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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

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Abstract. The effect of β-adrenergic receptor stimulation on Cl^- channel activation was investigated in alveolar epithelial cells grown in monolayer culture and in freshly isolated cells. Monolayers cultured under apical air interface conditions exhibited enhanced amiloride-sensitive Na^+ transport compared to apical liquid interface monolayers. Amiloride or benzamil inhibited most (66%) of the basal short circuit current (*Isc*) with half-maximal inhibitory concentration (IC_{50}) values of 0.62 μM and 0.09 μM respectively. Basolateral addition of terbutaline (2 μM) produced a rapid decrease in *Isc* followed by a slow recovery that exceeded the basal *Isc*. When Cl^- was replaced with methanesulfonate in either intact monolayers or basolateral membrane permeabilized monolayers, the response to terbutaline (2 μM) was completely inhibited. No effect of terbutaline on amiloride-sensitive Na^+ current was detected. β-Adrenergic agonists and 8-chlorothiophenyl cyclic adenosine monophosphate (8-ctp cAMP) directly stimulated a Cl^- channel in freshly isolated alveolar epithelial cells. The current was blocked by glibenclamide (100 μM) and had a reversal potential of −22 mV. No increase in amiloride-sensitve current was detected in response to terbutaline or 8-cpt cAMP stimulation. These data support the conclusion that β-adrenergic agonists produce acute activation of apical Cl^- channels and that monolayers maintained under apical air interface conditions exhibit increased Na^+ absorption.

Key words: CFTR — ENaC — Terbutaline — Cl absorption — Na absorption

Introduction

The adult alveolar epithelium plays an important role in facilitating gas exchange by maintaining a thin fluid layer along the apical surface of the alveolus through active absorption of Na^+ and fluid from the alveolar space (Cheek, Kim & Crandall, 1989, Kim, Cheek & Crandall, 1991, Russo, Lubman & Crandall, 1992, Yue, Shoemaker & Matalon, 1994, Matalon & O'Brodovich, 1999, Lazrak, Nielsen & Matalon, 2000). Sodium and fluid absorption by this epithelium is enhanced by administration of β-adrenergic receptor agonists such as terbutaline (Crandall et al., 1986, Saumon et al., 1987, Cheek et al., 1989, Kim et al., 1991, Johnson et al., 1993, O'Brodovich, 1996, Jiang, Ingbar & O'Grady, 1998, Minakata et al., 1998, Saldias et al., 1998, 1999, O'Grady, Jiang & Ingbar, 2000, Widdcombe, 2000). Previous isotopic flux measurements have shown 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 (*Isc*), consistent with anion absorption, followed by a slow increase in *Isc* that reached a new steady-state plateau that was greater than the basal current (Cheek et al., 1989). This sustained 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. The idea that stimulation of fluid absorption by β-adrenergic receptor agonists depends on an increase in net Na^+ absorption has been supported by *in vivo* experiments, using isolated perfused adult lungs (Crandall et al., 1986, Saldias et al., 1998, Saumon et al., 1987). A possible explanation for the increase in amiloride-sensitive *Isc* following terbutaline treatment may involve direct regulation of Na^+ channel activity (Lazrak et al., 2000, Matalon & O'Brodovich, 1999). In fetal distal lung epithelium, terbutaline was shown to activate amiloride-blockable nonselective cation channels (O'Brodovich, 1996). In freshly isolated adult alveolar type II cells, adrenergic receptor stimula-

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tion significantly increased the open probability (P_0) and the mean open time (τ) of amiloride-sensitive channels, but had no effect on channel conductance (Matalon & O'Brodovich, 1999, Lazrak et al., 2000). However, in alveolar epithelial cell monolayers the initial rapid decrease in *Isc* produced by terbutaline and terbutaline stimulation of active Cl^- absorption measured in flux experiments could not be explained by an increase in apical membrane Na^+ conductance (Jiang et al, 1998, O'Grady et al, 2000). In our previous studies, we found that terbutaline activated apical membrane Cl^- channels (Jiang et al, 1998). Our data suggested that apical membrane hyperpolarization produced by Cl^- influx increases the driving force for Na^+ uptake. This is consistent with the time-dependent, amiloride-sensitive increase in *Isc* that follows the rapid decrease in current produced by terbutaline. Amiloride-sensitive Na^+ channels were not directly affected by terbutaline stimulation (Jiang et al, 1998). At this time it is not known whether β-adrenergic receptor-regulated Cl^- channels are present in freshly isolated alveolar epithelial cells. The issue is important since properties of primary cells in monolayer culture may change with time, making it necessary to determine if Cl^- channels are expressed in freshly isolated alveolar cells and if terbutaline can produce Cl^- channel activation. In this study we address this issue by investigating the actions of terbutaline on Cl^- channel activation in both cultured alveolar epithelial cells and in freshly isolated cells using the perforated whole-cell patch-clamp recording technique.

Recently, it was discovered that the morphological properties of alveolar type II cells were maintained by using air-liquid interface culture, in which medium was only added to the basolateral side of the cells and the apical side was exposed to air (Dobbs et al., 1997). Alveolar epithelial cells cultured under apical air-interface conditions appeared to be more similar to type II cells observed under *in vivo* conditions. Previous studies using cultures of tracheal epithelial cells showed that monolayers grown under air-interface conditions exhibited higher rates of electrogenic Na^+ absorption compared to monolayers grown using apical liquid interface conditions (Johnson et al, 1993). In this study we investigated whether alveolar epithelial cells grown under apical air-interface conditions exhibited higher rates of amiloride-sensitive Na^+ absorption and if responses to β-adrenergic agonist stimulation were altered compared to those previously observed in monolayers grown with an apical liquid-interface.

