Hypoxia and $\beta_2$-Agonists Regulate Cell Surface Expression of the Epithelial Sodium Channel in Native Alveolar Epithelial Cells*

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Alveolar hypoxia may impair sodium-dependent alveolar fluid transport and induce pulmonary edema in rat and human lung, an effect that can be prevented by the inhalation of $\beta_2$-agonists. To investigate the mechanism of $\beta_2$-agonist-mediated stimulation of sodium transport under conditions of moderate hypoxia, we examined the effect of terbutaline on epithelial sodium channel (ENaC) expression and activity in cultured rat alveolar epithelial type II cells exposed to 3% $O_2$ for 24 h. Hypoxia reduced transepithelial sodium current and amiloride-sensitive sodium channel activity without decreasing ENaC subunit mRNA or protein levels. The functional decrease was associated with reduced abundance of ENaC subunits (especially $\beta$ and $\gamma$) in the apical membrane of hypoxic cells, as quantified by biotinylation. cAMP stimulation with terbutaline reversed the hypoxia-induced decrease in transepithelial sodium transport by stimulating sodium channel activity and markedly increased the abundance of $\alpha$- and $\gamma$-ENaC in the plasma membrane of hypoxic cells. The effect of terbutaline was prevented by brefeldin A, a blocker of anterograde transport. These novel results establish that hypoxia-induced inhibition of amiloride-sensitive sodium channel activity is mediated by decreased apical expression of ENaC subunits and that $\beta_2$-agonists reverse this effect by enhancing the insertion of ENaC subunits into the membrane of hypoxic alveolar epithelial cells.

The amiloride-sensitive epithelial sodium channel (ENaC)$^1$ located in the apical membrane of alveolar epithelial type II (ATII) cells constitutes a rate-limiting step for sodium absorption across alveolar epithelium (1–4). The critical role of ENaC in alveolar fluid homeostasis has been highlighted by the fact that newborn $\alpha$-ENaC knockout mice died shortly after birth, primarily from failure to clear their lungs of fluid (5). Despite its physiological importance, little is known about the regulation of ENaC processing, trafficking to, and stability at the cell surface of alveolar epithelial cells under physiological or pathological conditions and in response to hormonal and pharmacological stimuli. $\beta$-Adrenergic agonists have been reported to stimulate active sodium transport across alveolar epithelium in various species in vivo and in vitro (4), but the mechanism(s) whereby these agents increase apical sodium channel activity in native ATII cells remains unclear. Interestingly, a recent study performed in a model of Fischer rat thyroid epithelial cells cotransfected with $\alpha$, $\beta$, and $\gamma$-ENaC suggested that cAMP agonists stimulate sodium current by enhancing the translocation of ENaC subunits from intracellular pools to the plasma membrane, thus increasing the number of sodium channels at the cell surface (6).

The alveolar epithelium is normally exposed after birth to a mean alveolar oxygen ($O_2$) pressure of 100 mm Hg, but alveolar hypoxia may occur in many physiological or pathological conditions, such as ascent to high altitude, alveolar hypventilation, or pulmonary edema from heart failure or acute lung injury. Rapid ascent to high altitude may induce in some subjects the development of pulmonary edema. Although the initial cause of alveolar flooding is likely related to altered hemodynamics or increased lung microvascular permeability (7, 8), new data from human and animal studies support the concept that defective alveolar fluid clearance could have a pathogenic role in the development of high altitude pulmonary edema (HAPE) and could be a potential target for therapy. Sartori et al. (9) recently reported that HAPE-sensitive subjects at low altitude have a decrease in nasal transepithelial potential difference compared with HAPE-insensitive subjects, suggesting that these subjects may have a genetically determined impairment of transepithelial sodium and liquid clearance in the lungs. Interestingly, the risk of HAPE was clearly reduced in these susceptible subjects by prophylactic inhalation of $\beta_2$-agonists, which are known to up-regulate sodium-dependent alveolar fluid transport in the human lung (10). In accordance with these findings, a recent animal study revealed that in vivo hypoxia (8% $O_2$) reduced sodium and fluid transport across rat alveolar epithelium (11). This hypoxia-induced decrease in transepithelial sodium transport occurred with no concomitant decrease in the quantity of sodium transport proteins expressed in alveolar epithelial cells from hypoxic rats, suggesting that inhibition of sodium transport was related to post-translational mechanism(s) altering the trafficking, stability at the cell surface, or the biophysical properties of transport proteins. Moreover, in this rat model, intra-alveolar administration of the $\beta_2$-agonist terbutaline rapidly and completely reversed the hypoxia-induced decrease in transepithelial sodium transport and alveolar liquid clearance.

