon-P (Millipore, Bedford, MA). After washing, blots were reacted overnight in primary antibody (rabbit anti-mouse KCC1 and KCC4 from Chemicon, Temecula, CA, 1 μg/ml final concentration) in freshly prepared 1× Transfer Buffer (192 mM glycine, 25 mM Tris-base, 20% methanol) containing 5% milk. ECL substrate (Amersham, Piscataway, NJ) was used to identify protein bands by autoradiography.

Statistics

All values in figures 1 and 2 are presented as means ± SEM, n is the number of monolayers in each experiment. The differences between control and treatment means were analyzed using a t-test for paired and unpaired means where appropriate. A value of $P < 0.05$ was considered statistically significant.

Results and Discussion

The effect of β-adrenergic receptor stimulation with terbutaline on transepithelial Cl^- transport across cultured monolayers of adult alveolar epithelial cells is shown in Fig. 1. Under basal conditions, net Cl^- transport was not significantly different from zero. Treatment with terbutaline (2 μM) produced a significant increase in the apical-to-basolateral unidirectional Cl^- flux with no significant effect on the basolateral-to-apical unidirectional flux. The result was an increase in net Cl^- absorption from 0.19 ± 0.13 to 1.43 ± 0.31 $\mu mol \times cm^{-2} \times hr^{-1}$. These findings demonstrate that terbutaline activates a transcellular pathway for Cl^- absorption across the epithelium. In a second series of experiments, we investigated the effects of terbutaline on unidirectional Cl^- fluxes across the basolateral membrane under voltage-clamp conditions (the basolateral membrane was held at zero mV). In these studies, the apical membrane was permeabilized with the pore-forming antibiotic amphotericin and Cl^- fluxes were measured before and after treatment with 2 μM terbutaline, using paired monolayers with resistances that were not significantly different (Fig. 2*A*). Under conditions where an outward K^+ gradient was imposed across the basolateral membrane, treatment