Materials and Methods

Male pathogen-free Sprague-Dawley rats weighing 150–174 g were purchased from Harlan, Inc (Indianapolis, Indiana). 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) and penicillin-streptomycin 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). Phosphate buffered saline (PBS) was obtained from Celox Laboratories (Oakdale, MN). Amiloride was purchased from Merck Sharp & Dohme (West Point, PA). Benzamil, 5-Nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) and 8-(4-chlorophenylthio)-cAMP (8 cpt-cAMP) were obtained from RBI (Natick, MA). All other chemicals were obtained from Sigma. Anti-CFTR antibody and blocking peptide were gifts from J. Cohen, Duke University.

Cell Preparation and Culture

Alveolar epithelial cells were isolated from adult rat lungs using a modification of the protocol described by Borok et al. (1996). Rats were anesthetized with an intraperitoneal injection of pentobarbital. Lungs were perfused with solution A (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 solution B (in mM: 140 NaCl, 5 KCl, 2.5 NaH_2PO_4 6 glucose, 10 HEPES) and solution A to eliminate macrophages. The lungs were then filled with Elastase-containing solution (2.7 U/ml in solution A) and were incubated at 37°C for 30 min in a shaker bath. Elastase was neutralized by stop solution (2 mM EDTA, 1% BSA, 0.1% soybean trypsin inhibitor and 0.15/ml deoxyribonuclease I in a buffered saline solution (in mM: 136 NaCl, 2.2 Na_2HPO_4, 5.3 KCl, 10 HEPES, and 5.6 glucose). 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% non-essential 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 (WPI, New Haven, CT). To prepare the alveolar epithelial monolayers with an apical air-interface, cells were allowed to adhere to the filters, at which time the medium on the apical surface was removed. The transport properties of these monolayers were investigated on day 9 or 10 following isolation. All the other transport studies were performed on day 7 following isolation. For patch clamp experiments, individual cells were dissociated from the membrane filters with either 0.05% trypsin-0.53 mM EDTA or 2 mg/ml collagenase type I in serum free DMEM/F12 medium. Freshly isolated alveolar cells were also used in patch-clamp experiments.

Ussing Chamber Measurements

High-resistance monolayers on Transwell inserts were mounted in Ussing chambers and bathed on both sides with identical solutions (standard saline solution or Cl^--free saline solution (in mM: 120 $NaMeSO_4$, 10 $KMeSO_4$, 30 mannitol, 3 Ca-Gluconate, 0.7 $MgSO_4$, 20 $NaHCO_3$ and 0.3 NaH_2PO_4) at 37°C. The solutions were bubbled with 95% O_2-5% CO_2 on each side. Monolayer potential difference (luminal side as reference so that Na^+ influx produces an inward current and Cl^- influx produces an outward current), short circuit current (*Isc*), and resistance were measured with voltage-clamp circuitry from JWT En-

gineering (Overland Park, KS). Workbench data acquisition software (Kent Scientific, CT) was used to record the data. Measurements of apical membrane current responses to terbutaline were performed using amphotericin B (10 μM) to premeabilize the basolateral membrane. The apical membrane voltage was held at zero mV and the basolateral surface was bathed with intracellular solution containing (in mM): 120 $KMeSO_4$, 20 $KHCO_3$, 10 NaCl, 1 $CaCl_2$, 0.7 $MgSO_4$, 0.3 NaH_2PO_4, 1.3 Na_2HPO_4, 15 mannitol, pH 7.4).

Patch-Clamp Recording

The perforated whole-cell patch configuration was used in these experiments in order to retain regulatory compounds within the cells that are typically lost during standard whole-cell recordings. Pipette electrodes were pulled to a resistance of 2–4 MΩ from 7052 glass (Garner Glass, Claremont, CA). The pipette tip was filled with $KMeSO_4$ Ringer solution consisting of (in mM) 130 $KMeSO_4$, 5 KCl, 1 $CaCl_2$, 10 HEPES, pH 7.2. The pipette was then back-filled with the same solution containing 10 μM amphotericin B. High resistance seals (>5 GΩ) were formed between the pipette and the cell membrane, and amphotericin B was allowed to partition into the membrane to obtain the whole-cell configuration prior to recording currents. Bath solutions contained serum-free DMEM/F12 medium. Whole-cell currents were obtained in response to the voltage step protocols described in the figure legends. The mean capacitance of the cells used in this study was 14 ± 2 pF. An Axopatch 1D amplifier and TL-1 A-D interface were used (both from Axon Instruments). P-CLAMP software was used to generate the voltage step commands and record the resulting currents.

Confocal Immunocytochemical Localization of CFTR in Cultured Alveolar Cells

Rat alveolar epithelial cells were plated on plastic LabTek chamber slides (Miles). After 1–6 days in culture with Dulbecco's Modified Eagles Medium with 10% fetal calf serum (Gibco) and antibiotics in a 5% CO_2-95% air environment, the cells were fixed with 4% formalin in PBS, pH 7.4 at room temperature for ten minutes. After washing, indirect immunofluorescence was performed according to previously used protocols (Ingbar et al., 1995). The primary antibody used was a rabbit polyclonal anti-CFTR peptide antibody directed against amino acids 1468–1480 (KEETEEEVQDTRL) (gift of J. Cohn, Duke University). This antibody has previously been characterized (Marino et al., 1991). Controls included substitution of the following for the primary antibody: premixed CFTR peptide-antiCFTR peptide antibody; normal rabbit serum at the same dilution; or no primary antibody.

