Dexamethasone prevents transport inhibition by hypoxia in rat lung and alveolar epithelial cells by stimulating activity and expression of Na\(^+-\)K\(^+-\)ATPase and epithelial Na\(^+\) channels

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Güney S, Schuler A, Ott A, Höschele S, Zügel S, Baloğlu E, Bärtsch P, Mairbaurl H. Dexamethasone prevents transport inhibition by hypoxia in rat lung and alveolar epithelial cells by stimulating activity and expression of Na\(^+\)-K\(^+-\)ATPase and epithelial Na\(^+\) channels. Am J Physiol Lung Cell Mol Physiol 293: L1332–L1338, 2007. First published September 14, 2007; doi:10.1152/ajplung.00338.2006.—Hypoxia inhibits Na and lung fluid reabsorption, which contributes to the formation of pulmonary edema. We tested whether dexamethasone prevents hypoxia-induced inhibition of reabsorption by stimulation of alveolar Na transport. Fluid reabsorption, transport activity, and expression of Na transporters were measured in hypoxia-exposed rats and in primary alveolar type II (ATII) cells. Rats were treated with dexamethasone (DEX; 2 mg/kg) on 3 consecutive days and exposed to 10% O\(_2\) on the 2nd and 3rd day of treatment to measure hypoxia effects on reabsorption of fluid instilled into lungs. ATII cells were treated with DEX (1 μM) for 3 days before exposure to hypoxia (1.5% O\(_2\)). In normoxic rats, DEX induced a twofold increase in alveolar fluid clearance. Hypoxia decreased reabsorption (∼30%) by decreasing its amiloride-sensitive component; pretreatment with DEX prevented the hypoxia-induced inhibition. DEX increased short-circuit currents (ISC) of ATII monolayers in normoxia and blunted hypoxic transport inhibition by increasing the capacity of Na\(^+\)-K\(^+-\)ATPase and epithelial Na\(^+\) channels (ENaC) and amiloride-sensitive ISC. DEX slightly increased the mRNA of α- and γ-ENaC in whole rat lung. In ATII cells from DEX-treated rats, mRNA of α\(_1\)-Na\(^+\)-K\(^+-\)ATPase and α-ENaC increased in normoxia and hypoxia, and γ-ENaC was increased in normoxia only. DEX stimulated the mRNA expression of α\(_1\)-Na\(^+\)-K\(^+-\)ATPase and α, β, and γ-ENaC of A549 cells in normoxia and hypoxia (1.5% O\(_2\)) when DEX treatment was begun before or during hypoxic exposure. These results indicate that DEX prevents inhibition of alveolar reabsorption by hypoxia and stimulates the expression of Na transporters even when it is applied in hypoxia. Hypoxic pulmonary edema; alveolar fluid reabsorption; mRNA expression; ion transport

**ACTIVE TRANSPORT** of Na across alveolar epithelial cells plays an important role in the regulation of the volume of alveolar lining fluid and in the reabsorption of edema fluid in pathological conditions (25). Alveolar hypoxia causes pulmonary edema, in part, probably by inhibition of alveolar Na and fluid reabsorption (21, 30, 31, 34). Hypoxia has been shown to inhibit lung fluid reabsorption in hypoxia-exposed rats by decreasing its Na-depen-

dent component (34). Inhibition of Na transport by hypoxia was also found in cultured alveolar epithelial cells (4, 20, 21, 28, 29). Transport inhibition has been explained with a decreased mRNA expression (29) causing a decreased expression of Na\(^+\)-K\(^+-\)ATPase and epithelial Na\(^+\) channel (ENaC) protein in the plasma membrane of alveolar epithelial cells (5, 36). Internalization of transporters also seems to contribute to the decreased Na channel activity in hypoxic alveolar epithelial cells (6, 28). Results among alveolar epithelial cells obtained from hypoxia-exposed rats are discrepant since both decreased (36) and increased (34) expression of Na transporters have been observed. In humans exposed to high altitude hypoxia, a decreased capacity of alveolar Na transport indicated by a decreased nasal epithelial potential difference has been discussed as a possible reason for susceptibility to high altitude pulmonary edema (20, 32), whereas stimulation of Na transport with inhaled β-adrenergic agents such as terbutaline and glucocorticoids (for review, see Ref. 24).