Therefore, we hypothesized that one mechanism whereby hypoxia may reduce transepithelial sodium transport across

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1 The abbreviations used are: ENaC, epithelial sodium channel; ATII, alveolar epithelial type II; HAPE, high altitude pulmonary edema; rENaC, rat ENaC; DMEM, Dulbecco’s modified Eagle’s medium; AML, amiloride; TB, terbutaline; PBS, phosphate-buffered saline; BFA, brefeldin A; X, Xenopus laevis.
alveolar epithelial cells would be by decreasing the number of sodium channels at the cell surface of ATII cells and that β₂-agonists could reverse this effect by promoting insertion of new sodium channel subunits in the apical membrane. To test this hypothesis, we used an in vitro model of rat ATII cells in primary culture exposed to 3% O₂, equivalent to an O₂ pressure of 45 mm Hg in culture medium and corresponding to the degree of hypoxia used in the in vivo model of previously studied rats. The effects of hypoxia and β₂-agonist treatment on transepithelial sodium transport and specifically on rat ENaC (rENaC) expression and activity were evaluated. Using an approach of apical cell surface biotinylation, we also quantified, for the first time in native mammalian epithelial cells, the abundance of the three rENaC subunits in the plasma membrane of ATII cells and evaluated whether it was altered by moderate hypoxia or β₂-agonists.

**MATERIALS AND METHODS**

**Cell Isolation and Culture**—The procedure of ATII cell isolation from pathogen-free male Sprague-Dawley rats accorded with legislation currently in force in France and animal welfare guidelines (Ministère de la Pêche et de l’Agriculture, agreement 56689). ATII cells were isolated from adult rats (200–250 g) by elastase digestion of lung tissue followed by sequential filtration and differential adherence on bacteriological plates. Isolated cells, as previously described by Guo et al. (15), were bathed with an apical compartment solution containing 145 mM sodium chloride, 3.3 mM HPO₄²⁻, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 25 mM HCO₃⁻, 25 μM amiloride, and 5 mM glucose, 10 mM Hepes, 23.8 mM NaHCO₃, 2 μM t-glutamine, 10 mM fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin, and 10 μg/ml gentamycin. On day 3 after isolation, 1 μM dexamethasone was added to the culture medium, and fetal bovine serum concentration was reduced to 5%. The experiments were performed 4–6 days after isolation.

**Exposure to Hypoxia**—4–5 days after plating, the cell medium was removed and replaced by 0.2 ml/cm² of fresh medium to decrease the diffusion distance of the ambient gas. The cells were placed in a humidified airtight incubator with inflow and outflow valves, and the hypoxic gas mixture containing 3% O₂, 2% CO₂, and 95% air atmosphere in DMEM containing 25 mM glucose, 10 mM Hepes, 30 mM NaHCO₃, 2 mM l-glutamine, 10% fetal bovine serum, 50 μg/ml penicillin, 50 μg/ml streptomycin, and 10 μg/ml gentamycin was used as an internal standard because hypoxia did not significantly modify the level of actin mRNA (12). The results were expressed as the ratios of expression of the mRNA of interest to actin mRNA (arbitrary units).