Confocal laser scanning microscopy was performed as previously described (Ingbar et al., 1995) using a Bio-Rad argon dual wavelength laser and Olympus inverted phase contrast microscope. Images were digitized and transferred to a Silicon Graphics Iris-4D/240GTX power series computer for three-dimensional reconstruction with Voxel View (Plymouth, MN) software. More than 30 cells were examined from three separate cell isolations.

Statistics

All data are presented as mean ± SEM and n is the number of monolayers studied. The IC_{50} values for amiloride and benzamil were determined by using a four-parameter logistic function to fit the data. The concentration of each compound at 50% maximal effect was derived from the equation used to fit the concentration-response relationship. Differences between means were analyzed by using either paired or unpaired Student's t-test, as appropriate.

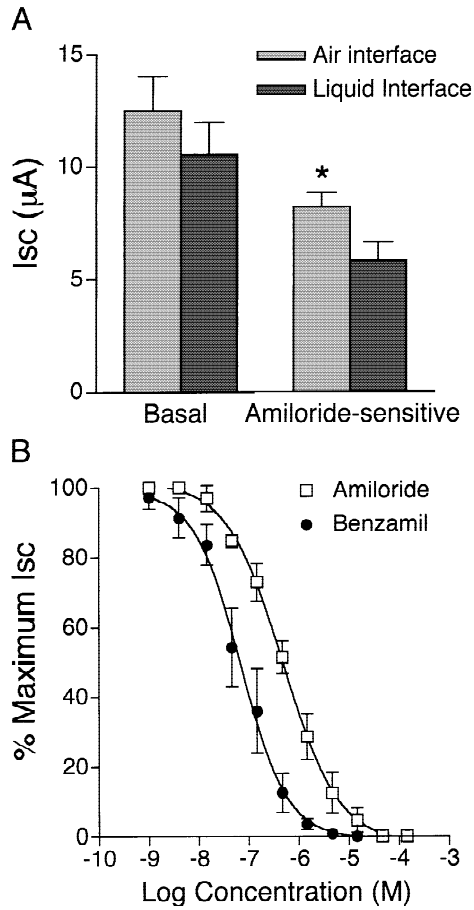

Fig. 1. Effect of sodium channel blockers on *Isc* of alveolar monolayers grown under apical air interface conditions. (*A*) Comparison of basal and amiloride-sensitive *Isc* between monolayers grown under apical air-interface and apical liquid-interface conditions. The amiloride-sensitive (20 μM) *Isc* was significantly greater in monolayers exposed to apical air-interface conditions. (*B*) In monolayers exposed to apical air-interface conditions, apical addition of sodium channel blockers decreased *Isc*, with benzamil (IC_{50} = 0.09 μM, n = 4) > amiloride (IC_{50} = 0.62 μM, n = 4).

Results

Effects of an Apical Air Interface on the Amiloride-Sensitive *Isc*

Monolayers maintained under apical air interface conditions had significantly greater amiloride-sensitive currents than those under liquid interface conditions (Fig. 1*A*). The total basal *Isc*, however, was not significantly different. The concentration-response relationships for amiloride and benzamil showed that the rank order of potency for inhibition was benzamil > amiloride, with

mean IC_{50} values of 0.09 μM, and 0.62 μM, respectively (Fig. 1*B*). These IC_{50} values were not significantly different from those previously measured in monolayers maintained under liquid-interface conditions (Jiang et al., 1998). Also in this previous study and in a subsequent investigation (Jiang, Ingbar & O'Grady, 2000), we found that most of the amiloride-insensitive *Isc* was due to the activity of a Na^+-dependent amino acid cotransport mechanism present in the apical membrane. Although we did not specifically measure the activity of this transport pathway in monolayers under air-interface conditions, we did not find any significant differences between the amiloride-insensitive *Isc* in air interface monolayers compared to monolayers grown under liquid-interface conditions.

Effects of Terbutaline on *Isc* of Monolayers Grown in the Presence of an Apical Air Interface

Addition of the selective β-adrenergic receptor agonist terbutaline (2 μM) to the basolateral bath of alveolar epithelial monolayers cultured under apical air interface conditions produced a rapid decrease in *Isc* followed by a time-dependent increase and overshoot of the basal current (Fig. 2*A*). The mean initial decrease in *Isc* stimulated by terbutaline was 2.84 ± 0.21 μA ($n = 5$). Subsequent addition of amiloride (20 μM) to the apical bathing solution blocked 60.5 ± 4.4% ($n = 5$) of the remaining current. The *Isc* response of monolayers cultured under apical air-interface conditions was similar to that observed under apical liquid-interface conditions. Pretreatment of monolayers with amiloride reduced the initial effect of terbutaline on *Isc* and eliminated the time-dependent increase in current following terbutaline stimulation (Fig. 2*B*). Replacement of Cl^- with methanesulfonic acid in both the apical and basolateral bathing solutions eliminated the initial decrease in *Isc* produced by terbutaline and abolished the time-dependent increase in amiloride-sensitive *Isc* (Fig. 2*C*). Inhibition of the terbutaline response under Cl^--free conditions was consistent with previously published data using monolayers grown with culture media continuously present on the apical surface.

Effects of Terbutaline on Apical Membrane Permeability

In amphotericin-permeabilized monolayers (10 μM amphotericin B added to the basolateral surface), terbutaline caused a rapid and sustained decrease in the apical membrane current (Fig. 3*A*). Subsequent addition of amiloride to the apical solution produced a further decrease in current. Replacement of Cl^- with methanesulfonate completely blocked the terbutaline effect on the current but did not alter the response to amiloride (Fig. 3*B*). This result indicates that the initial decrease in current produced by terbutaline is the result of Cl^- entry into the cell across the apical membrane.

