Postnatal glucocorticoids induce α-ENaC formation and regulate glucocorticoid receptors in the preterm rabbit lung

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Mustafa, Shamimunisa B., Robert J. DiGeronimo, Jean A. Petershack, Joseph L. Alcorn, and Steven R. Seidner. Postnatal glucocorticoids induce α-ENaC formation and regulate glucocorticoid receptors in the preterm rabbit lung. Am J Physiol Lung Cell Mol Physiol 286: L73–L80, 2004. First published August 29, 2003; 10.1152/ajplung.00342.2002.—At birth, lung fluid clearance is coupled to Na+ transport through epithelial Na+ channels (ENaC) in the distal lung epithelium. We evaluated the effect of postnatal glucocorticoids (GC) on lung α-ENaC expression in preterm 29-day gestational age (GA) fetal rabbits. Postnatal treatment of 29-day GA fetuses with 0.5 mg/kg of dexamethasone (Dex) iv resulted in a 2- and 22-fold increase in lung α-ENaC mRNA expression compared with saline-treated fetuses after 8 and 16 h, respectively. Lung α-ENaC protein levels in Dex-treated fetuses were also elevated compared with saline-treated counterparts. The extravascular lung water (EVLW)/dry lung tissue weight ratios of 29-day GA fetuses treated with either saline or Dex decreased over 24 h compared with that observed at birth; however, at 24 h, the EVLW/dry lung tissue weight ratios of saline- and Dex-treated fetuses were similar. Dex-induced α-ENaC mRNA and protein levels were attenuated by glucocorticoid receptor (GCR) antagonist RU-486 in fetal distal lung epithelial cells isolated from 29-day GA fetuses, indicating that GC-dependent augmentation of lung α-ENaC requires the presence of functional GCR. Lung GCR mRNA expression and protein levels were elevated in 29-day GA fetuses compared with fetuses at earlier GA. Exposure of 29-day GA fetuses to Dex for 16 h caused a 2.1-fold increase in lung GCR mRNA expression, but GCR protein levels were decreased in Dex-treated fetuses after 24 h. We conclude that postnatal treatment of preterm 29-day GA fetal rabbits with GC results in an elevation of lung α-ENaC accompanied by an autoregulation of pulmonary GCR.

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observed that there is a reduction in the expression of α-ENaC mRNA in the lungs of newborn GCR−/− mice (14).

Because GC, together with other hormones and growth factors, finely coordinate lung development in a timely fashion for the eventual transition to air breathing at birth, it is quite likely that these sequential, maturational changes in the lung are severely disrupted in preterm newborns. Postnatal treatment of premature human babies with synthetic GC analogs such as dexamethasone (Dex) has demonstrated several short-term improvements in lung function, including increased surfactant synthesis, inhibition of prostaglandins and leukotrienes, suppression of cytokine-mediated inflammation, and a reduction in pulmonary edema (2, 3, 7, 31, 35, 38, 43). Thus the in vivo regulatory effects of GC on the components involved in alveolar ion transport, particularly in preterm neonates, are of great importance.

The consequence of treating preterm animal models postnatally with exogenous GC on the level of expression of α-ENaC in the lung has yet to be clearly defined. Previous studies have documented that exogenous GC increase alveolar fluid clearance in adult rats (20). In light of these findings, we first tested the hypothesis that postnatal treatment of preterm rabbits with Dex causes an upregulation in α-ENaC expression in the lung. Secondly, as the level of cellular GCR present in the lung at a given time is thought to regulate GC-mediated signaling pathways, resulting in alterations in gene transcription, the effect of exogenous GC treatment on the expression and regulation of its receptors located in the preterm rabbit lung was also investigated.

