Differential Regulation of Glucocorticoid Receptor Expression by Ligand in Fetal Rat Lung Cells

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ABSTRACT

The glucocorticoid receptor (GR) mediates glucocorticoid stimulation of surfactant production by fetal mammalian lung. In many other tissues, glucocorticoids decrease expression of GR, thereby reducing responsiveness to these hormones. We therefore determined whether there is a similar effect of exogenous glucocorticoids on GR in fetal rat whole lung, and in the principal cell types involved in the stimulation of surfactant, the fibroblasts and the epithelial cells. The ontogeny of GR in late gestation lung differed between the two cell types, with maximal levels occurring in fibroblasts on gestational d 19, and on d 20 in epithelial cells. Administration of dexamethasone (1 mg/kg) to the mother on gestational d 18 or 19 (term = 22 d) increased specific GR binding activity in whole lung 24 h later. Furthermore, *in vitro*, incubation of cultured fibroblasts of gestational d 20 with 10^{-7} M cortisol increased GR immunoreactive protein and binding activity in a dose- and time-dependent manner, without affecting cellular levels of GR mRNA. However, identical treatment of d 20 distal airway epithelial cells was followed by decreased GR protein without significant change in cellular GR mRNA. Surfactant protein-A protein levels, taken as assessments of lung maturation, were increased in response to the same treatment. Our findings suggest that hormonal regulation of GR in fetal lung cells occurs at a posttranscriptional level, and is cell-specific. In the context of substantial increases in circulating glucocorticoid concentrations during late gestation, these findings may be of physiologic importance to the biochemical maturation of the antenatal lung. (*Pediatr Res* **38: 506–512, 1995**)

Abbreviations
GR, glucocorticoid receptor
FBS, fetal bovine serum
FPF, fibroblast-pneumocyte factor
MEM, minimal essential medium
SP-A, surfactant protein-A

Glucocorticoids accelerate the onset of mature levels of surfactant synthesis by fetal mammalian lung (1–3) via the classic GR mechanism (1, 2, 4–9). However, in many tissues, glucocorticoids decrease GR expression, thereby decreasing tissue responsiveness to glucocorticoid stimulation. The effect of glucocorticoids on GR in late gestation fetal lung around the time of the onset of augmented surfactant production is incompletely understood.

Administration of glucocorticoids decreases GR mRNA (10) and GR protein (11) in several adult rat organs, including lung. In various cell lines, glucocorticoids lower GR number by decreasing GR transcription rates without altering affinity for ligand (12, 13). However, it is recognized that the response of GR mRNA steady-state levels to glucocorticoid treatment can vary as a function of cell type. Pépin *et al.* (14) found dexamethasone caused a decrease in GR mRNA in hypothalamic neurones, but an increase in GR mRNA in cerebral cortex neurones. Treatment of a steroid-sensitive subclone of a human lymphoid cell line causes an increase in GR protein and in GR mRNA, but the same treatment brings about no change in GR protein or GR mRNA levels in a steroid-resistant subclone (15). Furthermore, it is also recognized that the response of GR to glucocorticoid stimulation can depend on stage of development. Kalinyak *et al.* (16) reported a glucocorticoid induced decrease in GR mRNA levels in postnatal brain and liver, but no change in the corresponding fetal tissues. There are parallel increases in late gestation fetal lung in both glucocorticoid hormone concentration (1) and GR number (without any apparent change in affinity for ligand) (17, 18), suggesting that glucocorticoids may not regulate fetal lung GR gene expression by a feedback down-regulatory mechanism.

In the present study of fetal rat lung during late gestation, we investigated the ontogeny and response to glucocorticoids of GR in whole lung, in fibroblasts, and in distal airway epithelial

cells. These cell types were of interest because production of surfactant by epithelial cells seems to be augmented by a differentiation factor elaborated by fetal lung fibroblasts (FPF) in response to glucocorticoids (19–21).