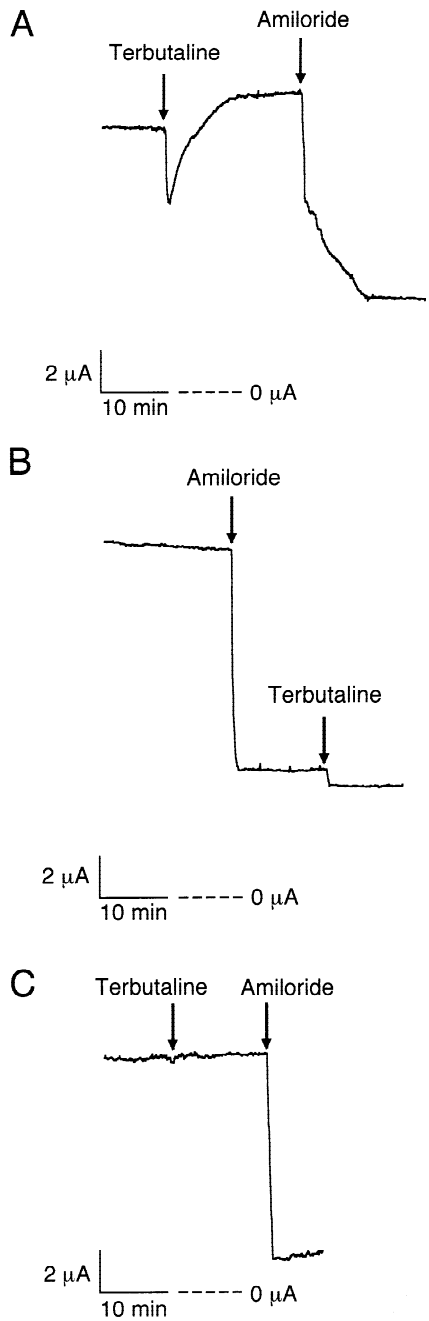

Fig. 2. Effects of terbutaline on *Isc*. (*A*) Addition of terbutaline (2 μM) produced a rapid decrease followed by a time-dependent increase in *Isc*. Subsequent addition of amiloride blocked approximately 70% of the total *Isc* ($n = 5$). (*B*) Pretreatment with apical amiloride (20 μM) reduced the *Isc* response produced by terbutaline and eliminated the time-dependent increase previously shown in part *A*. (*C*) Replacement of Cl^- with methanesulfonic acid blocked the effects of terbutaline but did not change the effects of amiloride (20 μM) on *Isc*.

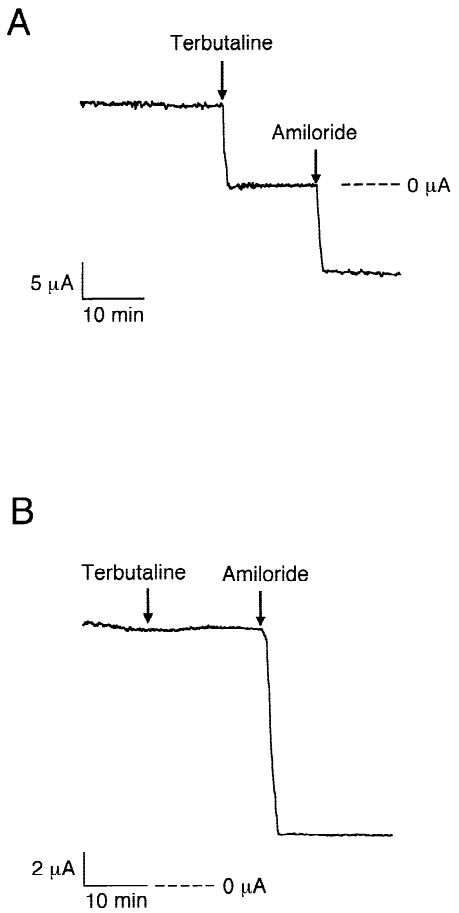

Fig. 3. Effects of terbutaline on apical air-interface monolayers treated with amphotericin (10 μM on the basolateral side) to determine the effects of terbutaline on apical membrane conductances. (*A*) The apical membrane was voltage-clamped at zero mV following replacement of the basolateral solution with high K intracellular solution. The apical surface was bathed with standard saline solution. Addition of terbutaline produced a rapid decrease in apical membrane current that did not exhibit any secondary increase as previously observed in measurements of *Isc* (Fig. 2*A*). Subsequent addition of amiloride produced an additional decrease in current. (*B*) Replacement of Cl^- with methanesulfonate abolished the decrease in apical membrane current produced by terbutaline but did not affect the response to amiloride (20 μM).

Effects of Terbutaline on Cl^- Current in Dissociated Alveolar Epithelial Cells

Amphotericin B-perforated whole-cell patch clamp recordings from cells isolated by either trypsin or collagenase dissociation of alveolar epithelial cell monolayers (at day 7 following isolation) are reported in Fig. 4. When the holding potential was set at −60 mV we observed an increase in outward current following depolarization of the cell from −50 to 0 mV. Saturation was observed at −10 mV and the reversal potential was −32 mV (Fig. 4*A*). The half-maximal activation voltage (V_{50}) for this current (−23 mV) was identical to that of a previously characterized voltage- gated K^+ channel (Kv) present in adult rat alveolar type II cells (DeCoursey, Jacobs & Silver, 1988). When the holding potential was set at zero mV and the command potential was stepped from −100 to +120 mV (20 mV increments) the Kv current was inactivated and a relatively small, slowly activating outward current was observed (Fig. 4*B*). In freshly isolated alveolar cells, we also observed basal whole-cell currents similar to that of the Kv potassium channel and a similar basal current when the holding potential was set at zero mV.