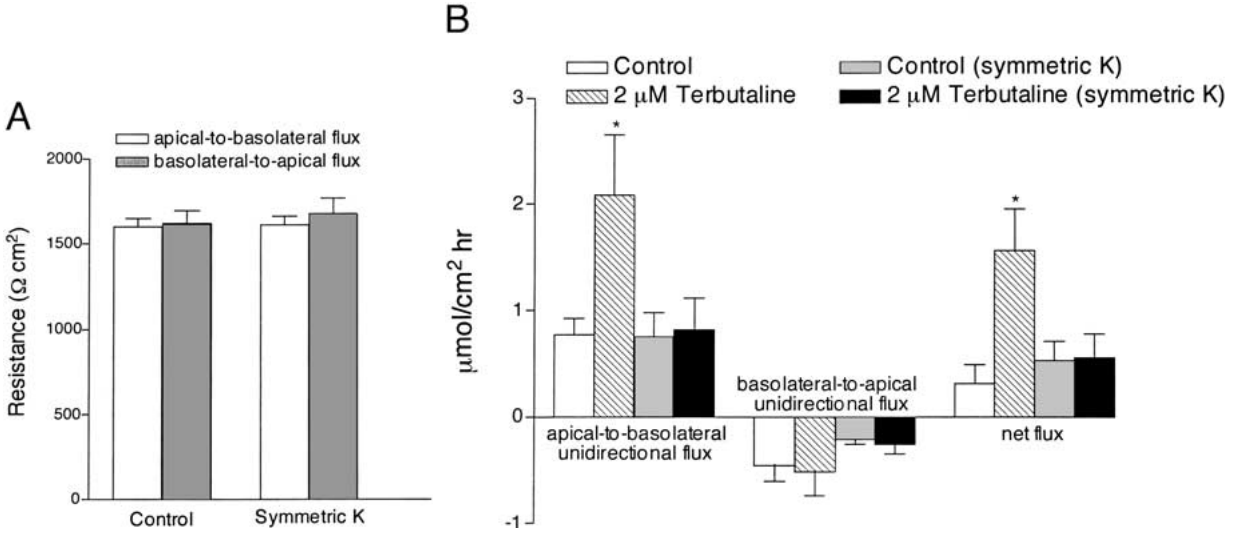

Fig. 2. Cl^- flux measurements across the basolateral membrane of apical-membrane-permeabilized monolayers. (*A*) Bar graph showing a comparison of transepithelial resistances of monolayers used for unidirectional flux measurements. (*B*) Unidirectional and net Cl^- fluxes before and after terbutaline treatment in the presence and absence of a $[K^+]$ gradient across the basolateral membrane (n = 8 paired monolayers using cells from 16 adult rats).

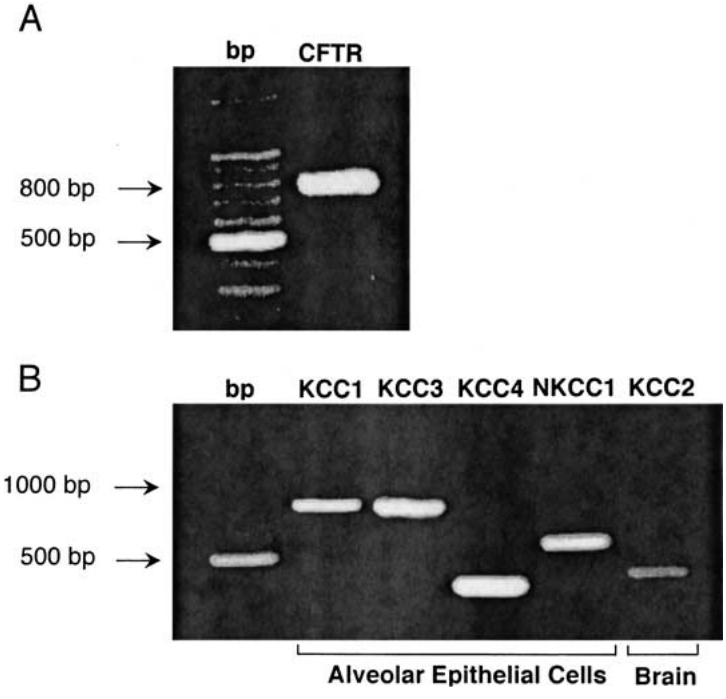

Fig. 3. Detection of rat CFTR, NKCC1 and KCC isoforms in rat alveolar cells. (*A*) RT-PCR amplification of rat CFTR with RNA isolated from rat alveolar epithelial cells. Amplified product (800 bp) was identified using a 1% agarose gel stained with ethidium bromide. *bp*, molecular size markers (number of base pairs). (*B*) RT-PCR amplification of rat KCC isoforms and NKCC1 with RNA isolated from rat alveolar epithelial cells (lanes 1–4) and rat brain (5th lane). Amplified product was identified using a 1% agarose gel stained with ethidium bromide: KCC1 (lane 1), KCC3 (lane 2), KCC4 (lane 3), NKCC1 (lane 4) and KCC2 (lane 5). Arrows indicate positions of molecular size markers (in bp). The predicted size of the bands is listed in Table 1.

with terbutaline increased the cell-to-basolateral solution Cl^- flux but had no significant effect on the basolateral-to-cell unidirectional flux. The result was a net stimulation of Cl^- efflux from the intracellular compartment across the basolateral membrane (Fig. 2*B*). Eliminating the outward K^+ concentration gradient by bathing both sides of the epithelium with symmetric K^+ saline solution blocked the increase in net Cl^- transport stimulated by terbutaline. The results of these experiments indicated that terbutaline activated an electroneutral, K^+-dependent, Cl^- transport pathway present in the basolateral membrane.