Glucocorticoids stimulate alveolar fluid clearance in a time-dependent manner. Noda et al. (27) have shown that reabsorption was stimulated 24 h after a single application of the glucocorticoid dexamethasone (DEX) to rats and observed a concomitant increase in the expression of Na transporters such as Na\(^+\)-K\(^+-\)ATPase and ENaC. Similar results were obtained in cultured alveolar epithelial cells (3, 7, 24). Whereas in human A549 cells, particularly, the expression of the β-

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γ-subunits of ENaC has been found to be upregulated by DEX (15), Dagenais et al. (7) reported also an increase in the mRNA expression of the α ENaC subunit on DEX treatment of primary alveolar type II (ATII) cells from adult rats.

Based on these results, it is conceivable that treatment with DEX might blunt the hypoxic inhibition of alveolar Na transport and fluid reabsorption by increasing expression and activity of Na transporters in alveolar epithelial cells and might thus prevent the formation and stimulate the clearance of pulmonary edema. This hypothesis appears to be supported by the prevention of pulmonary edema in high altitude hypoxia by prophylactic DEX in individuals with a history of high altitude pulmonary edema (16). It is not known whether hypoxia affects glucocorticoid hormone-dependent intracellular signaling. The main action of glucocorticoids on alveolar epithelial cell ion transport seems to be the synthesis of new transporters (15). Interference with hypoxia seems likely, since inhibition of protein synthesis is a common strategy to adjust to hypoxia (13). Also, in A549 cells, hypoxia has been shown to inhibit protein synthesis (21).

It was, therefore, studied whether DEX prevents the inhibition of alveolar reabsorption by hypoxia. To answer this question, fluid reabsorption was measured in rats treated with DEX before being exposed to hypoxia. Ion transport activity and the capacity of Na^+ -K^+ -ATPase and ENaC were measured in rat primary ATII cells. Human A549 alveolar cells were studied to test whether treatment with DEX before exposure to hypoxia was required to stimulate the expression of transporters.

**MATERIAL AND METHODS**

**Fluid Reabsorption in Hypoxia-Exposed Rats**

Male Sprague-Dawley rats with an average weight of 220 g with free access to standard rat chow and tap water were randomly assigned to treatment groups. DEX (2 mg kg^{-1} day^{-1}) or an equivalent volume of saline was administered intraperitoneally on 3 consecutive days. Beginning on the 2nd day of treatment, rats were continued in normoxia or exposed normobrac hypoxia (10% O_2, rest N_2) for 48 h while DEX/ shamt injections continued. Rats were anesthetized with sodium thiopental (100 mg/kg; Trapanal, Altana) and were anticoagulated with heparin-Na (1,500 IU/kg). For the measurement of alveolar reabsorption, rats were placed on a heating pad (39°C), and 2.5 ml of prewarmed medium (in mM: 135 NaCl, 5 KCl, 1 MgCl_2, 1 CaCl_2, 1 NaH_2PO_4, 5 glucose, 10 HEPES) containing 5% fatty acid-free BSA (Sigma) without or with 1 mM amiloride was instilled through a tracheal cannula. Thirty minutes after instillation, 250 μl of the instillate was removed to account for dead space, and a sample of instillate was collected and centrifuged (10,000 g for 10 s at room temperature) for protein measurements. Fluid reabsorption was calculated from the increase in the protein concentration in the instillate measured with a test kit from Bio-Rad. An aliquot of lung tissue was removed and lysed in lysis buffer and processed for measuring the mRNA expression of Na transporters. ATII cells were also prepared from untreated rats and cultured in normoxia (room air supplemented with 5% CO_2) on day 3 after preparation, cells were treated with DEX (24 h; 1 μM) and exposed to hypoxia (48 h; 1.5% O_2, 5% CO_2, rest N_2).