The preterm rabbit was utilized as our experimental animal model, as at full-term gestation the pattern of lung development in these animals is quite similar to that observed in humans versus the postnatal maturation of developing lungs in rats (1, 34). In the present study, day 29 of gestational age (GA, term = 31 days) was chosen as representative of the terminal sacular stage of rabbit lung development (34). More importantly, 29-day GA preterm rabbits can survive for at least 24 h in an incubator supplemented with 50% O2, whereas 28-day GA preterm rabbits require ventilator support and survive for only a few hours after birth. In general, the lungs of 29-day GA preterm rabbits have newly formed alveolar ducts and air spaces and are capable of secreting surfactant (34). Because 29-day GA preterm rabbits treated with postnatal surfactant showed no improvement in lung compliance despite low, but evidently sufficient, endogenous surfactant pools (26), this model is probably more useful for evaluating the effects of postnatal Dex on gene regulation than it is for evaluating therapeutic effects on lung physiology.

**METHODS**

**Animals.** The animals used during the course of this study were timed-pregnant New Zealand White rabbits. All animal experiments were undertaken with protocols approved by the University of Texas Health Science Center at San Antonio Animal Care and Use Committee. The mating date was defined as day 0 of gestation. Pregnant rabbits at 23, 27, 29, and 31 days’ GA were anesthetized with halothane and given supplemental oxygen by facemask. Local anesthesia with 1% lidocaine was given in the abdominal wall followed by exposure of the uterus and sequential delivery of the rabbit fetuses by cesarean section with the absence of preceding labor. Immediately after delivery, rabbit fetuses were killed by means of intrathecal 1% lidocaine, and the descending aorta was severed below the diaphragm.

The lungs were carefully removed for further analysis. For additional studies, 29-day GA preterm rabbits were weighed and placed in an incubator at 37°C with 50% supplemental oxygen. After 30 min, rabbit fetuses were treated with Dex (0.5 mg/kg, intracardiac) or saline for different periods of time. This particular dose of Dex was chosen as it has been previously employed clinically for the treatment of chronic lung disease in preterm infants (24, 44). Four to seven 29-day GA preterm rabbit fetuses were used at each time point for each of the two different conditions. At the end of each time point, rabbit fetuses were killed by means of intrathecal 1% lidocaine, and the descending aorta was severed below the diaphragm. The lungs were carefully removed and set aside for analysis of extravascular lung water (EVLW/dry lung tissue ratio measurements. A sample of the lung was frozen in liquid N2 for further RNA and protein analysis.

**Isolation and culture of FDLE cells.** After removal of the lungs from 29-day GA preterm rabbits (~6–8 fetuses), the tissue was chopped in to small pieces (<1 mm3) with a razor blade and incubated in a Ca2+- and Mg2+-free solution with trypsin-EDTA (0.125%/0.01%) for 15 min at 37°C. Ca2+ and Mg2+ (final concentration 1.9 and 1.3 mM, respectively) and DNase I (0.1 mg/ml) were added to the homogenate and incubated for an additional 15 min. The action of trypsin was stopped by the addition of 5% fetal bovine serum (FBS). The cell homogenate was filtered through two layers of NITEX (NITEX, Lakewuster, NY) gauze (150 and 20 μm) and centrifuged at 400 g for 10 min. The cells were resuspended in RPMI 1640 medium supplemented with 10% FBS, 100 U/ml sodium penicillin G, and 100 μg/ml streptomycin and plated in tissue culture dishes at 37°C for no longer than 1 h to allow fibroblasts to adhere. After 1 h, the unattached cells were gently removed and washed in RPMI medium. The resuspended cells were cultured again for no longer than 1 h to maximize the efficient separation of nonadherent epithelial cells from fibroblasts. Subsequently, the enriched epithelial cell supernatant was gently removed, and cells were resuspended and cultured in the same medium. Cells were allowed to grow in tissue culture dishes for 2–3 days before experimental use. During this period it was observed that epithelial cells proliferated into several small monolayers. The medium was replaced daily, thereby removing any remaining nonadherent cells and other cellular debris.