METHODS

Materials. [6,7-^{3}H(N)]Triamcinolone acetonide (9 Ci/mmol) was obtained from Dupont Canada (NEN Research Products) (Mississauga, Ontario). D-[α-^{32}P]CTP (3000 Ci/mmol), nylon (Hybond N) membranes, and random primer labeling systems were purchased from Amersham Canada (Oakville, Ontario), and restriction enzymes, dextran sulfate, prepackaged disposable (Sephadex) columns, and blue Sepharose 6B-CL from Pharmacia (Baie D'Urfé, Quebec). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). A construct named pRd N93, containing the open reading frame for the 2.3-kb rat GR cDNA (except for the 19-glutamine repeat, which was excised) inserted into the *Bam*HI site of the plasmid pSP64, was a gift of Dr. Yamamoto (University of California, San Francisco). A 1.5-kb β-actin cDNA derived from the 2-kb cDNA (A1) of Dr. D. W. Cleveland *et al.* (21a) was used to monitor evenness of loading and transfer of RNA in Northern blot analyses. MEM (glucocorticoid-free) and FBS were obtained from GIBCO/BRL (Burlington, Ontario).

Animals. Wistar rats were obtained from Charles River (St. Constant, Quebec) and were bred in the Lab Animal Services facilities of The Hospital for Sick Children, Toronto. Pregnant dams of known gestational age (d 0 = mating; term = d 22) were killed with diethylether. The fetuses were immediately removed from the uterus, and the fetal lungs dissected out. For whole lung studies, they were then flash frozen in liquid nitrogen, and stored at −70°C until used for RNA isolation and cytosol generation. Cell culture was performed as described below. In comparing the data from fetal lung with data from adult lung, binding assays were performed on nonpregnant adult rat lung rather than on the maternal lung of the fetal samples to avoid the possibility of confounding effects from the altered hormonal milieu of the pregnant state.

Cell culture. Fibroblasts and epithelial cells were isolated from the fetal lungs as described in detail previously (22). Briefly, the lung tissue was digested for 20 min in 0.0625% trypsin and 0.4 mg/mL DNAse. Then these enzymes were neutralized with MEM + 10% FBS, and the mixture was filtered through a 100-μm mesh nylon bolting cloth and centrifuged. The pellet was resuspended in MEM containing 0.1% collagenase. After 15 min of incubation, the collagenase activity was neutralized by adding MEM + 10% FBS. Fibroblasts were allowed to adhere to tissue culture flasks during two incubations of 1 h each. The nonadherent cells were transferred to new culture flasks and incubated overnight for attachment of epithelial cells. Nonadherent cells were removed from all cell cultures after overnight incubation. Cells were grown to confluence in MEM + 10% FBS, and then thoroughly rinsed in MEM (serum- and glucocorticoid-free). For studies of ontogeny, or for controls in studies of the effects of hormones, the cells were then incubated in MEM alone. Cells

incubated in MEM plus a specified concentration of cortisol were in all other respects handled exactly as were the MEM controls. All experiments were performed 24–48 h after isolation. Viability and purity of the cultures were comparable to previously published data (22). In previous studies, we have shown that the epithelial cells express phenotypic features of type II cells, and possess antigenic determinants of mature type II cells (22).

Glucocorticoid receptor binding assay. Most studies now support the hypothesis that the GR is cytosolic, translocating to the nucleus after binding to specific ligand (9). Therefore, we measured the amount of specific GR binding activity in cytosol, according to the method of Wrange *et al.* (23). Briefly, fetal lung tissue or cells were homogenized in an equal volume of 1 mM EDTA, 10% (wt/vol) glycerol, 10 mM DTT, 20 mM sodium phosphate (pH 7), and 50 mM sodium chloride (pH 7.0) using a Dounce homogenizer. Cytosol was prepared by centrifuging the homogenate at 100 000 × g for 60 min at 4°C. Avoiding the floating layer of fat, the clear supernatant was retained as cytosol. Protein content of the cytosol was assayed by the method of Bradford (24). Cytosol (200 μL) was incubated for 16 h at 4°C with 800 μL of H$_2$O, 5 μL of [^{3}H]triamcinolone acetonide, and 50 μL of either DMSO (total binding) or 2 mg of triamcinolone acetonide/mL DMSO (nonspecific binding). The excess of unbound [^{3}H]triamcinolone acetonide was then removed by incubation for 5 min at 4°C with a one-third volume of aqueous 3% (wt/vol) activated charcoal in 0.3% (wt/vol) dextran sulfate, followed by centrifugation at 12 000 × g at 4°C for 15 min. An aliquot of the supernatant was then counted in a Rackβeta 1219 liquid scintillation counter with automated correction for quenching (LKB, Bromma, Sweden). Specific cytosolic GR binding activity (expressed as femtomoles per mg of protein) was saturable, with little additional specific binding noted above 20 nM [^{3}H]triamcinolone acetonide (Fig. 1). Nonspecific binding was <10% in both control and dexamethasone-treated groups. Incubation of the cytosol with [^{3}H]triamcinolone acetonide at 4°C for 16 h increased binding by 10–20% compared with incubation for 4 h, in good agreement with the findings of Carbone *et al.* (25), who studied GR binding in d 21 fetal rat epiphyseal chondrocytes using the same radioligand.