**Cell isolation and culture.** Experiments were performed on primary cultures of ATII cells isolated from lungs of normoxic male rats (Sprague-Dawley; 150–200 g) as described elsewhere (19). Briefly, lungs from rats anesthetized (intraperitoneal injection with 100 mg/kg pentobarbital; Trapanal, Byk Gulden) were perfused with PBS while being ventilated with air. ATII cells were isolated by elastase digestion, mincing of lung tissue, filtration, and differential adhesion on IgG-coated plates (21). Nonadherent cells were dissolved in RLT (Qiagen) for isolation of total RNA or suspended in DMEM supplemented with 10% neonatal calf serum, glutamine (4 mM), and gentamycin (50 μg/ml) and plated on tissue culture-treated Nucleopore filters (pore size 0.4 μm, diameter 12 mm, Transwell, Costar, Cambridge, MA) at a seeding density of 1 x 10^5 cells/cm^2 for functional analysis. Both purity and viability of ATII cells were >85%. For tissue culture, cells were maintained in normoxia (room air/5% CO_2) until they had reached confluence (typically on day 3 after plating). Formation of tight monolayers was tested by measuring transepithelial resistance using the epithelial volto-meter (EVOM) device and chopstick electrodes (World Precision Instruments, Sarasota, FL).

**Ussing chamber measurements.** For measuring transport activity, cell monolayers were typically used on day 3–5 after plating. After mounting in modified Ussing chambers, the monolayers were bathed with media composed of (in mM) NaCl 141, KCl 0.78, NaHPO_4, 1.8 CaCl_2, 0.6 MgCl_2, 5 glucose, and 15 HEPES, pH 7.4, at 37°C. After equilibration to the medium (10 min; 37°C; open-circuit conditions), short-current circuit (ISC) was recorded with an automated voltage clamp unit (W. Nagel, Munich, Germany) by clamping the transepithelial potential to 0 mV and stored in a computer for offline analysis. Amiloride (final concentration 10 μM) was used to inhibit the activity of apical Na channels (ISC_amiloride).

To assess changes in the number of transport proteins in the plasma membrane of primary ATII cells, we chose to measure the capacity of the Na^+ -K^+ -ATPase (ISC_Na,K) and of amiloride-sensitive current (ISC_amiloride) after permeabilization of the apical and basolateral membrane, respectively (19). This method provides a measure of the number of active transporters, whereas Western blots show total protein without reference to its activity. To measure ISC_Na,K in the basolateral membrane, ISC was recorded after permeabilization of the apical membrane with amphotericin B (final concentration 7.5 μM) using the above described bathing medium. To measure ISC_amiloride, monolayers were bathed with the above mentioned medium at the apical membrane, whereas the basolateral side was bathed with this medium, but the concentration of Na^+ was reduced to 25 mM by replacement with N-methyl-d-glucamine to establish a Na gradient before permeabilization of the basolateral membrane (19).

**Experiments on A549 Cells**

Since the action of DEX on Na reabsorption seems to depend mainly on stimulation of the expression of Na transport proteins (7, 27), it was tested on the human A549 alveolar epithelial cell line whether pretreatment with DEX is required to blunt hypoxia effects or whether DEX also exerts its action when it is applied during hypoxia. Although the basal mRNA expression of ENaC is lower in A549 cells than in primary ATII cells, these cells appear a valid model since they show many characteristics of alveolar epithelial cells, and they also show inhibition of ion transport by hypoxia similar to primary rat ATII cells (21, 36). It has not been studied, however, whether human A549 and primary rat ATII cells respond quantitatively similar to DEX exposure to hypoxia. DEX (1 μM) was added to the culture medium on days 4 and 6 after plating. On day 7, cells were exposed to hypoxia

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(1.5% O₂, 5% CO₂, rest N₂) in an O₂- and CO₂-controlled tissue culture incubator (Nunc); control cells continued to stay in normoxia for 24 h. In a second series of experiments, cells were exposed to hypoxia for 48 h. After 24 h of exposure, cells were treated with DEX (1 μM). Cells were then lysed for isolation of total RNA using the TriFast reagent (PeqLab) for RT-PCR.