Identification of Cl^- transport pathways present in cultured adult alveolar epithelial cells is presented in Fig. 3. RT-PCR results indicate the presence of mRNA for CFTR Cl^- channels (Fig. 3*A*) as well as multiple electroneutral cotransport systems for Cl^- (Fig. 3*B*). Previous studies by Jiang et al. (1998) and Jiang, Ingbar & O'Grady (2001) provided functional

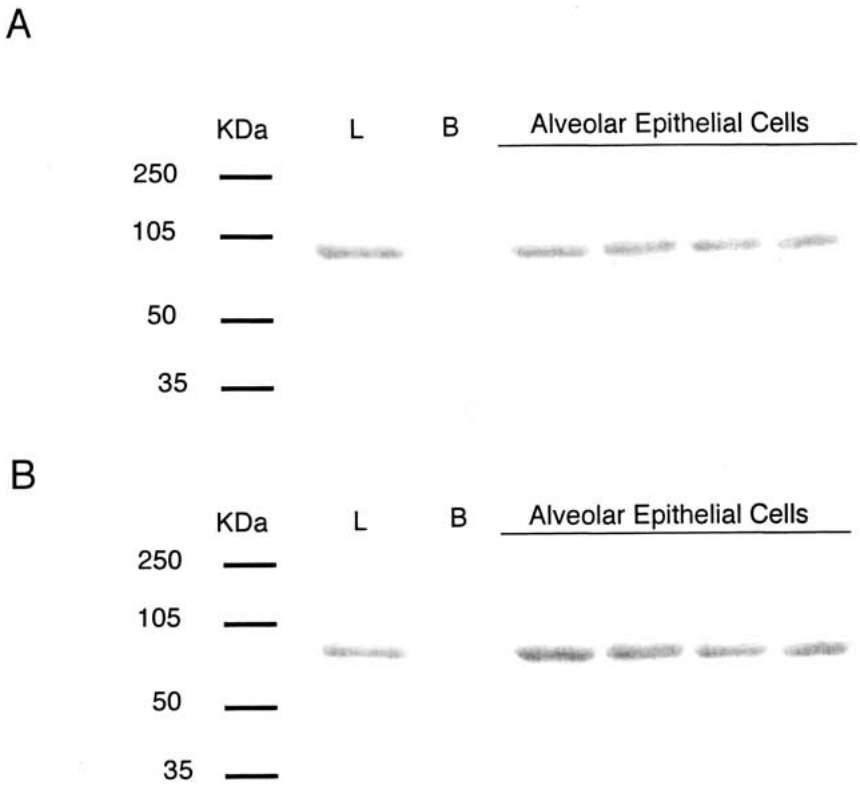

Fig. 4. Western blot showing proteins labeled with either KCC1 (*A*) or KCC4 (*B*) antibodies. (*L* = whole lung lysate, *B* = brain, lanes 3–6 show labeling results from 4 separate monolayers of adult rat alveolar epithelial cells).

evidence for the presence of CFTR in the apical membrane of rat alveolar epithelial cell monolayers and in freshly isolated cells using whole-cell perforated-patch clamp recording techniques. The PCR results from the present study are thus consistent with previous functional data for CFTR. In addition to detecting transcripts for CFTR, we discovered that monolayers of adult alveolar cells also contained mRNA for the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1). This cotransport system is known to play a role in anion secretion in fetal alveolar epithelial cells (O'Brodovich, Rafi & Post, 1990, McCray et al., 1993).

An interesting result of the present study was the identification of mRNA for multiple KCl cotransport isoforms present in cultured monolayers of adult alveolar epithelial cells (Fig. 3*B*). Three isoforms of the KCl cotransporter were identified (KCC1, KCC3 and KCC4), but the brain-specific isoform (KCC2) was not detected. As a positive control, the PCR primers designed to detect KCC2 were used to identify this isoform in samples of rat brain mRNA. The gel shows a PCR product corresponding to the correct size predicted for KCC2, and subsequent sequence analysis confirmed that the primers used to detect KCC2 in alveolar cells were able to identify the appropriate KCC sequence from rat brain.