**Western Blot Analysis**—Cells in 24-mm Transwell filters were washed twice, scraped off the filters in ice-cold PBS, and centrifuged at 1500 rpm for 5 min at 4 °C. The pellet was resuspended in 0.5 ml of ice-cold lysis buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 7.6), 1% Triton X-100, 0.1% SDS, and protease inhibitors and kept on ice for 1 h. The cell lysates were then centrifuged (12,000 rpm, 15 min) at 4 °C. Samples of the supernatants (10–15 μg of protein in 1 volume of sample buffer containing 10% glycerol, 12.5% 0.5 M Tris-HCl (pH 6.8), 10% of 20% SDS, 5% β-mercaptoethanol, and 2.5% of 0.05% (w/v) bromphenol blue) were resolved through 10% acrylamide gels, electroblotted, electrically transferred to nitrocellulose paper, and subsequently probed for α-, β-, and γ-rENaC subunits. Rabbit polyclonal anti-γ-rENaC, anti-β-rENaC, and anti-α-rENaC antibodies were used at 1:2000 dilution, and mouse monoclonal anti-α-rENaC antibodies were used at 1:5000 dilution. The specificity of antibodies against α-, β-, and γ-rENaC has previously been demonstrated by the displacement of the immunoprecipitated product with anti-α-, β-, or γ-rENaC fusion proteins, respectively (14). The anti-rabbit IgG secondary antibody (Amersham Biosciences) was used at dilution 1:5000, and the signal was developed with the ECL Plus system (Amersham Biosciences). Quantification of rENaC and actin levels was obtained using NIH image software.

**Biotinylation of Apical rENaC Subunits**—Biotinylation and recovery of apical membrane proteins were performed with a method adapted from Gottardi et al. (15) and Hanwell et al. (16). Briefly, ATII cells grown on Transwell filters were placed on ice and washed three times with ice-cold PBS-Ca²⁺–Mg²⁺ (PBS with 0.1 mM CaCl₂ and 1 mM MgCl₂). Apical membrane proteins were then biotinylated by a 15-min incubation at 4 °C with NHS-SS-biotin at a 1:50 dilution against α-, β-, and γ-rENaC, respectively (14). The anti-rabbit IgG secondary antibody (Amersham Biosciences) was used at dilution 1:5000, and the signal was developed with the ECL Plus system (Amersham Biosciences). Quantification of rENaC and actin levels was obtained using NIH image software.

**Table I**

| Condition          | Rsc     | Base line | AML-sensitive |
|--------------------|---------|-----------|---------------|
| Normoxia (21% O₂)  | 738 ± 93.4 | 7.1 ± 0.73 | 5.8 ± 0.69   |
| Hypoxia (3% O₂)    | 914 ± 70.1 | 4.5 ± 0.37⁺ | 3.2 ± 0.32⁺  |

*Significantly different from normoxic control (p < 0.01).
absence of leakage of biotin into the cells, we systematically verified the absence of the intracellular protein actin in biotinylated extracts.

**Statistical Analysis**—The results are presented as the means ± S.E. For functional data, one-way or two-way variance analyses were performed, and when allowed by the F value, the results were compared by the modified least significant difference. For Western blot experiments, differences between groups were evaluated with paired t tests. p < 0.05 was considered significant.

### RESULTS

**Effect of Hypoxia on ATII Cell Sodium Transport Properties**—The effects of hypoxia (3% O2) on sodium transport across ATII cell monolayers were evaluated by electrophysiological studies. Snapwell filters were mounted immediately at the end of normoxic/hypoxic exposure into a voltage clamp system, and electrophysiological measurements were performed. The mean electrophysiological values obtained from filters exposed to normoxia or hypoxia are given in Table I. Hypoxia significantly decreased base-line Isc and amiloride-sensitive Isc by 37 and 45%, respectively, but did not change the amiloride-insensitive part of Isc. To determine whether hypoxia affected apical sodium channel activity, Isc was measured in the presence of a sodium concentration gradient across the monolayers (mucosal to serosal: 145:25 mM) after permeabilization of the basolateral membrane with amphotericin B. The addition of amphotericin B to the basolateral bath rapidly increased Isc to a peak value (Iampho,max), as shown in Fig. 1. The peak value Iampho,max and the amiloride-sensitive part of this current (Iampho,AML), reflecting sodium influx through amiloride-sensitive channels located in the apical membranes, were both significantly decreased in cells exposed to hypoxia as compared with normoxic controls (Fig. 1).