**RNA Isolation and RT-PCR**

Total RNA was isolated from the TriFast lysates according to the manufacturer’s instructions, and 1 μg of RNA was transcribed with SuperScript II RT (Life Technologies) using random hexamer primers (Roche, Mannheim, Germany). Real-time quantitative PCR was performed in the LightCycler using the LC Fast Start PCR Mix (Roche) and the primers (MWG Biotech) listed in Table 1. To test for the specificity of PCR amplification, PCR products were separated by agarose gel electrophoresis and stained with Gelstar (BMA). PCR products showed single bands of the predicted size (data not shown). Sequencing (MWG Biotech) of bands eluted from agarose gels of PCR products confirmed the predicted base sequence.

Standards for quantification of mRNA expression were prepared by conventional PCR using the primers listed in Table 1 and eluates of PCR products from agarose gels. To control for differences in the efficacy of reverse transcription, 28S rRNA and β-actin were used. The mRNA expression of these markers did not change during exposure to hypoxia and DEX (data not shown).

**Statistical Evaluation**

Results are shown as means ± SD or SE as indicated. Data were analyzed by ANOVA followed by Tukey’s test for pairwise comparisons. Level of significance was *P* ≤ 0.05.

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**Table 1. Primers used for real-time PCR measurement of mRNA in A549 cells and rat lung**

<table>
<thead>
<tr>
<th>Product</th>
<th>Sequence</th>
<th>Length, bp</th>
<th>Acc. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primers used for A549 cells</td>
<td>ATG GGG AAG GGG GTT GGA GCT GAT AA</td>
<td>530</td>
<td>NM_000701</td>
</tr>
<tr>
<td>Primers used for rat lung</td>
<td>TAT GTC TGA GCG TCA TGA GTT</td>
<td>237</td>
<td>NM_012504</td>
</tr>
<tr>
<td>Primers used for rat lung</td>
<td>TGG CGG CAG TCT GTC GAC</td>
<td>202</td>
<td>NM_013113</td>
</tr>
<tr>
<td>Primers used for rat lung</td>
<td>GTC TGG CAG TTG GAC ACA GA</td>
<td>365</td>
<td>NM_031548</td>
</tr>
<tr>
<td>Primers used for rat lung</td>
<td>GTC CTC CCA AAA AAG TCC TT</td>
<td>193</td>
<td>NM_026684</td>
</tr>
<tr>
<td>Primers used for rat lung</td>
<td>ACA AAG ACC TGA ACC TTA GAG T</td>
<td>286</td>
<td>NM_017046</td>
</tr>
</tbody>
</table>

The quality of the PCR was tested by analyzing melting curves and agarose gels of PCR products (data not shown). Accession numbers are from the National Center for Biotechnology Information nucleotide GenBank (http://www.ncbi.nlm.nih.gov/blast/). ENaC, epithelial Na⁺ channel; GAPD, glyceraldehyde phosphate dehydrogenase.

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**RESULTS**

**DEX Effects on Alveolar Fluid Reabsorption and Gene Expression in Rat Lung**

Reabsorption of fluid instilled into the lungs of anesthetized rats was measured after treatment with DEX before the animals were exposed to hypoxia (10% O₂). Results are summarized in Fig. 1. Treatment of rats with DEX for 3 days stimulated alveolar reabsorption by ~60% (*P* = 0.002) in normoxia. Exposure of rats without DEX to hypoxia for 48 h decreased reabsorption significantly by ~30% (*P* = 0.001). When rats were treated with DEX and then exposed to hypoxia for 48 h with continued DEX treatment, only a slight stimulation of reabsorption with DEX was detectible relative to hypoxic control rats (*P* = 0.07), and the reabsorption rate was significantly lower than after DEX treatment in normoxia (*P* = 0.03). Thus, after treatment with DEX and exposure to hypoxia, the reabsorption rate was not different from untreated rats in normoxia (*P* = 0.73). In normoxic control rats, amiloride inhibited ~60% of reabsorption. Significant inhibition of fluid reabsorption by amiloride was seen in all conditions except for hypoxia-exposed rats without DEX. The amiloride-insensitive portion of reabsorption was not affected by DEX and hypoxia.