To determine whether the KCC cotransport isoform mRNAs were expressed as protein in alveolar cell monolayers, we used commercially available antibodies for all four murine KCC cotransporters in an attempt to detect their presence using western blots (Fig. 4*A* and *B*). We could identify a major band between 95–100 kDa for KCC1 and one of similar size (between 85–90 kDa) for KCC4 isoforms but were unable to identify any specific bands for the KCC2 and KCC3 isoforms. The observation that the KCC3 antibody failed to detect a specific band corresponding to KCC3 cotransporter may reflect species differences in the sequence between rat and mouse isoforms or lack of translation of the KCC3 mRNA transcript in alveolar cells. Preabsorption controls for KCC1 and KCC4 antibodies using peptide antigen blocked labeling with these antibodies (*data not shown*). Attempts to use KCC1 and KCC4 antibodies for immunolocalization experiments with cultured alveolar cell monolayers were unsuccessful, thus it was not possible to determine the cellular distribution of these transporters by this approach. The molecular weight estimates for KCC1 and KCC4 isoforms obtained from western blots shown in Fig. 4 are lower than the predicted molecular weights from full-length mRNA transcripts of these cotransporter isoforms (Gillen et al., 1996, Mount et al., 1999, Mercado et al., 2000, Sangan et al., 2000). One possible explanation for this could be that rat alveolar cells express lower-molecular-weight splice variants for the KCC1 and KCC4 cotransport isoforms, or perhaps some combination of alternative splicing and

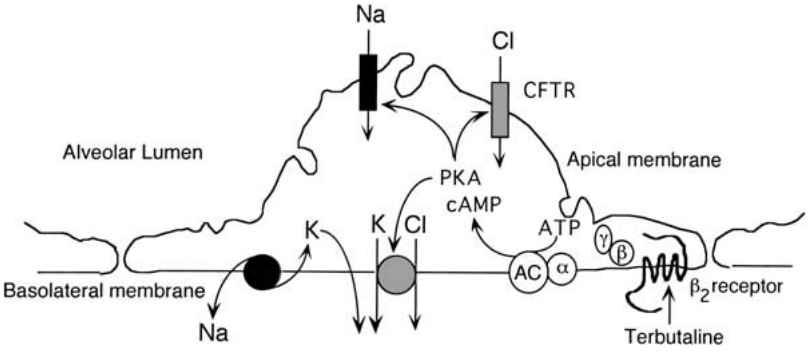

Fig. 5. Cell model indicating the proposed role for KCl cotransport in transepithelial Cl^- absorption across cultured alveolar epithelial cells.

reduced glycosylation of these isoforms occurs in alveolar epithelial cells.

A model showing a possible role of KCl cotransport in transepithelial Cl^- absorption is presented in Fig. 5. The unique aspect of this model is that Cl^- uptake occurs by an electrogenic transport mechanism that is activated by terbutaline and that there is coordinate regulation of an electroneutral, K^+-dependent Cl^--transport mechanism in the basolateral membrane that is necessary for transcellular Cl^- absorption. This organization of transport pathways contrasts with the generally accepted model for fetal lung Cl^- secretion, where Cl^- uptake across the basolateral membrane is mediated by an electroneutral Na^+-K^+-$2Cl^-$ cotransporter and Cl^- efflux across the apical membrane is dependent on Cl^- channels. A possible advantage for having an electroneutral mechanism for K^+ and Cl^- efflux across the basolateral membrane in alveolar epithelial cells is that this transport mechanism would not produce membrane hyperpolarization, and thus would not decrease the driving force for Cl^- uptake across the apical membrane.