**Effect of Hypoxia on rENaC mRNA Expression**—Fig. 2A shows that transcripts encoding for the three rENaC subunits were detected by RNase protection assays in ATII cells exposed to either normoxia or moderate hypoxia (3% O2 for 24h). Exposure to hypoxia did not significantly modify the levels of α-, β-, or γ-rENaC mRNAs in ATII cells, as compared with normoxic controls (Fig. 2B).

**Effect of Hypoxia on rENaC Subunit Protein Levels**—The protein levels of the three rENaC subunits were determined in whole ATII cell extracts grown on filters by Western blot experiments using rabbit polyclonal antibodies (Fig. 3A). The α-rENaC antibody revealed two bands, a main band at 85 kDa (the expected size of α-rENaC) and a smaller band that migrated at 65 kDa. The β- and γ-rENaC subunits were detected
as two single bands that migrated at 100 and 90 kDa, respectively, which represent the expected sizes of β- and γ-rENaC proteins. The protein levels of α- and γ-rENaC subunits (normalized to the corresponding actin signal) were not significantly modified by exposure to 3% O₂ hypoxia, but the quantity of β-rENaC subunit was decreased by almost 30% (p < 0.05) as compared with normoxic controls (Fig. 3B).

**Effect of Hypoxia on the Abundance of rENaC Subunits Expressed at the Cell Surface**—Biotinylation of ATII cell apical membranes was used to estimate the effect of moderate hypoxia on the amount of rENaC subunit proteins expressed at the plasma membrane. The absence of any labeling of actin, an abundant intracellular protein, in biotinylated extracts indicated that biotin did not enter the cells during the experimental procedures. As shown in Fig. 4A, biotinylated α-rENaC subunit protein was detected on Western blot as a single band of 65 kDa, a molecular mass clearly lower than that of the α-rENaC protein detected in the corresponding intracellular pool of proteins (85 kDa), as previously reported in A6 cells derived from Xenopus laevis kidney (17). The specificity of the signal was assessed by the absence of any signal with the preimmune serum or the specific antisera at 100 μg/ml. No band was visible in the presence of 3% O₂. The band visible in surface extracts in the presence of the antisera was displaced when the antisera was preincubated with α-rENaC fusion protein. Cell surface expression of α-, β-, and γ-rENaC subunits in ATII cells exposed to 21 or 3% O₂ was quantified as the ratios of biotinylated rENaC/actin and represent the means ± S.E. of four or five separate experiments (Fig. 4B). * and **, significantly different from normoxic control (p < 0.05).
Regulation of ENaC Cell Surface Expression in Alveolar Cells

Fig. 5. Effect of terbutaline on $I_{sc}$ of rat ATII cells exposed to moderate hypoxia or normoxia. Rat ATII cells grown on Snapwell filters were exposed to either 21 or 3% O$_2$ for 24 h before the bioelectric measurements were performed. A, representative traces of normoxic (black squares) and hypoxic (white squares) filters treated with TB (100 μM into the apical bath). The addition of terbutaline increased the short circuit current ($I_{sc}$), which reached a plateau ($I_{sc,max}$) after 35–40 min, and AML (10 μM) was then added into the apical bath. B, effect of 21 and 3% O$_2$ exposure on mean values of baseline $I_{sc}$ and $I_{sc,max}$. The stimulation of $I_{sc}$ was of greater magnitude in hypoxic filters than in normoxic controls so that $I_{sc,max}$ was not significantly different in normoxic and hypoxic cells. The values are the means ± S.E. of six or seven filters for each condition. *, $p < 0.05$; **, $p < 0.01$; NS, not significantly different from the normoxic value.