When cultured cells were stimulated with terbutaline (2 μM), we observed an increase in outward current that exhibited a slight degree of outward rectification and had a reversal potential of −22 mV (Fig. 5). Activation was not time-dependent and no inactivation was detected in response to depolarizing voltage steps. The terbutaline-activated current was inhibited by the Cl^- channel blocker NPPB (60 μM) and the NPPB-sensitive current was not significantly different from the terbutaline activated current. Addition of the epithelial Na^+ channel blocker amiloride (20 μM) had no effect on either the basal or the terbutaline activated whole-cell current. Stimulation with 20 μM 8-cpt cAMP resulted in activation of whole-cell currents that were similar to those obtained following terbutaline stimulation.

In freshly isolated alveolar cells, terbutaline also activated a current with properties that were nearly identical to those observed in cells obtained from monolayer cultures at day 7 following isolation (Fig. 6). The terbutaline-activated current was inhibited by glibenclamide, a known blocker of CFTR Cl^- channels at a concentration consistent with its effects on apical Cl^- channels previously identified in cultured monolayers of adult rat alveolar epithelial cells.

Confocal Immunocytochemical Localization of CFTR in Cultured Alveolar Cells

A rabbit polyclonal anti-CFTR peptide antibody directed against amino acids 1468–1480 of the CFTR sequence was used to obtain immunohistochemical evidence for the expression of CFTR-like Cl^- channels in adult rat alveolar epithelial cells. In Fig. 7, immunofluorescence labeling was detected in the apical membrane. Essentially no immunoreactivity was observed in the basolateral portions of the cells. No labeling was detected in CFTR peptide pre-absorption control experiments or in cells pretreated with secondary antibody alone.

Discussion

Alveolar type II cell monolayers maintained under apical air interface conditions, in the presence of added cell matrix components, have been previously shown to ex-

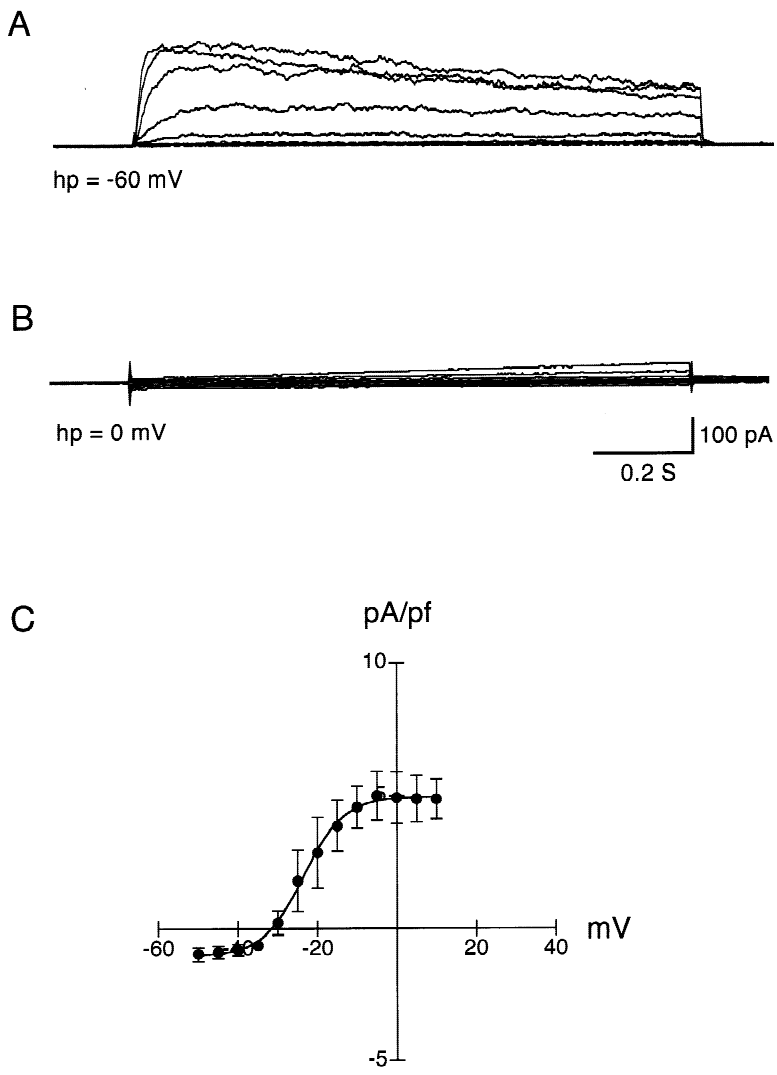

Fig. 4. Whole-cell currents from alveolar epithelial cells obtained from cultured monolayers at day 7 following isolation. Current tracings from dissociated cells recorded using the amphotericin B-perforated whole-cell patch-clamp technique. Cells were bathed in serum-free DMEM/F12 medium. The pipette solution contained (in mM) 130 mM $KMeSO_4$, 10 NaCl, 10 HEPES, pH = 7.4. (*A*) Basal whole-cell currents obtained in response to voltage steps from −60 mV to +10 mV (5 mV increments) at a holding potential of −60 mV. (*B*) Basal whole-cell currents from the same cell as in part *A* were elicited by voltage steps from −100 to +120 mV (20 mV increments) from a holding potential of 0 mV. (*C*) *I-V* relationships for basal outward currents observed in dissociated cells from monolayers at a holding potential of −60 mV. The reversal potential of the Kv-type current (*shown in A*) was −31 mV (V_{50} = −23 ± mV, n = 7).