The importance of terbutaline-activated Cl^- transport in alveolar fluid clearance was recently demonstrated in both mouse and human lung. Studies by Fang et al (2002) showed that in wild-type mice, Cl^- substitution and Cl^- channel inhibitors such as glibenclamide (a known blocker of CFTR), inhibited isoproterenol-stimulated fluid absorption. In contrast to these results, isoproterenol had no effect in ΔF508 CFTR mice. Experiments using ex vivo human lung at 37°C showed that pretreatment with glibenclamide completely inhibited terbutaline-stimulated fluid absorption. It is worth noting that glibenclamide treatment did not affect the basal rate of fluid absorption and that it had no effect on amiloride-sensitive Na^+ channels in cultured rat alveolar epithelial cells (Jiang et al., 1998). These observations substantially reduce the probability that the effect of glibenclamide could be explained by nonspecific inhibition of ENaC channels located in the apical membrane. The conclusion that CFTR was important in mediating isoproterenol-activated transcellular Cl^- absorption was consistent with earlier studies in cultured adult rat alveolar epithelial cells where terbutaline was shown to activate apical membrane Cl^- channels with functional properties similar to that of CFTR (Jiang et al., 1998). In the present study, PCR results shown in Fig. 3*A* confirmed the presence of CFTR mRNA in cultured alveolar epithelial cells from adult rats.

In conclusion, the results of this study provide the first demonstration that terbutaline can activate a Cl^- transport mechanism in the basolateral membrane. This transport pathway is electroneutral and dependent on an outward K^+ concentration gradient to drive Cl^- efflux from the intracellular compartment. RT-PCR and western blot analyses indicate that multiple KCl cotransport isoforms are present in adult alveolar epithelial cells. These findings suggest the possibility that one or more KCl cotransport isoforms are activated by terbutaline and mediate Cl^- exit across the basolateral membrane following adrenergic stimulation. KCl cotransport would also provide a mechanism for K^+ recycling across the basolateral membrane, which would help to sustain the activity of the Na^+-K^+ ATPase.

This work was supported by an SCOR grant from the National Institutes of Health (HL50152).

References

Borok, Z., Hami, A., Danto, S.I., Lubman, R.L., Kim, K.J., Crandall E.D. 1996. Effects of EGF on alveolar epithelial junctional permeability and active sodium transport. *Am. J. Physiol.* **270:**L559–L565

Cheek, J.M., Kim, K.J., Crandall, E.D. 1989. Tight monolayers of rat alveolar epithelial cells: bioelectric properties and active sodium transport. *Am. J. Physiol.* **251:**C688–C693

Crandall, E.D., Heming, T.A., Palombo, R.L., Goodman, B.E. 1986. Effects of terbutaline on sodium transport in isolated perfused rat lung. *J. Appl. Physiol.* **66:**289–294

Fang, X., Fukuda, N., Barbry, P., Sartori, C., Verkman, A.S. Matthay, M.A. 2002. Novel role for CFTR in fluid absorption from the distal airspaces of the lung. *J. Gen. Physiol.* **119:**199–207

Fiedler, M.A., Nemecz, Z.K., Shull, G.E. 1992. Cloning and sequence analysis of rat cystic fibrosis transmembrane conductance regulator. *Am. J. Physiol.* **262:**L779–784

Gillen, C.M., Brill, S., Payne, J.A., Forbush, B. 1996. Molecular cloning and functional expression of the K-Cl cotransporter from rabbit, rat, and human. A new member of the cation-chloride cotransporter family. *J. Biol. Chem.* **271:**16237–16244

Jiang, X., Ingbar, D.H., O'Grady, S.M. 1998. Adrenergic stimulation of Na transport across alveolar epithelial cells involves activation of apical Cl channels. *Am. J. Physiol.* **275:**C1610–1620

Jiang, X., Ingbar, D.H., O'Grady, S.M. 2001. Adrenergic regulation of ion transport across adult alveolar epithelial cells: Effects on Cl channel activation and transport function in cultures with an apical air interface. *J. Membrane Biol.* **181:**195–204