Effects of DEX and hypoxia on the mRNA expression of Na transporters were evaluated on whole lung tissue and on primary ATII cells isolated from rats after the respective treatment. Results are summarized in Table 2. In whole lung, hypoxia did not affect the expression of the α-, β-, and γ-subunit of ENaC and of α₁-Na⁺-K⁺-ATPase. In normoxia, DEX caused a significant increase in α-ENaC mRNA (+65%;
Results shown in Fig. 2B were obtained in symmetric, high Na\(^+\) medium after permeabilization of the apical membrane. It shows that hypoxia decreased ISC\(_{C-Na/K}\) by \(-33\% (P = 0.035\). In normoxic cells, DEX increased ISC\(_{C-Na/K}\) (+48%; \(P = 0.006\)). A 24-h DEX treatment before exposure to hypoxia increased ISC\(_{C-Na/K}\) by \(60\% (P = 0.015\). Results in Fig. 2C were obtained with high Na\(^+\) medium on the apical and low Na\(^+\) medium on the basolateral side and permeabilization of the basolateral membrane to obtain a measure of the capacity of amiloride-sensitive Na transport across the apical plasma membrane (ISC\(_{Damil}\). Hypoxia decreased ISC\(_{Damil}\) by \(-25\% (P = 0.03\). DEX stimulated ISC\(_{Damil}\) by \(75\% in normoxic (\(P = 0.001\) and by \(-50\% in hypoxic cells (\(P = 0.05\). However, neither component of ISC of nonpermeabilized and permeabilized cells of hypoxia-plus-DEX-treated cells significantly exceeded normoxic control values.

**mRNA Expression in A549 Cells**

A twofold (\(P = 0.005\); data not shown) increase in the expression of glyceraldehyde phosphate dehydrogenase (GAPD) mRNA confirmed that the exposure of the cultured cells to hypoxia resulted in an upregulation of typically hypoxia-induced genes. DEX did not affect the hypoxia-induced increase in GAPD mRNA.

DEX is known to stimulate alveolar Na transport by stimulating the expression of transport proteins (24). Since it is not known whether hypoxia affects DEX-stimulated gene expression, the mRNA expression of ENaC and Na\(^+\)-K\(^+\)-ATPase was measured in two sets of experiments: 1) A549 cells were treated with DEX before hypoxia; and 2) cells were exposed to hypoxia before DEX treatment. The results are summarized in Table 3. In both experimental conditions, DEX stimulated the mRNA expression of all three ENaC subunits and of Na\(^+\)-K\(^+\)-ATPase.

### Table 2. Effects of hypoxia and DEX on the mRNA expression of ENaC and Na\(^+\)-K\(^+\)-ATPase in whole rat lung and primary rat alveolar epithelial cells

<table>
<thead>
<tr>
<th>Control</th>
<th>DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole Rat Lung, fg/pg β-actin</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normoxia</td>
</tr>
<tr>
<td>α-ENaC</td>
<td>5.8±1.4</td>
</tr>
<tr>
<td>β-ENaC</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>γ-ENaC</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>α(_1)-Na(^+)-K(^+)-ATPase</td>
<td>13±2</td>
</tr>
<tr>
<td>β(_1)-Na(^+)-K(^+)-ATPase</td>
<td>2.6*</td>
</tr>
</tbody>
</table>

| Freshly Prepared ATII Cells, fg/pg β-actin | | |
| | Normoxia | Hypoxia | Normoxia | Hypoxia |
| α-ENaC | 8.9±2.3 | 8.3±2.4 | 13.5±9.3 | 12.6±2.4* |
| β-ENaC | 0.5±0.2 | 0.5±0.1 | 0.9±0.6 | 0.4±0.1 |
| γ-ENaC | 0.7±0.2 | 0.9±0.1 | 0.9±0.1* | 1.0±0.2 |
| α\(_1\)-Na\(^+\)-K\(^+\)-ATPase | 50.3±5.5 | 32.1±5.5* | 64.0±14.0* | 53.6±6.2* |
| β\(_1\)-Na\(^+\)-K\(^+\)-ATPase | 10.9±1.3 | 8.7±1.8 | 11.9±0.8 | 8.3±0.8 |

Rats were injected with dexamethasone (DEX; 2 mg·kg\(^{-1}\)·day\(^{-1}\)) on 3 consecutive days. A subgroup of animals was exposed to normobaric hypoxia (10% O\(_2\)) on the 2nd and 3rd day. Total RNA was extracted from whole lung and from freshly prepared primary alveolar type II (ATII) cells for the measurement of mRNA expression of Na transporters by real-time PCR. Values are means ± SD. *\(P < 0.05\) between DEX and control at the respective oxygenation level; †\(P < 0.05\) between normoxia and hypoxia.