ATII cells grown on filters. As shown in Fig. 5A, the addition of 100 μM terbutaline into the apical bath induced a transient decrease in $I_{sc}$ that was of greater magnitude in normoxic than in hypoxic filters (0.9 ± 0.10 versus 0.4 ± 0.07 μA/cm$^2$, respectively; $p < 0.001$). This decrease was followed by a gradual increase in $I_{sc}$ to steady state levels ($I_{sc,max}$) after 35–40 min, surpassing the base line in both normoxic and hypoxic monolayers. The TB-induced increase in $I_{sc}$ was of greater magnitude in hypoxic cells than in normoxic cells because $I_{sc,max}$ represented 142 ± 4.8% versus 117 ± 3.8% of baseline $I_{sc}$ value in hypoxic and normoxic filters, respectively ($p < 0.01$). As a consequence, although base-line $I_{sc}$ was significantly lower in hypoxic filters, $I_{sc,max}$ was not significantly different in normoxic and hypoxic filters (Fig. 5B). Indeed, the amiloride-sensitive component of $I_{sc,max}$ was comparable in cells exposed to 3% O$_2$ and in control cells (5.1 ± 0.26 versus 5.9 ± 0.51 μA/cm$^2$, respectively, non-significant), indicating that the β$_2$-agonist completely reversed the hypoxia-induced decrease in sodium transport across ATII cell monolayers.

To determine whether the TB-induced increase in $I_{sc}$ was associated in hypoxic ATII cells with an increase in apical sodium channel activity, hypoxic ATII cell monolayers treated for 40 min with 100 μM TB (or vehicle) in the presence of a mucosal to serosal sodium concentration gradient were basolaterally permeabilized with amphotericin B. Terbutaline induced as expected a progressive increase in $I_{sc}$, and basolateral application of amphotericin B further increased $I_{sc}$. Terbutaline increased by 48% $I_{sc,AML}$ of the amiloride-sensitive part of $I_{sc}$, peak value following amphotericin addition, which reflects sodium influx through apical amiloride-sensitive channels, as compared with vehicle $I_{sc,AML}$. 8.7 ± 0.56 versus 5.8 ± 0.87 μA/cm$^2$ for TB and vehicle, respectively; $n = 5–6$ filters for each condition. $p < 0.05$). This result indicates that the reversal by TB of the hypoxia-induced decrease in transepithelial sodium transport is, at least in part, accounted for by an increase in amiloride-sensitive sodium channel activity.

To determine whether the reversal of the hypoxia-induced decrease in amiloride-sensitive sodium transport by β$_2$-agonists could partly result from the translocation of sodium transport proteins from cytoplasmic storage to the plasma membrane (6), we studied the effect of brefeldin A (BFA), which inhibits intracellular trafficking of membrane proteins from the cytosolic pool to the cell surface, on TB-induced $I_{sc}$ response in hypoxic ATII cells. Alveolar type II cells exposed to 3% O$_2$ for 24 h were incubated for 20 min with or without BFA (1 μg/ml) before TB (or vehicle) was added to the apical bath (Fig. 6A). Brefeldin A had no significant effect per se on the basal $I_{sc}$ or on amiloride-sensitive $I_{sc}$ measured in the absence of TB (Fig. 6B). Pretreatment with BFA completely abolished the stimulatory effect of TB on $I_{sc}$. Indeed, amiloride-sensitive $I_{sc}$ in hypoxic cells treated with BFA and TB was not significantly different from $I_{sc}$ in control hypoxic cells or in hypoxic cells treated with BFA alone. Additional experiments showed that preincubation with BFA also completely inhibited the TB-induced $I_{sc}$ increase in normoxic cells (data not shown).

Effect of Terbutaline on the Abundance of rENaC Subunits Expressed at the Cell Surface—Incubation of hypoxic ATII cells with TB immediately at the end of the 3% O$_2$ exposure significantly increased the amount of biotinylated β- and γ-rENaC subunit proteins by 1.9- and 2.9-fold, respectively, as compared with unstimulated hypoxic controls (Fig. 7). Terbutaline did not significantly increase the amount of biotinylated α-rENaC subunit protein in hypoxic cells. In normoxic ATII cells, cell surface expression of rENaC subunits was not significantly modified by TB, because biotinylated α-, β-, and γ-rENaC in treated cells represented 114 ± 16, 126 ± 20, and 126 ± 36% of untreated normoxic controls, respectively ($n = 3–4$, non-significant).