**Northern blot analysis.** Total RNA was isolated from frozen whole lung tissue or isolated FDLE cells with TRIZol reagent (GIBCO-BRL, Gaithersburg, MD) according to the manufacturer’s instructions. RNA (20–30 μg) was size fractionated by electrophoresis on a 0.8% agarose-2.2 M formamide gel under denaturing conditions and transferred using a PosiBlot (Stratagene, La Jolla, CA) onto a Magna nylon membrane (Microns Separations, Westborough, MA). RNA was immobilized by ultraviolet cross-linking. cDNA probes used in this study were 565-bp rabbit α-ENaC PCR and the full-length rabbit GCR cDNA. cDNA probes were labeled with a multiprime DNA labeling system using [α-32P]dCTP (specific activity, 3,000 Ci/mmol). Northern blot hybridizations were performed in 50% formamide, 1 M NaCl, 10% dextran sulfate, 50 mM Tris-HCl, pH 7.5, 0.1% sodium pyrophosphate, and 0.2% Denhardt’s solution at 42°C for 16 h. The membranes were washed once in 2× SSC, 0.1% SDS at room temperature for 10 min and then in 2× SSC, 0.1% SDS at 65°C for 5 min. Radioactivity was visualized with a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA). Control hybridizations were performed using an α-32P end-labeled oligonucleotide complementary to rat 18S rRNA. Signal intensity was quantified by densitometry.

**Western blot analysis.** Frozen lung tissue or cultured FDLE cells were homogenized in lysis-buffered saline [50 mM Tris-HCl, pH 7.4, Tris-buffered saline (TBS)] containing 5 mM EDTA, 5 mM EGTA, leupeptin (1 μM), pepstatin A (1 μM), aprotinin (1 μM), and p-nitromethylsulfonyl fluoride (1 μM). Protein concentrations were determined with the Bio-Rad DC protein assay (Bio-Rad, Hercules, CA), with BSA used as the standard. Total protein (50–60 μg) was subjected to SDS-PAGE (7.5% gel) by the buffer system of Laemmli.
in preterm rabbit lung. A: representative Northern blot analysis of whole lung α-ENaC mRNA expression in fetal rabbits of varying gestational age (GA) (n = 3–4 fetal rabbits at each different GA). After total lung RNA was isolated and purified, samples were hybridized with a cDNA probe for α-ENaC (top) and then with a probe for 18S rRNA (bottom). B: representative Northern blot analysis of 2 independent experiments describing the developmental sequence of α-ENaC mRNA expression (top) in freshly isolated rabbit fetal distal lung epithelial (FDLE) cells of varying GA. Levels of 18S rRNA were used to assess RNA integrity and loading (bottom).

Fig. 1. Ontogeny of α-subunit of epithelial Na⁺ channel (α-ENaC) mRNA expression in preterm rabbit lung. A: representative Northern blot analysis of whole lung α-ENaC mRNA expression in fetal rabbits of varying gestational age (GA) (n = 3–4 fetal rabbits at each different GA). After total lung RNA was isolated and purified, samples were hybridized with a cDNA probe for α-ENaC (top) and then with a probe for 18S rRNA (bottom). B: representative Northern blot analysis of 2 independent experiments describing the developmental sequence of α-ENaC mRNA expression (top) in freshly isolated rabbit fetal distal lung epithelial (FDLE) cells of varying GA. Levels of 18S rRNA were used to assess RNA integrity and loading (bottom).

RESULTS

Developmental expression of α-ENaC mRNA and protein formation in rabbit fetal lungs. In this study the 29-day GA preterm rabbit was utilized as our experimental animal model of prematurity. In Fig. 1A, Northern blot analysis of total lung RNA isolated from fetal 23-day GA preterm rabbit killed immediately after birth indicates that α-ENaC mRNA expression is very low. By densitometric analysis, α-ENaC mRNA expression in preterm rabbit lung was increased twofold from 23 to 27 days’ GA and 3.4-fold from 23 to 29 days’ GA. A higher level of α-ENaC expression was detected in the lung of term (i.e., 31-day GA) newborn fetal rabbits. Additional studies were performed to demonstrate the developmental regulation of α-ENaC mRNA in the epithelium lining the distal air spaces of the lung. Figure 1B shows that in primary cultures of FDLE cells isolated from 23-, 27-, and 29-day GA fetal lungs, α-ENaC mRNA is expressed in a pattern similar to that observed in whole lung studies.