**Effects of DEX on Ion Transport in ATII Cells**

Primary rat ATII cell monolayers were treated with DEX and hypoxia to test whether DEX blunts the hypoxic transport inhibition we described earlier (19). Figure 2A shows that total ISC (ISC\(_{tot}\) was inhibited by hypoxia by \(-30\% (P = 0.008\). Also, ISC\(_{Damil}\) was decreased by \(-44\% (P = 0.046\). In normoxia, a 3-day treatment with DEX increased ISC\(_{tot}\) by almost \(50\% (P = 0.001\) and ISC\(_{Damil}\) by \(63\% (P = 0.022\). A 24-h DEX treatment before exposure to hypoxia increased transport activity above hypoxic values as indicated by an increase in ISC\(_{tot}\) by \(43\% (P = 0.029\), whereas ISC\(_{Damil}\) was not affected (\(P = 0.486\).

Experiments on amphotericin B-treated cells were performed to evaluate the capacity of Na transport as described earlier (19).
DISCUSSION

Our results show that DEX blunts the hypoxia-induced inhibition of alveolar fluid clearance of hypoxia-exposed rats as well as the inhibition of ion transport activity across alveolar epithelial monolayers by increasing activity and capacity of transepithelial Na transport. Although quantitatively different, DEX stimulation of transport of A549 cells occurs regardless of whether treatment started before or during exposure to hypoxia. Whereas the stimulation of Na transport and fluid reabsorption by DEX in normoxia is well-documented (7, 11, 15, 27), the results on stimulation in hypoxia are new and indicate a possible therapeutic approach to prevent or treat alveolar edema in diseases associated with alveolar hypoxia.

DEX Stimulates Alveolar Ion Transport in Normoxia

Our results in DEX-induced stimulation of rat alveolar fluid clearance confirm earlier findings obtained on rats pretreated by intramuscular injections on 2 consecutive days (11). Also, other results indicate that pretreatment might be required for stimulation of alveolar transport by DEX since it occurred with a time lag of ∼24–48 h on a single DEX application (27). Here, we show that repeated injections of DEX also increased reabsorption (Fig. 1), although the degree of stimulation of fluid reabsorption was not as high as that reported by Noda et al. (27). Most of the DEX effect appears to be due to stimulation of Na reabsorption, since the amiloride-insensitive component of alveolar fluid clearance was not affected. Our results using primary cultured rat alveolar epithelial cells support this notion since DEX treatment of normoxic cells stimulates ISCtot and ISCamil. Also, an increased amiloride-sensitive component of the transepithelial potentials (7) across ATII cell monolayers by DEX points to a stimulation of Na transport. Taken together, these results clearly show an increase in alveolar fluid and epithelial Na reabsorption on stimulation with DEX.

Several mechanisms may account for the stimulated transport activity. Patch-clamp analysis indicates that DEX increases the open probability of Na channels (15). We show here that DEX increased the capacity of amiloride-sensitive Na transport (ISCamil) across the apical membrane of ATII cell monolayers (Fig. 2C), which is consistent with not only an increased open probability of Na channels, but also with an increased expression and an increased insertion of endogenous Na channels. The parallel increase in the expression of Na+-K+-ATPase and ENaC in the lung and the stimulation of alveolar clearance found by Noda et al. (27) implies that a stimulation of expression is required for DEX-stimulated transport activity (15, 22, 23, 27). Our results show that DEX treatment increased the mRNA expression of α- and γ-ENaC both in whole lung tissue and in ATII cells isolated from DEX-treated rats (Table 2). However, changes in mRNA levels of ENaC were not as pronounced as those reported by Noda et al. (27).