hibit differentiated characteristics of type II cells for at least 3 weeks in culture (Dobbs et al., 1997). Although measurements of electrolyte transport were not conducted in this previous investigation, earlier studies in cultured canine bronchial epithelia showed that Na^+ transport was significantly enhanced in air-liquid interface cultures compared to standard liquid-liquid interface conditions (Johnson, et al., 1993). In the present study, we investigated the effects of air-interface conditions on alveolar epithelial cell electrolyte transport in cultures where cell matrix components were not added. We observed that the basal *Isc* was not significantly different, but the amiloride-sensitive current was significantly increased in monolayers under apical air-interface conditions, indicating enhanced Na transport compared to apical liquid interface monolayers (Jiang et al., 1998, O'Grady et al., 2000). We examined the effects of amiloride and benzamil on basal *Isc* of the monolayers with an apical air interface and observed that the rank order of potency was benzamil > amiloride. The IC_{50} values and rank order of potency for these inhibitors were similar to results of monolayers under apical liquid-interface conditions reported in a previous study (Jiang et al., 1998) and indicated the presence of high-affinity, amiloride-sensitive Na^+ channels active under both air- and liquid-interface conditions.

When the effects of terbutaline on alveolar epithelial cell monolayers cultured with an apical air-interface were investigated, we found that terbutaline produced a transient decrease in *Isc* followed by a gradual increase in current that often exceeded the initial value. A similar result was previously reported in monolayers grown under apical liquid-interface conditions (Jiang et al., 1998). The effects of terbutaline on basolateral membrane-permeabilized monolayers cultured with an apical air-interface were similar to those observed in monolayers with an apical liquid-interface (Jiang et al., 1998). When monolayers were pretreated with amiloride, the time-

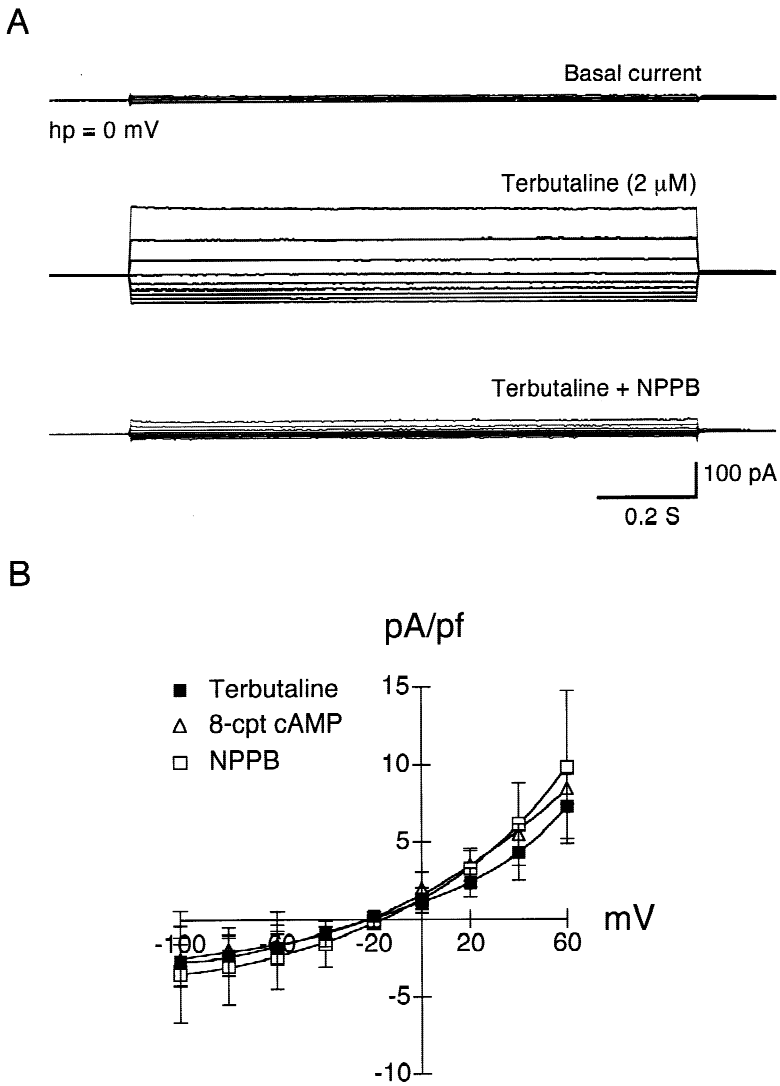

Fig. 5. Effects of terbutaline and NPPB on whole-cell current of alveolar epithelial cells cultured on Transwell membrane filters for seven days. Recording conditions were identical to those stated in Fig. 4 legend. Currents were obtained in response to a series of voltage step commands from −100 mV to +60 mV in 20 mV increments from a holding potential of 0 mV. (*A*) Top trace, basal currents; middle trace, currents after addition of terbutaline (2 μM); bottom trace, currents remaining after addition of 60 μM NPPB. (*B*) Steady state *I-V* relationships for the terbutaline-activated current, 8-cpt cAMP-activated current and the NPPB-inhibited current. Mean reversal potentials for the terbutaline-activated current ($n = 5$), 8-cpt cAMP-activated current ($n = 5$) and NPPB-inhibited current ($n = 5$) were −22 ± 3 mV, −22 ± 2 mV, and −19 ± 2 mV, respectively.

dependent recovery of the *Isc* following terbutaline stimulation was completely inhibited, a result that was previously observed in monolayers grown under apical liquid interface conditions (Jiang et al., 1998, O'Grady et al., 2000). When Cl^- was replaced in both apical and basolateral solutions with methanesulfonate, the effects of terbutaline in both intact and basolateral membrane-permeabilized monolayers were eliminated. Subsequent addition of apical amiloride blocked the *Isc* and apical membrane current, similar to its effects on monolayers cultured under apical liquid-interface conditions. Thus the results of these studies demonstrated that although basal amiloride-sensitive Na^+ transport was enhanced under apical air-interface conditions, the actions of terbutaline on *Isc* were unaffected compared to apical liquid-interface conditions.