Kim, K.J., Cheek, J.M., Crandall, E.D. 1991. Contribution of active Na^+ and Cl^- fluxes to net ion transport by alveolar epithelium. *Respir. Physiol.* **85:**245–256

Lauf, P.K., Adragna, N.C. 2000. K-Cl cotransport: properties and molecular mechanism. *Cell Physiol. Biochem.* **10:**341–354

Lazrak, A., Nielsen, V.G., Matalon, S. 2000. Mechanisms of increased Na^+ transport in ATII cells by cAMP: we agree to disagree and do more experiments. *Am. J. Physiol.* **278:**L233–L238

Matalon, S., O'Brodovich, H. 1999. Sodium channels in alveolar epithelial cells: molecular characterization, biophysical properties, and physiological significance. *Annu. Rev. Physiol.* **61:**627–661

McCray, P.B., Bettencourt, J.D., Bastacky, J., Denning, G.M., Welsh, M.J. 1993. Expression of CFTR and a cAMP-stimulated chloride secretory current in cultured human fetal alveolar epithelial cells. *Am. J. Respir. Cell. Mol. Biol.* **9:**578–585

Mercado, A., Song, L., Vazquez, N., Mount, D.B., Gamba, G. 2000. Functional comparison of the K^+-Cl^- cotransporters KCC1 and KCC4. *J. Biol. Chem.* **275:**30326–30334

Mount, D.B., Mercado, A., Song, L., Xu J., George, A.L. Jr., Delpire, E., Gamba, G. 1999. Cloning and characterization of KCC3 and KCC4, new members of the cation-chloride cotransporter gene family. *J. Biol. Chem.* **274:**16355–16362

O'Brodovich, H., Rafii, B., Post, M. 1990. Bioelectric properties of fetal alveolar epithelial monolayers. *Am. J. Physiol.* **258:**L201–206

O'Brodovich, H. 1996. Epithelial ion transport in fetal and perinatal lung. *Am. J. Physiol.* **261:**C555–564

O'Grady, S.M., Jiang, X., Ingbar, D.H. 2000. Cl^- channel activation is necessary for stimulation of Na^+ transport in adult alveolar epithelial cells. *Am. J. Physiol.* **278:**L239–L244

Payne, J.A., Stevenson, T.J., Donaldson, L.F. 1996. Molecular characterization of a putative K-Cl cotransporter in rat brain. A neuronal-specific isoform. *J. Biol. Chem.* **271:**16245–16252

Race, J.E., Makhlouf, F.N., Logue, P.J., Wilson, F.H., Dunham, P.B., Holtzman, E.J. 1999. Molecular cloning and functional characterization of KCC3, a new K-Cl cotransporter. *Am. J. Physiol.* **277:**C1210–1219

Reuss, L. 1983. Basolateral KCl co-transport in a NaCl-absorbing epithelium. *Nature* **305:**723–726

Saldias, F., Lecuona, E., Friedman, E., Barnard, M.L., Ridge, K.M., Sznajder, J.I. 1998. Modulation of lung liquid clearance by isoproterenol in rat lungs. *Am. J. Physiol.* **274:**L694–L701

Sangan, P., Brill, S.R., Sangan, S., Forbush, B., Binder, H.J. 2000. Basolateral K-Cl cotransporter regulates colonic potassium absorption in potassium depletion. *J. Biol. Chem.* **275:**30813–30816

Saumon, G., Basset, G., Bouchonnet, F., Crone, C., 1987. cAMP and β-adrenergic stimulation of rat alveolar epithelium: Effects on fluid absorption and paracellular permeability. *Pfluegers Arch.* **410:**464–470

Warnock, D.G., Eveloff, J., 1989. K-Cl cotransport systems. *Kidney Int.* 412–417

Widdcombe, J.H. 2000. How does cAMP increase active Na absorption across alveolar epithelium? *Am. J. Physiol.* **278:**L231–L232