α-ENaC protein content in preterm and term rabbit lung was studied by Western immunoblotting with a custom-made polyclonal anti-rabbit-α-ENaC antibody. This antibody recognizes a membrane protein of ~94–96 kDa in whole rabbit lung and cultured rabbit FDLE cells in similar agreement with a recently published study (27). As depicted in Fig. 2, a detectable level of α-ENaC protein was observed in preterm 23-day GA rabbit fetal lung. By densitometric analysis, α-ENaC protein expression in preterm rabbit lung was increased 2.2-fold from 23 to 27 days’ GA and 5.8-fold from 23 to 29 days’ GA. Lung α-ENaC protein levels markedly improved as the GA of the preterm rabbit increased. Whole lung α-ENaC protein was sustained at a higher level of expression in full-term fetal rabbits.

Regulation of α-ENaC mRNA expression by Dex in 29-day GA rabbit fetal lung. Treatment of 29-day GA rabbits with Dex (0.5 mg/kg iv) resulted in a significant increase in expression of lung α-ENaC mRNA over a time frame of 24 h (Fig. 3A). After 8 h of exposure to Dex, α-ENaC mRNA levels were elevated twofold and increased to 2.2-fold after 16 h of exposure in fetal rabbit lungs compared with their saline-treated counterparts (summarized in Fig. 3B). After 24 h of treatment with Dex, expression of α-ENaC mRNA in rabbit fetal lungs was markedly decreased compared with that observed at 16 h of exposure (Fig. 3A).

Regulation of α-ENaC protein formation by Dex in 29-day GA rabbit fetal lung. As shown in the representative Western blot depicted in Fig. 4A (and summarized in the bar graph below, Fig. 4B), whole lung α-ENaC protein was detectable in 29-day GA fetuses killed 30 min after delivery (0 h). Treatment of 29-day GA preterm rabbits with Dex as described earlier resulted in a 2.2-fold elevation of whole lung α-ENaC protein after 12 h and 3.2-fold after 16 h of treatment compared with the level of α-ENaC protein detected in saline-treated counterparts. Whole lung α-ENaC protein in 29-day GA fetuses remained elevated for up to 24 h post-Dex exposure. Basal levels of α-ENaC protein in saline-treated rabbits remained relatively unaltered over the 24-h time frame.

Effect of Dex treatment on EVLW/dry lung tissue weight ratio in 29-day GA rabbit fetal lung. The clearance of fetal lung fluid at the time of birth is an important step in establishing the transition to air breathing (3). Extensive studies have documented the key role of Na⁺ transport via ENaC located in the distal lung epithelium during lung fluid clearance at the
time of birth (35, 36). To assess the effects of exogenous Dex treatment on lung liquid removal in 29-day GA preterm rabbits, we measured the postnatal change in EVLW relative to the weight of the dry lung tissue. The changes in EVLW/dry lung tissue ratios measured over a period of 24 h subsequent to saline or Dex exposure are depicted in Fig. 5. EVLW, relative to the weight of dry lung tissue, was depressed by 8 h in both saline-treated and Dex-treated fetal rabbits compared with that observed in 29-day GA preterm rabbits killed immediately at birth (0 h). There was little change from 8–24 h, but by 24 h the EVLW/dry weight ratio in both saline-treated and Dex-treated fetal rabbits was significantly decreased compared with that observed in fetal rabbits at 0 h. Interestingly, at each of the time points observed, there was no significant difference in EVLW/dry weight ratios between saline-treated or Dex-treated 29-day GA preterm rabbits.