DISCUSSION

The present study shows that reduction of amiloride-sensitive sodium channel activity in native rat ATII cells by moderate hypoxia is related to decreased expression of rENaC subunits, especially that of β- and γ-rENaC, at the apical plasma membrane. The β$_2$-agonist terbutaline rapidly reversed the hypoxia-induced inhibition of amiloride-sensitive sodium channel activity, at least in part by increasing the abundance of β- and γ-rENaC subunits in the apical membrane of hypoxic ATII cells. These results provide direct evidence that moderate hypoxia reduces the apical expression of sodium channels in ATII cells, either by decreasing the translocation of rENaC subunits from cytoplasmic pools to the plasma membrane or by increasing their rate of internalization. The results also suggest that β$_2$-agonists stimulate sodium absorption by increasing the delivery of rENaC subunits into the plasma membrane of alveolar epithelial cells.

A previous study reported that in vivo exposure of rats to moderate hypoxia (8% O$_2$ for 24 h, equivalent to an alveolar O$_2$
pressure of 45–50 mm Hg) reduced alveolar fluid clearance through a decrease of active sodium transport across alveolar epithelium (11). This hypoxia-induced decrease in alveolar transepithelial sodium transport occurred with no change in gene expression or protein abundance of α-, β-, and γ-rENaC subunits in ATII cells, suggesting that hypoxia modifies rENaC expression at the post-translational level. Interestingly, intra-alveolar administration of the β2-agonist terbutaline rapidly and completely reversed the decrease in transepithelial sodium transport in that in vivo study.

In the present in vitro study, rat ATII cell monolayers were exposed to a moderate hypoxia (3% O2, equivalent to an O2 pressure of 45 mm Hg in culture medium) for 24 h. Hypoxia resulted in an inhibition of active transepithelial sodium transport as assessed by a 45% decrease in amiloride-sensitive I_sc caused, at least in part, by a decrease in sodium channel activity because the amiloride-sensitive part of I_sc in basolaterally permeabilized monolayers decreased by −40% in hypoxic ATII cells as compared with normoxic controls. In this condition of moderate hypoxia, α-, β-, and γ-rENaC mRNA levels as well as α- and γ-rENaC subunit proteins in whole ATII cell extracts were unchanged, and only a mild decrease in the amount of β-rENaC subunit was observed. These in vitro results mimicked those obtained in vivo in rats exposed to 8% O2 (11). They strongly suggest that inhibition of amiloride-sensitive sodium channels by moderate hypoxia occurs at the post-translational level and may involve either a decrease in the number of sodium channels in the apical membrane of hypoxic ATII cells or direct alteration of intrinsic sodium channel properties.

To assess rENaC subunit protein expression at the plasma membrane of normoxic and hypoxic ATII cells, we performed experiments of biotinylation of proteins expressed at the apical cell surface. This approach was original inasmuch as, to our knowledge, it constitutes the first example of direct quantification of rENaC subunit apical expression in mammalian epithelial cells endogenously expressing ENaC. In normoxic ATII cells under steady state conditions, the apical expression of α-rENaC represented 20–25% of total α-rENaC cellular pool, a proportion similar to that obtained in renal amphibian A6 cells (18), and apical expression of β- and γ-rENaC subunits was only 5% of total cellular pools. Although biotinylated β- and γ-rENaC subunit bands migrated at the same level as intracellular β- and γ-rENaC, biotinylated α-rENaC had an apparent molecular mass (65 kDa) lower than intracellular α-rENaC (85 kDa). A similar observation has been previously made in X. laevis kidney-derived A6 cells in which the prominent fraction of α-xENaC expressed at the cell surface also had an apparent molecular mass of 65 kDa (17). This finding has been attributed to the fact that during its maturation, α-xENaC is prone to form disulfide bridges resistant to reducing agents, a phenomenon that induces a faster migration of the protein on SDS-PAGE and changing the apparent molecular mass to 65 kDa.