GR RIA. Cellular GR protein content was measured by RIA as previously described in detail (11, 15). The RIA uses a rabbit antiserum (14B2) raised against a 14-amino acid peptide (14-mer) of the amino terminus domain of the human GR. It is a simple and specific RIA to quantitate both human and rat GR, which detects both activated and inactivated GR (11). Briefly, after dissociation from monolayer, cell suspensions were collected by centrifugation, washed, and resuspended in ice-cold homogenate buffer containing 25 mM Tris-HCl (pH7.4), 10 mM NaCl, 2 mM MgCl$_2$, 1 mM EDTA, 5 mM EGTA, 10 mM leupeptin, and 5 mM DTT in 0.1% (vol/vol) Triton. Cells were homogenized using a ground glass homogenizer. Homogenates were centrifuged at 100 000 × g for 20 min at 4°C, and the supernatants were frozen at −70°C until assayed. Total protein content was using a Bio-Rad protein assay (Bio-Rad, Richmond, CA). The standard buffer of RIA consists of PBS containing 0.1% nonimmune rabbit serum. The RIA was per-
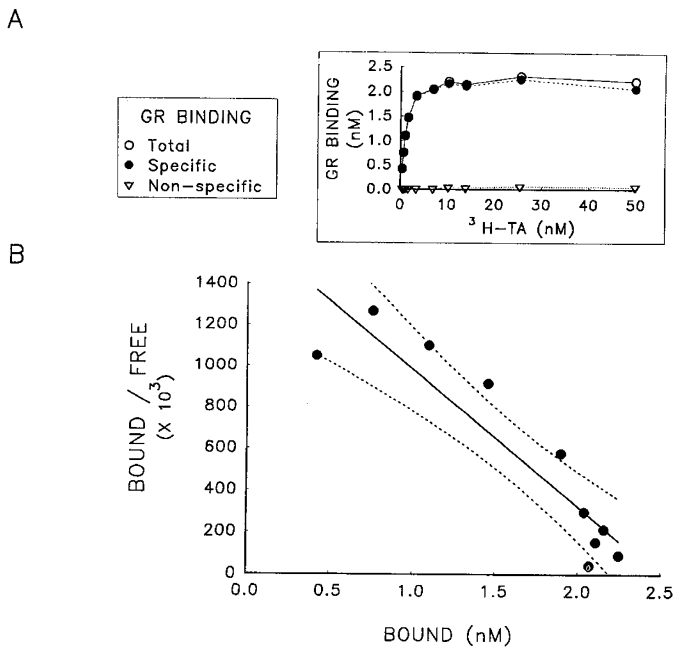
A

B

Figure 1. Scatchard analyses of GR binding activity in d 21 fetal rat whole lung. Representative data, with least squares' regression and 95% confidence intervals. *Inset*, GR binding as a function of ligand concentration. Apparent dissociation constant (k_d): (1.5 ± 1) nM (d 21). Ligand: triamcinolone acetonide (*TA*).

formed in polystyrene tubes; 50 μL of 14-mer GR standards were added to 100 μL of standard buffer containing 20 000 cpm of [125]I-14-mer and 100 μL of the diluted anti-14-mer. After incubation at 22°C for 1 h, 100 μL of goat anti-rabbit antiserum (Biomega, Montreal) tested at various dilutions were added. The tubes were incubated at 4°C overnight (14–18 h), then 1 mL of ice-cold standard buffer was added before centrifugation at 5 000 $\times$ g for 30 min at 4°C. The supernatant was discarded, the radioactivity of the precipitate left in the tubes was counted in an LKB-Wallac (Sweden) Compu-gamma, and RIA data were calculated by the RIA program provided with the counter.