**Dex-induced α-ENaC upregulation occurs via binding to GCR in FDLE cells.** To confirm that the elevated level of lung α-ENaC expression observed in the intact preterm animal occurred as a result of exposure to exogenous Dex acting through the GCR, we isolated FDLE cells from 29-day GA preterm rabbits. After 2–3 days in culture, cells were treated with Dex (0.1 μM) in either the absence or presence of the specific GCR antagonist RU-486 (10 μM). It has been previously reported that RU-486 (10 μM) treatment of cultured adult rat lung epithelial cells does not induce α-ENaC mRNA expression (12). Figure 6, A and B, shows that exposure of FDLE cells to Dex for 24 h resulted in a threefold elevation in α-ENaC mRNA expression compared with untreated cells. The stimulatory effects of Dex were abrogated 1.7-fold in the presence of RU-486 compared with Dex treatment alone. In addition, we observed that α-ENaC protein levels were also elevated threefold in FDLE cells following exposure to Dex compared with untreated cells. A similar pattern of inhibition (a 2.8-fold decrease) of α-ENaC protein in FDLE cells in the presence of RU-486 compared with Dex alone was observed by Western blot analysis (Fig. 6, C and D).

**GCR mRNA expression and protein formation in fetal rabbit lung during development.** It has been well documented that maternal circulating GC levels are elevated toward the end of pregnancy, but the potential role of these hormones in fetal lung liquid management is poorly understood. In this study, we have demonstrated that exogenous Dex treatment results in a decrease in lung liquid clearance in 29-day GA preterm rabbits, which is consistent with decreased GCR mRNA expression in fetal rabbit lung. To determine if this decrease in lung liquid clearance is due to decreased GCR expression, we have measured the EVLW/dry weight ratio in saline-treated control fetuses at the same time point. *P < 0.05 was considered significantly different from saline-treated control fetuses at the same time point.

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**Fig. 2.** Ontogeny of α-ENaC protein levels in rabbit fetal lung. Representative Western blot analysis of whole lung α-ENaC protein levels in fetal rabbits of varying GA (α = 3–4 fetal rabbits at each GA). Whole lung tissue was subjected to SDS-PAGE followed by immunoblot analysis using the anti-α-ENaC antibody as described in METHODS.

**Fig. 3.** Regulation of α-ENaC mRNA expression in 29-day GA rabbit fetal lung following treatment with dexamethasone (Dex). A: typical Northern blot analysis demonstrating the upregulation of lung α-ENaC mRNA expression (top) in 29-day GA preterm rabbits following exposure to Dex (α = 4–7 29-day GA preterm rabbits at each time point and condition). Fetuses that were killed immediately after delivery are designated 0 h (n = 3). RNA loading was similar among all conditions, as indicated by equivalence of signal following hybridization with an 18S rRNA probe (bottom). B: densitometric analysis of all Northern blots are expressed in arbitrary units as means ± SE for each time point. *P < 0.05 and #P < 0.01 were considered significantly different from saline-treated control fetuses at the same time point.

**Fig. 4.** Representative Western blot analysis depicting the time course of the increase in α-ENaC protein in 29-day GA preterm rabbit lung following treatment with Dex over a period of 24 h (α = 4–7 29-day GA preterm rabbits at each time point and condition). A: tissue extracts were subjected to SDS-PAGE followed by immunoblot analysis using the anti-α-ENaC antibody. B: bar graph showing densitometric values expressed as means ± SE for each time point. *P < 0.05 was considered significantly different from saline-treated control fetuses at the same time point.

**Fig. 5.** Changes in lung extravascular lung water (EVLW)/dry lung tissue ratios after treatment with either saline or Dex at the indicated time points. Values are expressed as means ± SE; n = 4–6 29-day GA preterm rabbits at each time point studied. *P < 0.05 was considered significantly different from 0 h.
gestation to enhance the final stages of lung development in preparation for the onset of air breathing. These particular responses of GC in the preterm lung shortly before parturition are dependent on the presence of functional GCR (8, 15, 17). Accordingly, our findings demonstrate that, in 29-day GA rabbit killed immediately after birth, lung GCR mRNA expression was demonstrable and increased 1.3 ± 1.1 fold in full-term rabbit lung (densitometric analysis, Fig. 7A). Western blot analysis of the α-subunit of GCR detected, as previously reported, the 94-kDa protein in rabbit lung samples (8, 40). GCR lung protein levels were evident in 23- and 27-day GA preterm rabbits and were elevated 1.8-fold (by densitometric analysis) from 27 to 29 days’ GA (Fig. 7B). In addition, GCR protein levels in term rabbits were also increased 1.8-fold compared with that observed in 29-day GA rabbit fetal lung. The preterm and term rabbits used for the above analysis were all killed immediately after birth.