Previous whole-cell patch-clamp measurements demonstrated the existence of low-affinity amiloride-sensitive Na^+ channels in both freshly isolated and cultured rat alveolar type II cells (Saumon et al., 1987). In single-channel recordings, terbutaline significantly increased P_0 and τ without affecting single-channel conductance in cells cultured for 24 hours (Matalon & O'Brodovich, 1999, Lazrak et al., 2000). Using the cell-attached patch technique, the same lab reported that a very small fraction of adult rat alveolar type II cells expressed Cl^- channels (O'Brodovich, 1999), but they were not stimulated by cAMP. In contrast to these results, we found that addition of terbutaline stimulated the basal current in freshly isolated alveolar epithelial cells. The terbutaline-activated current was inhibited by glibenclamide (an inhibitor of the CFTR Cl^- channel), but not amiloride. The reversal potentials for terbutaline and the glibenclamide-sensitive currents were similar to those of terbutaline and 8-cpt-cAMP stimulated Cl^- currents in cultured alveolar epithelial monolayers (Jiang et al, 1998, O'Grady et al., 2000). A similar terbutaline-activated current was also observed in patch-clamp stud-

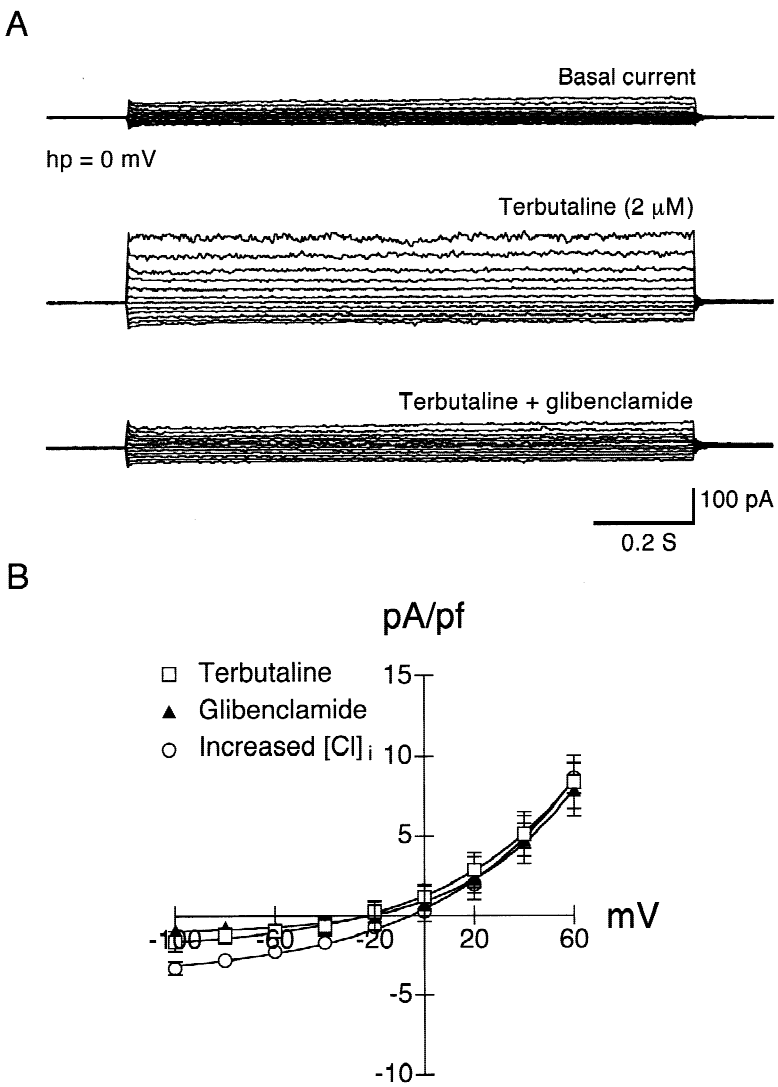

Fig. 6. Effects of terbutaline and glibenclamide on whole-cell currents from freshly isolated alveolar epithelial cells. Recording conditions were identical to those stated in Fig. 5 legend. (*A*) Top trace: basal currents; middle trace, currents after addition of terbutaline (2 μM); bottom trace, currents remaining after addition of 100 μM glibenclamide. (*B*) Steady-state *I-V* relationships of terbutaline-activated or glibenclamide-inhibited currents and elevated-intracellular-[Cl^-] currents from the voltage step protocol described in Fig. 5 legend. Mean reversal potentials for currents in terbutaline ($n = 5$), glibenclamide ($n = 5$) and elevated-intracellular-[Cl^-] (65 mM) conditions ($n = 5$) were −22 ± 3 mV, −21 ± 2 mV, and −6 ± 2 mV, respectively.

ies of cultured alveolar epithelial cells after 7 days in culture. Further evidence supporting the presence of cAMP-dependent Cl^- channels in the apical membrane of alveolar epithelial cells comes from the results of immunocytochemical localization experiments using an anti-peptide antibody to CFTR (Fig. 7). This finding is consistent with the results of an earlier study with alveolar epithelial cell monolayers, which showed that the permeability ratios for Cl^-, Br^-, I^- and SCN^- were similar to those reported for CFTR (Jiang et al., 1998).

