Dexamethasone upregulates the Na-K-ATPase in rat alveolar epithelial cells

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Barquin, Nora, David E. Ciccolella, Karen M. Ridge, and J. Iasha Sznejder. Dexamethasone upregulates the Na-K-ATPase in rat alveolar epithelial cells. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L825–L830, 1997.— Previous studies in kidney, heart, and liver cells have demonstrated that dexamethasone regulates the expression of Na-K-ATPase. In the lungs, Na-K-ATPase has been reported in alveolar epithelial type II (ATII) cells and is thought to participate in active Na+ transport and lung edema clearance. The aim of this study was to determine whether Na-K-ATPase would be regulated by dexamethasone in cultured rat ATII cells. Regulation of the Na-K-ATPase by dexamethasone could lead to a greater understanding of its role in active Na+ transport and lung edema clearance. Rat ATII cells were isolated, plated for 24 h, and exposed to 10−7 and 10−8 M dexamethasone. These cells were harvested at 0, 3, 6, 12, and 24 h after dexamethasone exposure for determination of steady-state Na-K-ATPase mRNA transcript levels, protein expression, and function. The steady-state Na-K-ATPase β1-mRNA transcript levels increased in ATII cells 6, 12, and 24 h after dexamethasone exposure (P < 0.05). However, the steady-state α1-mRNA transcript levels were unchanged. The protein expression for the α1- and β1-subunits increased in ATII cells exposed to dexamethasone compared with controls in association with a temporal increase in Na-K-ATPase function after dexamethasone exposure. These results suggest that dexamethasone regulates Na-K-ATPase in ATII cells possibly by transcriptional, translational, and posttranslational mechanisms.

alveolar type II cells; sodium-potassium-adenosinetriphosphatase; regulation of sodium-potassium-adenosinetriphosphatase in lungs

THE NA-K-ATPASE is a transmembrane heterodimer comprised of an α- and β-subunit (16). The α-subunit is the catalytic subunit with an intracellular ATP binding site, a phosphorylation site, and a ouabain binding site. The β-subunit is a smaller glycosylated subunit (26) that may play a role in the anchoring of the enzyme to the plasma membrane (25, 29). The Na-K-ATPase plays an important role in the maintenance of osmotic and electrochemical cell gradients (20). It couples the hydrolysis of ATP to the transport of Na+ and K+ against their cellular concentration gradients and is inhibited by cardiac glycosides such as ouabain (38, 40).

Three isoforms of the α- and β-subunit coded by different genes have been described (17, 39). In the rat lung, two isoforms of the α-subunit and one isoform of the β-subunit Na-K-ATPase have been described (32, 35, 37). Previous studies have reported on the Na-K-ATPase α- and β-subunits in alveolar epithelial type II (ATII) cells and adult lung (28, 30, 31, 36).

Na-K-ATPase function has been reported to be hormonally modulated (14, 44, 45). In the lungs, glucocorticoid receptors are present in alveolar epithelial cells (15). Corticosteroids have been shown to bind to specific response elements in the promoter regions of the Na-K-ATPase genes (33). In previous studies, dexamethasone has been shown to increase the β1-mRNA of the Na-K-ATPase in rat liver cells (3). Hence, we postulated that dexamethasone would regulate the Na-K-ATPase in lung epithelial cells and tested this hypothesis in rat ATII cells. We found that dexamethasone increased steady-state Na-K-ATPase β1-mRNA transcripts, α1- and β1-isoform proteins, and Na-K-ATPase function in cultured ATII cells. These findings further support the hypothesis that dexamethasone regulates Na-K-ATPase by transcription and possibly translation and posttranslational mechanisms.

MATERIALS AND METHODS

Cell isolation and experimental protocol. ATII cells were isolated from pathogen-free male Sprague-Dawley rats (200–225 g) by standard techniques (8, 31). Briefly, the lungs were perfused via the pulmonary artery, lavaged, and digested with 30 U/ml elastase (Worthington Biochemical). The ATII cells were purified by differential adherence to immunoglobulin G-pretreated dishes. Cells were suspended in Dulbecco's modified Eagle's medium (DMEM; Irvine Scientific) containing 10% charcoal-stripped fetal bovine serum with 2 mM l-glutamine, 40 mg/ml gentamicin, 100 U/ml penicillin, and 100 µg/ml streptomycin. For studies of alveolar epithelial cell transport in intact cells, 1 × 106 cells were plated into each well of 24-well tissue culture plates (Becton-Dickinson). For preparation of membranes, assay of Na-K-ATPase activity, and mRNA preparation, 7 × 106 cells/well were plated in 10-cm² tissue culture plates (Becton-Dickinson). Cells were incubated in a humidified atmosphere of 5% CO2-95% air at 37°C. After 24 h, nonadherent cells were removed by rinsing the monolayers. Plating efficiency was ~60%. The cells were then cultured for 3, 6, 12, and 24 h in serum-free medium consisting of DMEM-Ham's F-12 medium containing 0.5 mg/ml bovine serum albumin (BSA)-linoleic acid, 4 ng/ml selenic acid, 5 µg/ml transferrin, and 2 mM glutamine in the absence or presence of 10−7 and 10−8 M dexamethasone.