Regulation of GCR mRNA expression by Dex in 29-day GA fetal rabbit lung. In the lung, GC bound to its intracellular receptor acts in a manner that alters the rate of transcription of specific genes (17). One particular gene regulated by GC is the GCR gene itself (17, 28, 29, 49). A change in the expression of basal levels of GCR in either whole tissue or isolated cell culture by exogenous GC is described as autoregulation (28–30, 49). In these particular studies we observed that treatment of 29-day GA preterm rabbits with Dex for 8 h did not greatly alter whole lung GCR mRNA expression. However, a 16-h exposure of fetuses to Dex resulted in a 2.1-fold increase in lung GCR mRNA expression compared with saline-treated counterparts (Fig. 8, A and B).

Regulation of GCR protein by Dex in 29-day GA preterm rabbit lung. Time-course studies in 29-day GA preterm rabbits treated with Dex or saline as previously described above were performed to analyze the effect of exogenous GC on the preexisting levels of GCR protein in the lung. As depicted by Fig. 9, A and B, lung GCR protein levels in 29-day GA preterm rabbits killed immediately after birth (0 h) were clearly detectable. In Dex-treated fetuses, lung GCR protein levels were slightly elevated after 8 h of exposure to Dex compared with saline-treated animals. However, lung GCR protein levels started to decrease significantly by 12 h (1.2-fold compared with saline-treated counterparts) and remained depressed for up to 24 h compared with saline-treated counterparts. These in vivo findings (Figs. 8, and 9) support previously described studies in fetal and adult rat lung demonstrating that exogenous GC can either up- or downregulate the level of their own receptor by a variety of mechanistic pathways including transcriptional, posttranscriptional, and translational (28, 29, 49).

DISCUSSION

The presence of functional ENaC that is primarily responsible for Na⁺ transport is now known to play a key role during the clearance of fetal lung fluid at the time of birth (5, 6, 35, 36). Hummler and coworkers (25) showed that amiloride-sensitive Na⁺ transport in the airway epithelia is completely abolished in α-ENaC knockout mice, resulting in their death within 40 h of birth due to respiratory distress. In contrast, β- and γ-ENaC knockout mice did not die of respiratory distress but from a urinary electrolyte imbalance (52). These indepen-

Fig. 6. Glucocorticoid receptor (GCR) antagonist RU-486 inhibits Dex-induced α-ENaC expression in FDLE cells. FDLE cells were isolated from 29-day GA preterm rabbits and cultured for 2–3 days before exposure to Dex (0.1 μM) in the absence and presence of RU-486 (10 μM) for 24 h. A: after total RNA was isolated from FDLE cells and purified, samples were hybridized with a cDNA probe for α-ENaC (top) and then with a probe for 18S rRNA (bottom). B: densitometric analysis of the corresponding blot. Values are expressed as means ± SD. C: cell protein lysates were isolated from FDLE cells and subjected to SDS-PAGE followed by immunoblot analysis using the anti-α-ENaC antibody. D: densitometric analysis of the corresponding blot. Values are expressed as means ± SD.
dent studies highlight the importance of the \( \alpha \)-subunit of ENaC responsible for \( \text{Na}^+ \) transport particularly in the lung. The ontogeny of \( \alpha \)-ENaC mRNA and protein expression in fetal lung is consistent with the need to promote fetal lung fluid secretion to foster fetal lung growth in utero and then to promote lung fluid absorption at the time of birth. As a result, the more developmentally immature the lungs are, the less their ability to adapt to perinatal transition and postnatal adaptation. This observable fact has been confirmed by a previous study that showed that the very immature fetal lamb lung, in contrast to its mature counterparts, could not convert from fluid secretion to fluid reabsorption in response to \( \beta \)-agonists (9). The present study demonstrates that \( \alpha \)-ENaC is developmentally regulated in fetal rabbit lung. This phenomenon has been clearly demonstrated in previous experiments utilizing fetal rats, mice, and human lung explants (16, 21, 45, 50, 51).