DEX has also been reported to increase the activity of Na+-K+-ATPase (27) in whole lung, which indicates an increased capacity to remove Na taken up by the cells via Na channels. We confirm this result by showing an increased capacity of Na+-K+-pumping (ISCamil) in ATII cell mono-
layers after permeabilizing the apical plasma membrane with amphotericin B (Fig. 2B). Also, in this case, it is not possible to discriminate between stimulated membrane insertion and increased expression. The increased mRNA levels of the α1-subunit of the Na+/K+-ATPase we found in alveolar epithelium on DEX treatment support the latter (Table 2). Thus our results indicate an increase in alveolar clearance by stimulation of Na reabsorption, which is due to an increase in the capacity of apical, amiloride-sensitive Na uptake and Na removal across the basolateral membrane by Na+/K+-ATPase.

**DEX Stimulates Transport in Hypoxia**

Hypoxia has been shown to decrease alveolar fluid clearance of hypoxia-exposed rats (34) and to inhibit ion transport activity of cultured alveolar epithelial cells (21, 29, 30). Vivona et al. (34) reported an almost 60% inhibition of alveolar fluid clearance in rats exposed to 8% O2, whereas inhibition was only 25% in our experiments (Fig. 1) where rats were exposed to 10% O2. The difference probably relates to the different degree of hypoxia. In both experimental settings, mainly the amiloride-sensitive component of alveolar fluid clearance was affected by hypoxia, possibly due to a decreased transport activity (19), decreased expression (29), and decreased membrane insertion (28). In primary alveolar epithelial monolayers (Fig. 2), not only amiloride-sensitive transport, but also the capacity of the Na+/K+-ATPase is decreased by hypoxia as shown previously (19). It has to be pointed out that a higher degree of hypoxia was used in tissue culture experiments than in the in vivo rat experiments and that changes induced by hypoxia become smaller with increased oxygenation (19).

Hypoxic transport inhibition in cultured alveolar epithelial cells has been associated also with the inhibition of expression of Na transporters (29, 36). Since hypoxia decreases overall protein synthesis in lung alveolar epithelial cells (12, 21), it was not clear whether DEX, for which stimulation of fluid clearance and Na transport seems to depend mainly on the synthesis of new Na transporters (15, 27), would increase the expression of transport proteins also in hypoxia. Our results indicate that treatment with DEX of rats before exposure to hypoxia stimulated alveolar fluid clearance in hypoxia. This result was verified in primary rat ATII cells, where we found an increase in ISClot and ISCabmi by DEX. However, DEX-stimulated reabsorption and transport in hypoxia just gained the values measured without treatment in normoxia both in the intact lung and in cultured ATII cells, and in neither system was the normoxic maximal level of activity achieved. This is in contrast to results on terbutaline stimulation of alveolar fluid clearance, where the same maximal values were reached in either oxygenation state (34). Similar to normoxia, also in hypoxia, DEX stimulation of reabsorption and transport activity can be explained by an increased capacity of the Na+/K+-ATPase and of amiloride-sensitive transport (Fig. 2). The change in transport activity and capacity is paralleled by an increase in the mRNA expression of the α1-subunit of Na+/K+-ATPase and of the α-ENaC subunits, whereas β- and γ-ENaC expression was not affected. These results indicate that DEX stimulates alveolar Na transport and fluid clearance also in hypoxia and thus blunts the hypoxic inhibition of reabsorption, which can, in part, be explained by increased expression of Na transporters.

Pathological conditions associated with alveolar hypoxia might favor the formation of pulmonary edema by decreasing the rate of alveolar Na transport and fluid reabsorption (18, 20, 26, 32, 35). It might, therefore, be of clinical significance to prevent the hypoxia-induced transport inhibition to reduce the risk of alveolar edema by prophylaxis or treatment with DEX. This appeared reasonable since prophyllactic DEX intake has recently been shown to completely prevent high altitude pulmonary edema (16). Similarly, inhaled β-adrenergics reduced the risk of high altitude pulmonary edema presumably by stimulation of alveolar reabsorption (32).