RNA isolation and Northern analysis. Total RNA was isolated by guanidine thiocyanate-phenol-chloroform extraction methods as previously described (6). Quantity and purity were determined spectrophotometrically. Five micrograms per sample were size fractionated in 2.2 M formaldehyde-1% selenic acid, 5 µg/ml transferrin, and 2 mM glutamine in the absence or presence of 10−7 and 10−8 M dexamethasone.

Ethidium bromide staining of ribosomal 28S and 18S bands on the gel was visualized with ultraviolet (UV) light and was recorded photographically to assure equal lane loading. RNA was electrotransferred (20 V, 6 h, 4°C) to nylon membranes. The blotting was verified as uniform across the paper by UV transillumination of the nylon filter and by recording the 18S...
and 28S ribosomal bands photographically. Membranes were baked for 2 h at 80°C and were UV cross-linked. [32P]CTP-labeled riboprobes (∼1.0 kb) were prepared by SP6-mediated in vitro transcription from cDNAs spanning the phosphorylation and ATP-binding site sequences of each subunit that had been subcloned in pGEM3Z (Promega) generously supplied by J. E. Emanuel (12). Membranes were prehybridized in 50% formamide, 10% dextran sulfate, 0.5% nonfat dry milk, 1% sodium dodecyl sulfate (SDS), 250 μg of sheared salmon sperm DNA, and 250 μg of yeast tRNA in 6× standard sodium citrate (SSC) overnight followed by hybridization at 57°C. They were then washed at 65°C in 0.1× SSC and 0.1% SDS for 1 h and were exposed to X-ray film at −70°C. Multiple exposures of the autoradiograms were made to ensure that signals were within the linear range of the film. Bands on autoradiograms were quantitated with a Hoeffer Scientific GS300 scanning densitometer, and the area of the peak was determined with Lakeshore Technologies Gelscan Software. In all cases, triplicate RNA samples from dexamethasone-treated cells and time-matched controls were analyzed simultaneously on the same nylon membrane.

Transport measurements. Ouabain-sensitive [86Rb] uptake was used to assess the rate of K+ transport by the Na-K-ATPase in alveolar epithelial cells. ATII cells were incubated at 37°C with and without 1 mM ouabain for 5 min. This medium was then removed, and otherwise identical fresh medium containing 1 μCi/ml [86Rb] was added. After a 5-min incubation, uptake was terminated by aspirating the assay medium and by washing the plates in ice-cold MgCl2. Plates were then washed at 65°C in 0.1× SSC and 0.05% Tween 20 in tris(hydroxymethyl)aminomethane-buffered saline (TBS) followed by incubation with Na-K-ATPase subunit antibodies for 12 h. The antibodies used were α1-specific mouse monoclonal anti-rat antibody C464-6B, generously provided by M. Caplan, and rabbit polyclonal anti-rat β1-antibody FPβ1, generously provided by Alicia McDonough. Blots were washed three times with wash buffer (TBS and 0.05% Tween 20) and then incubated with horseradish peroxide-conjugated goat anti-mouse or anti-rabbit secondary antibody (Bio-Rad) for 1 h. Blots were washed three times with wash buffer, developed as previously described using the enhanced chemiluminescence detection system (Amersham), and analyzed by densitometry (4).

Data analysis. When multiple comparisons were made, a one-way analysis of variance and Duncan’s means comparison test were used. The results were expressed as means ± SE. Results were considered significant at P < 0.05.

RESULTS

Effect of dexamethasone on Na-K-ATPase steady-state mRNA transcript levels in ATII cells. The steady-state Na-K-ATPase α1-mRNA transcript levels in ATII cells exposed to dexamethasone were not different from time-matched control ATII cells at either dexamethasone concentration (Fig. 1A). However, the steady-state Na-K-ATPase β1-mRNA transcript levels were significantly increased after incubation with 10−8 M dexamethasone at 6, 12, and 24 h compared with control levels (Fig. 1B). A representative Northern blot is shown for the β1 Na-K-ATPase mRNA (Fig. 2A).