Hypoxia significantly decreased the abundance of α-, β-, and γ-rENaC subunits expressed at the apical membrane of ATII cells. Interestingly, the decrease was greater for β- and γ-rENaC subunits than for the α-rENaC subunit. Although moderate, the decrease in apical α-rENaC might participate per se in the functional impairment of sodium transport, inasmuch as the expression of α-rENaC subunit is required to obtain significant channel activity in Xenopus oocytes (2). In-
deed, the dramatic decrease in β- and γ-ENaC subunit expression at the cell surface suggests that the trafficking of these proteins is highly sensitive to hypoxia and that the density of β- and γ-ENaC subunits at the apical membrane of ATII cells could become critical when O₂ availability is reduced. Insufficient abundance of β- and γ-ENaC subunits may lead to a decreased number of highly selective sodium channels typical of ENaC, inasmuch as the presence of the three subunits is required to form complete ENaC channels with optimal activity (2). In support of this hypothesis, a previous patch-clamp study (19) reported that in cultured rat ATII cells, the level of oxygenation positively influenced the expression of highly selective sodium channels over nonselective sodium channels presumably made of α-ENaC subunits alone or in association with proteins other than β- and γ-ENaC subunits (20).

In the present study, the β₂-agonist terbutaline added at the end of hypoxic exposure rapidly and completely reversed the hypoxia-induced decrease in transepithelial sodium transport by increasing apical sodium channel activity, in line with results obtained in hypoxic rats in vivo (11). Up to now, most studies trying to address the mechanism(s) whereby β₂-agonists increase sodium absorption in native epithelial cells have been limited to a functional approach, because the very small number of ENaC channels expressed at the plasma membrane has hampered the use of traditional biochemical techniques. In native ATII cells, functional studies have provided conflicting results, attributing the effect of β₂-agonists to either an increase in the number of highly selective sodium channels in the apical membrane (21), an increase in sodium channel open probability (22, 23), or an indirect stimulation of transcellular sodium movement through the stimulation of apical chloride conductance (24). Recently, using a novel approach based on the covalent modification of cotransfected ENaC subunits at the cell surface of Fischer rat thyroid epithelial cells, Snyder (6) provided convincing evidence that the cAMP-mediated stimulation of Iₑ × resulted from an increase in the ratio of translocation versus internalization of ENaC subunits to the cell surface. That such a phenomenon could also take place in alveolar epithelial cells endogenously expressing rENaC was supported herein by biotinylation experiments and functional data. In hypoxic ATII cells, terbutaline largely increased the abundance of ENaC, inasmuch as the presence of the three subunits is required to form complete ENaC channels with optimal activity (2). In support of this hypothesis, a previous patch-clamp study (19) reported that in cultured rat ATII cells, the level of oxygenation positively influenced the expression of highly selective sodium channels over nonselective sodium channels presumably made of α-ENaC subunits alone or in association with proteins other than β- and γ-ENaC subunits (20).

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The present study, the β₂-agonist terbutaline added at the end of hypoxic exposure rapidly and completely reversed the hypoxia-induced decrease in transepithelial sodium transport by increasing apical sodium channel activity, in line with results obtained in hypoxic rats in vivo (11). Up to now, most studies trying to address the mechanism(s) whereby β₂-agonists increase sodium absorption in native epithelial cells have been limited to a functional approach, because the very small number of ENaC channels expressed at the plasma membrane has hampered the use of traditional biochemical techniques. In native ATII cells, functional studies have provided conflicting results, attributing the effect of β₂-agonists to either an increase in the number of highly selective sodium channels in the apical membrane (21), an increase in sodium channel open probability (22, 23), or an indirect stimulation of transcellular sodium movement through the stimulation of apical chloride conductance (24). Recently, using a novel approach based on the covalent modification of cotransfected ENaC subunits at the cell surface of Fischer rat thyroid epithelial cells, Snyder (6) provided convincing evidence that the cAMP-mediated stimulation of Iₑ × resulted from an increase in the ratio of translocation versus internalization of ENaC subunits to the cell surface. That such a phenomenon could also take place in alveolar epithelial cells endogenously expressing rENaC was supported herein by biotinylation experiments and functional data. In hypoxic ATII cells, terbutaline largely increased the abundance of ENaC, inasmuch as the presence of the three subunits is required to form complete ENaC channels with optimal activity (2). In support of this hypothesis, a previous patch-clamp study (19) reported that in cultured rat ATII cells, the level of oxygenation positively influenced the expression of highly selective sodium channels over nonselective sodium channels presumably made of α-ENaC subunits alone or in association with proteins other than β- and γ-ENaC subunits (20).
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