Experimental evidence strongly suggests that perinatal adaptations in the newborn, e.g., fluid clearance in the lung, are primarily regulated by the rapid increase in circulating maternal levels of GC and catecholamines (9, 19, 22, 41, 48). It has been well documented that maternal antenatal GC administered to humans and animal subjects before imminent premature delivery accelerates the maturation of many organ systems, most importantly, the lungs (3, 10, 31, 34). Therefore, it could be likely that the very low levels of circulating maternal GC could possibly contribute to the inability of the preterm infant to successfully adapt to air breathing at birth. Recent clinical studies have documented that a single dose of Dex administered to preterm infants soon after delivery resulted in improved respiratory and cardiovascular outcomes (10, 18, 31, 43, 53). The improved pulmonary adaptation of preterm infants reported in the above studies who were treated immediately after birth with Dex could perhaps be partly attributed to increases in lung \( \alpha \)-ENaC expression and consequent function, which would help to improve lung fluid clearance. The present study is the first to demonstrate that postnatal treatment of 29-day GA preterm rabbits with Dex shortly after delivery (within 30 min) results in an upregulation of lung \( \alpha \)-ENaC mRNA expression and consequently an increase in total lung \( \alpha \)-ENaC protein over a period of 24 h. This could possibly be a previously unrecognized mechanism of action of Dex on lung function when administered postnatally to preterm infants. We did not observe a significant difference in EVLW/dry lung weight ratios in 29-day GA preterm rabbits exposed to Dex for 24 h compared with their saline-treated counterparts at the same time despite increased amounts of lung \( \alpha \)-ENaC protein in the fetal rabbits that were treated with Dex (Figs. 4 and 5). It is important to note here that fetuses treated with saline for 24 h displayed a significant decrease in EVLW/dry lung weight ratios compared with that observed in fetal rabbits at 0 h, without any change in the level of \( \alpha \)-ENaC protein in the lung (Figs. 4 and 5). This decrease was similar to that seen during postnatal lung liquid clearance in full-term rabbits delivered by cesarean section without labor, a process that neared equilibrium/completion by 6 h of age (5). Therefore, these relatively mature newborns are ideal for studying changes and mechanisms of gene expression in response to early postnatal GC but are unlikely to show changes in physiological parameters, since protein levels are just starting to elevate at a time when lung liquid clearance is nearing completion. Similarly, 29-day GA preterm rabbits treated with postnatal surfactant showed no improvement in lung compliance despite low, but evidently sufficient, endogenous surfactant pools (26).