Effect of dexamethasone on Na-K-ATPase protein levels. The subunit protein levels were evaluated in ATII cells exposed to dexamethasone by Western blot analysis. The signal at ∼96 kDa (corresponding to the Na-K-ATPase α1-subunit) increased in ATII cells after 6 h of exposure to dexamethasone compared with time-matched ATII control cells (Fig. 3A). A representative autoradiogram is shown for the α1-protein expression in Fig. 3B. Evaluation of the Na-K-ATPase β1-subunit protein revealed a band of ∼55 kDa that increased by 6, 12, and 24 h after incubation of ATII cells with dexamethasone compared with time-matched controls (Fig. 3B).
A representative autoradiogram is shown for the $\beta_1$-protein expression (Fig. 4A). A representative autoradiogram is shown for the $\beta_1$-protein expression (Fig. 4A).

Effect of dexamethasone on Na-K-ATPase hydrolytic activity and $^{86}$Rb$^+$ uptake. As shown in Fig. 5A, the Na-K-ATPase hydrolytic activity increased in ATII cells 12 and 24 h postincubation with $10^{-8}$ M dexamethasone ($P < 0.05$). Figure 5B depicts that the $^{86}$Rb$^+$ uptake in ATII cells increased two- and fourfold when incubated for 12 h with $10^{-7}$ and $10^{-8}$ M dexamethasone, respectively ($P < 0.05$).

DISCUSSION

The Na-K-ATPase has been shown to be regulated by corticosteroids in different organs in vivo and in vitro. In the thick ascending limb of Henle of the developing rat kidney, increased levels of serum corticosterone were associated with increased Na-K-ATPase activity and increased Na$^+$ concentration capacity (34). Also, $10^{-8}$ M dexamethasone restored the Na-K-ATPase activity in the medullary thick ascending limbs of Henle's loop from adrenalectomized rats (9). This effect was inhibited by cyclohexamide and actinomycin D, indicating the participation of transcriptional and translational mechanisms. In adrenalectomized rats, corticosteroids increased the $\alpha_1$ and $\beta_1$ Na-K-ATPase mRNA transcripts and renal Na-K-ATPase activity (22).

In the lungs, glucocorticoids have been shown to modulate the timing of lung development and to prevent respiratory distress syndrome in premature infants (2). Also, glucocorticoids have been shown to modulate fibroblasts to stimulate ATII cell surfactant secretion (12) and to decrease lung damage induced by hyperoxia in piglets (7).

Glucocorticoid receptors have been reported in lung fetal fibroblasts and epithelial cells, suggesting that both cell types could be potential targets of these hormones and that corticosteroids may play a role in the maturation of the lung (15). Orlowski and Lingrel (33) reported a rapid increase in Na-K-ATPase $\alpha_1$- and $\beta_1$-mRNA transcript levels in the rat lung during neonatal development; mRNA transcript levels continued to increase until 2 days after birth and then declined to basal levels. These changes possibly respond to the need of the lung to clear fluid during the neonatal stage and to maintain the dry alveoli for adequate gas exchange (30, 33). In a recent study, dexamethasone upregulated alveolar epithelial liquid clearance in anesthetized ventilated rats, which supports our present findings that these effects may be a result of increased Na-K-ATPase activity in ATII cells (13). Finally, steady-state Na-K-ATPase $\alpha_1$- and $\beta_1$-mRNA transcript levels and Na-K-ATPase protein expression and activity in rat ATII cells increased in two
different models of hyperoxic injury in parallel with changes in lung edema clearance (28, 31, 41).

In the present study, the steady-state Na-K-ATPase β₁ mRNA transcript levels increased by 2-, 3-, and 1.5-fold after 6, 12, and 24 h of incubation with dexamethasone without change in the steady state of α₁ mRNA transcript levels. These results are consistent with previous studies in liver cells of adrenalectomized rats, in which an increase in β₁ Na-K-ATPase mRNA was observed after stimulation with dexamethasone without modifying the expression of the α₁-subunit (3). Another example of differential regulation of the β₁-subunit mRNA was demonstrated in LLC-PK1/Cl₂ cells incubated in a low concentration of K⁺; the β₁ Na-K-ATPase mRNA transcript levels increased 1.9-fold over control levels at 6 h, resulting in a 2-fold increase of the α₁- and β₁-proteins and increased Na-K-ATPase hydrolytic activity (24). These studies suggest that the β₁-subunit could be rate limiting for the assembly of the Na-K-ATPase heterodimer. Other studies also support the hypothesis that the β₁-subunit is critical for the intracellular transport of the newly synthesized Na⁺ pump molecules to the plasma membrane and that it plays an important role in the correct assembly of the α₁-subunit into the membrane for the formation of the Na-K-ATPase heterodimers (29) and normal functional properties (1). The dexamethasone-mediated regulation of the β₁ Na-K-ATPase mRNA observed in this report is compatible with the previous studies and could be due to increased gene transcription as shown in infant rat kidney in which glucocorticoids directly stimulated transcription of the α₁ and β₁ Na-K-ATPase subunits (45). However, interpretation of the effect of glucocorticoids in isolated ATII cells is limited by changes that may have been induced during the isolation procedure and cell culture.