Since stimulation of reabsorption and Na transport in hypoxia was observed when treatment with DEX was begun before exposure to hypoxia, it was important to know whether treatment is also effective when cells are already hypoxic. We used the human alveolar epithelial A549 cell line to test this effect. These cells show hypoxic inhibition of Na transport (21) and stimulation of Na transport with DEX (15) similar to that found in primary cells despite the fact that their basal level of expression of Na transporters appears very low. The results of these measurements show that DEX increased the amounts of β- and γ-ENaC mRNA much more than α-ENaC mRNA. Most importantly, we found that the stimulation of mRNA expression was seen regardless of whether cells were DEX-treated before exposure to hypoxia or whether DEX was applied to already hypoxic cells, although the degree of stimulation of mRNA formation was smaller in the latter protocol.

In summary, our results indicate that DEX prevents the hypoxia-induced inhibition of alveolar fluid clearance and Na

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**Table 3. Effects of hypoxia and DEX on the mRNA expression of ENaC and Na⁺-K⁺-ATPase in A549 cells**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control Cells</th>
<th>DEX in Normoxia</th>
<th>DEX and then Hypoxia</th>
<th>Hypoxia and then DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells collected in</td>
<td>Hypoxia (n=10)</td>
<td>Normoxia (n=6)</td>
<td>Hypoxia (n=6)</td>
<td>Hypoxia (n=4)</td>
</tr>
<tr>
<td>α-ENaC</td>
<td>1.19 ± 0.5*</td>
<td>5.07 ± 3.3†</td>
<td>4.61 ± 1.82†</td>
<td>3.95 ± 2.31†</td>
</tr>
<tr>
<td>β-ENaC</td>
<td>5.73 ± 1.10*</td>
<td>200.20 ± 155.97†</td>
<td>173.51 ± 85.37†</td>
<td>78.54 ± 38.80‡</td>
</tr>
<tr>
<td>γ-ENaC</td>
<td>1.35 ± 0.47*</td>
<td>233.04 ± 147.36†</td>
<td>368.08 ± 213.68†</td>
<td>78.54 ± 38.80‡</td>
</tr>
<tr>
<td>α₁-Na⁺-K⁺-ATPase</td>
<td>1.22 ± 0.31</td>
<td>6.41 ± 2.06†</td>
<td>5.81 ± 1.93†</td>
<td>4.31 ± 1.43†</td>
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<tr>
<td>β₁-Na⁺-K⁺-ATPase</td>
<td>3.85 ± 1.29*</td>
<td>0.95 ± 0.41</td>
<td>0.74 ± 0.35†‡</td>
<td>0.9 ± 0.4†</td>
</tr>
</tbody>
</table>

Fold change in ENaC and Na⁺-K⁺-ATPase mRNA of A549 cells treated with DEX (1 μM) and/or exposed to hypoxia (1.5% O2). DEX and then Hypoxia refers to 3-day DEX treatment followed by 24 h of hypoxia in presence of DEX. Hypoxia and then DEX refers to exposure to hypoxia for 24 h before 24-h treatment with DEX with continued exposure to hypoxia. Amounts of mRNA measured by real-time PCR were corrected for 28S rRNA before normalizing to normoxic controls. Values are means ± SD of fold change relative to normoxic control cells. Level of significance was determined as P < 0.05.* difference between normoxia and hypoxia at respective treatment; † effect of DEX; ‡ difference between DEX 1st vs. hypoxia 1st at respective condition. Numbers in parentheses represent the number of experiments per condition.
transport and stimulates the mRNA expression of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase and ENaC, which leads to an increased capacity of alveolar epithelial amiloride-sensitive Na\textsuperscript{+} uptake and Na removal by Na\textsuperscript{+}-K\textsuperscript{+}-ATPases not only in normoxia, but also in hypoxia. These results also indicate that glucocorticoid-dependent signaling and gene expression are not impaired by hypoxia. If this is also the case in vivo in humans, stimulation of alveolar fluid clearance might, in part, explain the prevention of hypoxic edema at high altitude by prophylactic DEX (16) and might point to a possible therapeutic approach to prevent or treat pulmonary edema in pathologic states of alveolar hypoxia. The results presented here as well as results on gene therapy aimed to increase alveolar fluid reabsorption are in support of this hypothesis (1, 10, 33).

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