GC play a major role during embryonic lung development and maturation (8, 14, 15, 17, 21, 23, 49). The physiological effects of GC are mediated by intracellular GCR that function as ligand-dependent transcription factors (8, 14, 17, 49). Cellular GCR levels are thought to be a limiting factor in the signal transduction pathway, resulting in transcriptional responses to GC (17, 28–30, 42). Furthermore, the cellular sensitivity to endogenous and/or exogenous GC is directly proportional to...
intracellular GCR concentrations (40, 42). Successful adaptation of the neonatal lung during the perinatal situation requires that there be a sufficient response of the immature lung in late gestation to elevated levels of circulating maternal GC, which in turn, acting via GCR, accelerate lung maturation in preparation for birth (22, 29, 48, 49). Thus an elevation in lung GCR expression toward the end of gestation in neonates is not only an absolute necessity for GC to exert their functional effects but is also a developmental adaptation. Our current findings using rabbits as a preterm animal model are consistent with these observations and previously published studies in rats demonstrating that lung GCR mRNA expression and protein levels are elevated toward the end of gestation (8, 14, 28, 49). Furthermore, the considerable importance of functional GCR during fetal development was highlighted in a study by Cole and coworkers (14). These researchers showed that GCR−/− mice develop to term but die shortly after birth due to respiratory distress (14). Interestingly, the GCR−/− mice exhibited a very low level of expression of α-ENaC mRNA in the lung compared with their wild-type counterparts. The lack of lung α-ENaC expression in GCR−/− mice could have possibly contributed to their respiratory distress. A possible explanation for the lack of lung α-ENaC mRNA expression in GCR−/− mice could be the fact that analysis of the rat α-ENaC gene has indicated the presence of GC response elements in its promoter sequence (39). In concurrence with these previously published studies we have demonstrated that, in the presence of the specific GCR antagonist RU-486, Dex-induced α-ENaC mRNA expression and protein formation are markedly reduced in FDLE cells isolated from 29-day GA preterm rabbits (Fig. 6). These findings further highlight the important intrinsic link between functional GCR and α-ENaC gene regulation, particularly in the lung (14, 25).

Autoregulation of GCR (both up and down) by endogenous and/or exogenous GC has been reported to occur in whole lung tissue and cultured cells derived from the lung (8, 28–30, 40, 42, 49). To this end, we observed that in experiments carried out to 24 h, exposure of 29-day GA preterm rabbits to Dex after 12 h actually caused a decrease in basal lung GCR protein levels that was apparent up to 24 h. The data presented herein suggesting receptor downregulation by its cognate ligand in whole lung are in similar agreement with another published study that reported a downregulation of GCR protein levels by Dex in cultured human airway epithelial cells (40). Further analysis of our data demonstrates that postnatal treatment of 29-day GA preterm rabbits with Dex over a period of 16 h resulted in an elevation of lung GCR mRNA expression that was concurrent with the decline in protein levels, suggestive of a transcriptional mode of upregulating gene expression in response to the posttranslational mechanism of downregulating existing protein levels.

During the last 20 years or so, neonatologists and researchers have persevered in their attempts to alleviate and treat a variety of respiratory disorders in preterm neonates. Owing to its potent anti-inflammatory properties, Dex has been widely used to ameliorate the severity of and/or prevent the onset of respiratory distress in preterm neonates (3, 10, 18, 35, 43, 53). Despite the improvements in pulmonary functions associated with treatment of preterm neonates with Dex, the short- and possible long-term adverse side effects associated with this particular therapeutic regime are an exceedingly important concern (10). The short-term unfavorable side effects reported to date include hypertension, gastrointestinal bleeding, hypertrophic obstructive cardiomyopathy, limited weight gain, and poor growth of head circumference (10, 18, 53). Several of these anomalies have been attributed to the high dose of Dex (0.5 mg/kg) used. In the present study we used this particular dose of Dex as we were assessing only α-ENaC changes in the lung and EVLW/dry weight ratios and not any other physiological parameters. This pharmacological dose of Dex commonly used in clinical trials is usually 10 to 15 times higher than the estimated physiological concentration of endogenous GC in preterm neonates (33). A recent study evaluated the effects of low doses of Dex (0.1–0.25 mg/kg) on improvements in pulmonary functions in preterm neonates (18). These researchers report that preterm neonates treated with low doses of Dex displayed improvements in pulmonary functions comparable to those observed in neonates exposed to the higher dose of Dex (0.5 mg/kg) and that low doses of Dex may help minimize the short- and long-term adverse side effects of exogenous GC (18).

In conclusion, we have shown that early (within 30 min of birth) postnatal treatment of 29-day GA preterm rabbits with Dex induces lung α-ENaC formation that is accompanied by an upregulation in GCR mRNA expression. Further studies are warranted in experimental animals to clarify the appropriate timing, dose, and preparation of GC therapy used postnatally in preterm infants to better understand its effects on α-ENaC expression and regulation of lung fluid clearance.

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