We also found that the Na-K-ATPase α₁-isoform protein was increased in ATII cells incubated with dexamethasone for 6 h after incubation and that the β₁-subunit increased at 6, 12, and 24 h (see Figs. 3 and 4). The increase in Na-K-ATPase α₁-protein was not associated with increases in the α₁-isofrom mRNA, suggesting that posttranslational events may be involved in the regulation of Na-K-ATPase in this model.

The dynamics of the events that occur in the Na-K-ATPase synthesis and transport to the plasma membrane have been previously studied. After their synthesis in polysomes that become bound to the membrane of the rough endoplasmic reticulum, the α₁- and β₁-subunits of the Na-K-ATPase are transported through
A network of membrane compartments to their functional site in the cell surface. Tamkun and Fambrough (42), in pulse chase experiments with cultured chick neurons, found that this process is achieved within 50 min of synthesis. The delivery of newly synthesized Na-K-ATPase to the plasma membrane continues for several hours, and 20% of the cells’ pulse-labeled Na+ pump appears to be retained intracellularly. The synthesis of proteins that we analyzed by Western blot in the membranes obtained from ATII cells was done without a subcellular fractionation, so we could not differentiate between endoplasmic reticulum, Golgi, or plasma membrane. Thus a limitation of our study is the uncertainty of whether the Na-K-ATPase protein subunits are in the plasma membrane or in the intracellular pool (19, 27). Conceivably, the increase in α1-isofrom protein in our model could be due to mobilization of this protein from intracellular pools by translocation to the plasma membrane or other posttranslational events (19).

Our study shows an increase in the expression of β1-mRNA that corresponds to an increase in the protein expression of the β1-subunit at the same time points. The α1-mRNA did not increase when ATII cells were incubated with dexamethasone; however, the α1-protein abundance increased at 6 h. Therefore, we reason that dexamethasone could have increased the Na-K-ATPase function in ATII cells as a result of transcription, translation, and posttranslational events. This is shown in Figs. 3 and 4. The 86Rb+ uptake in ATII cells was increased after 12 h of exposure compared with control ATII cells and decreased to control values by 24 h after exposure (see Fig. 5A), whereas the hydrolytic activity of the Na-K-ATPase was increased for up to 24 h after exposure to dexamethasone (see Fig. 5B). The observed differences between the two Na-K-ATPase assays can be explained by the somewhat different aspects of the Na-K-ATPase function that these assays measure. The 86Rb+ uptake measures transport across the ATII cell, whereas the hydrolytic assay measures the release of Pi, under maximal velocity conditions in isolated microsomes from ATII cells. Finally, the effect of dexamethasone in ATII cells appears to be biphasic. This may be a result of dexamethasone consumption or the glucocorticoid receptors may have become desensitized before the end of the incubation period.

The increase in Na-K-ATPase activity induced by dexamethasone in ATII cells could be explained as an increase of transport efficiency or as an increase in the number of active pumps in the surface of the cell. Chapman et al. (5) demonstrated that the cation transport by rabbit lung epithelial cells changes at birth and that ouabain-sensitive Rb uptake is greater in lung epithelial cells obtained from newborn rabbits than in cells obtained from pups. There is also a postnatal increase in ouabain-sensitive Rb uptake that reached adult values at 30 days after birth. They calculated the turnover rate of the enzyme, concluding that there is an increase in the Na-K-ATPase turnover near birth, whereas the number of Na+ pumps increases postnataIly (5).

In summary, this study demonstrates that the exposure of rat ATII cells in culture to dexamethasone results in increased steady-state Na-K-ATPase β1-mRNA transcript levels and increased α1- and β1-protein abundance that is associated with increased Na-K-ATPase function measured by two functional assays. We thus reason that corticosteroids could be utilized as a strategy to regulate Na-K-ATPase in the alveolar epithelium.

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