In contrast to the effects of terbutaline on Cl^- channels, we found no evidence that amiloride-sensitive Na^+ channels were directly activated by terbutaline in freshly isolated alveolar cells or cultured alveolar epithelial cells. Moreover, there was also no detectable amiloride-sensitivity associated with the basal current. The absence of amiloride-sensitive Na^+ currents reported in this study may be due to the isolation procedures used to obtain cells for whole-cell recording. It should be noted however, that cells in monolayer culture were dissociated using two different enzymatic dissociation protocols, one involving the use of trypsin and the other using collagenase (*see* Methods) and freshly isolated cells were dissociated with elastase. Na^+ currents were not detected under any of these conditions. Although we cannot exclude the possibility that some damage to Na^+ channels may have occurred by using these dissociation protocols, the cells were still able to respond to β-agonist stimulation by increasing Cl^- conductance, and basal K^+ channel activity was similar under each of the isolation conditions. An alternative explanation for the absence of Na^+ current could be that following dissociation, selective downregulation of Na^+ channels from the cell surface occurred as a means to limit constitutive Na^+ influx into the cells. Such a mechanism would reduce ATP utilization by the Na^+-K^+ ATPase and thus conserve metabolic energy under conditions where vectorial Na^+ transport was not possible.

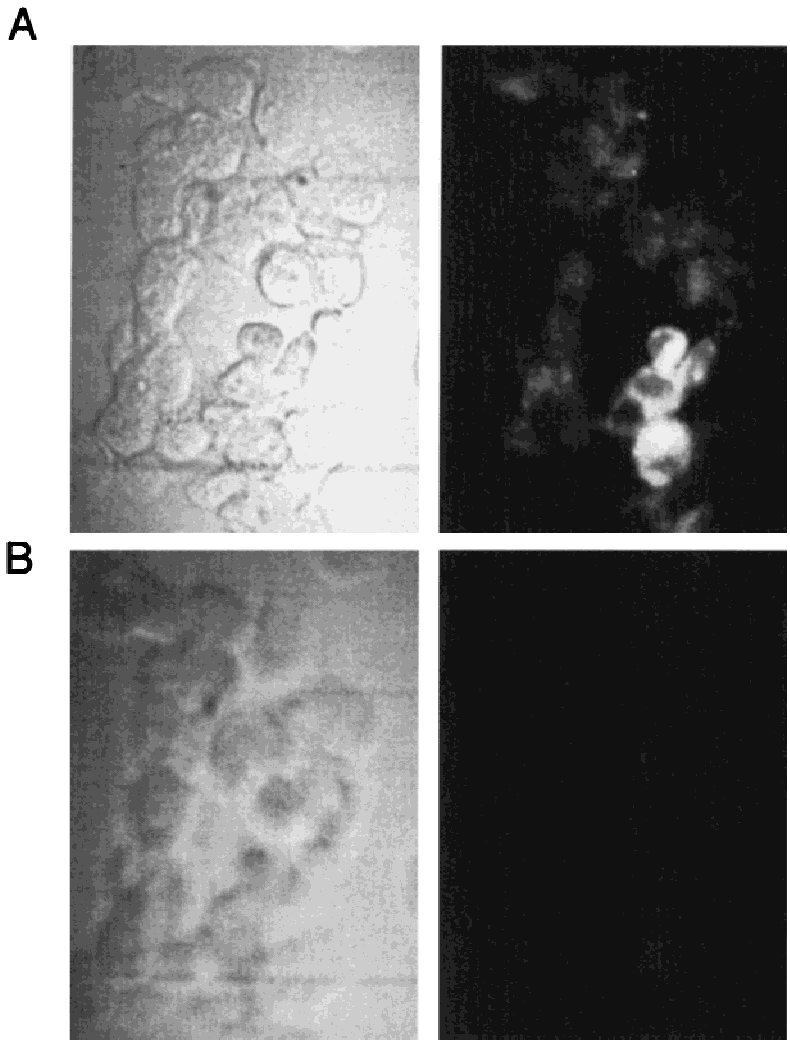

Fig. 7. An anti-peptide antibody to CFTR labels the apical surface of cultured adult rat alveolar epithelial cells. (*A*) Confocal microscopy showing the apical surface of antibody treated cells. (*B*) Optical sections showing the absence of antibody-labeling in the basolateral membrane.

In conclusion, we have found that alveolar epithelial monolayers cultured under apical air-interface conditions exhibit enhanced amiloride-sensitive Na^+ transport and that the response to terbutaline was identical to that observed in apical liquid-interface monolayers. Our results also demonstrated that terbutaline directly stimulates Cl^- channels in both freshly isolated alveolar cells and in cells dissociated from monolayers in culture for 7 days. This result indicates that the Cl^- channels detected in cultured monolayers and cells isolated from these monolayers are present in native alveolar epithelial cells and are not a consequence of time in culture. Terbutaline-activated currents were found to be blocked by the Cl^- channel blockers NPPB and glibenclamide, which were previously shown to block the actions of terbutaline on *Isc* in intact alveolar epithelial cell monolayers. An anti-peptide antibody to CFTR labeled the apical membrane, but not the basolateral membrane of cultured alveolar epithelial cells suggesting that a CFTR-like Cl^- channel was present in the apical membrane. Amiloride-sensitive Na^+ channels were not observed as part of the basal current in freshly isolated alveolar cells and were not activated by terbutaline.

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