Rat SP-A isolation. Fetal lung GR levels were correlated with levels of SP-A, a glucocorticoid-responsive gene in developing rat lung (26–28) which is a marker of epithelial type II cell development.

SP-A was isolated from bronchoalveolar lavage fluid of rats. Routinely, 20 adult rat lungs were lavaged twice with 10 mL of 0.9% (wt/vol) NaCl per rat. The lavage fluid was centrifuged at 300 $\times$ g for 10 min, and the supernatant was collected and spun at 20 000 $\times$ g for 20 min at 4°C. The supernatant was decanted, and the pellet was washed in 2 mL of 0.15 M NaCl, 0.1 mM EDTA, 5 mM Tris, pH 7.4, 0.2 mM DTT, 1 mM phenylmethylsulfonyl fluoride, and then extracted with 100 mL of *n*-butanol. After 1 h of stirring at room temperature, the butanol extract was centrifuged at 3 000 $\times$ g for 10 min. The supernatant was removed, and the remaining pellet (crude surfactant) was washed several times with 100 mM NaCl, 5 mM Tris, pH 7.4, 20 mM octyl-β-D-glucopyranoside and then resuspended in 5 mM HEPES, pH 7.4. The suspension was dialyzed for 48 h against HEPES, pH 7.4, and centrifuged at 100 000 $\times$ g for 60 min, and the supernatant was collected.

The extracted proteins were eluted through pd10 columns followed by blue Sepharose CL-6B columns to remove any residual serum proteins. The filtrate, which contained mainly rat SP-A as determined by SDS-gel electrophoresis, was concentrated and stored in small aliquots at −70°C.

Determination of SP-A content. A polyclonal antibody raised against rat SP-A was produced by inoculating rabbits with 200 μg of crude surfactant protein in Freund's complete adjuvant. Boosts with 100 μg of purified SP-A were given on a 2-wk basis in incomplete Freund's adjuvant. Serum from injected rabbits was tested by Western blotting. One-dimensional SDS-PAGE was performed by the method of Laemmli (29) using 1-mm thick gels containing 15% polyacrylamide. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose was performed according to Towbin *et al.* (30), after immunostaining with rabbit anti-rat SP-A (1:200), goat anti-rabbit IgG (1:150), and rabbit peroxidase anti-peroxidase (1:500). The IgG fraction of this antiserum was used for ELISA, and it recognized only rat SP-A molecules with molecular masses of approximately 28, 32, and 38 kD on Western blotting (Fig. 2). Similar rat SP-A sizes have been published previously (31). This antibody does not recognize any fetal liver or intestinal epitopes similar to SP-A, but does recognize epithelial type II cells in the adult rat lung (Fig. 2). The method for the ELISA was similar to that described by Ballard *et al.* (32). Standard curves were constructed from the absorbance of known amounts of rat SP-A, and sample values were calculated from the dilutions that fell on a linear part of the standard curve. As little as 0.5 ng of SP-A in a sample could reliably be detected by this technique. ELISA were performed in triplicate from three different samples, and data are expressed per milligram of total protein.

RNA analysis. Total (nuclear and cytoplasmic) RNA was isolated by lysing the tissue in 4 M guanidinium isothiocyanate followed by centrifugation on a 5.7 M cesium chloride cushion to pellet RNA (33). After extraction with phenol:chloroform: isoamyl alcohol (25:24:1, vol/vol) and reextraction with chlo-
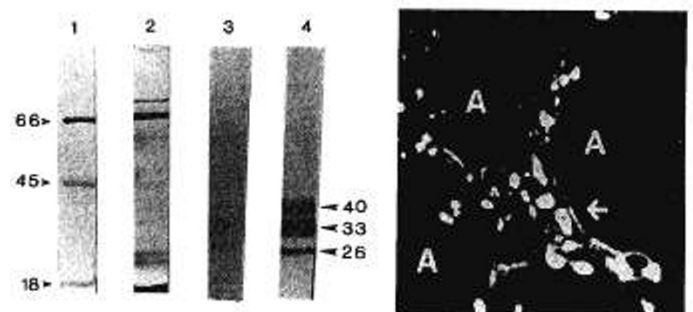

Figure 2. Specificity of rat SP-A antibody. (*Left panel*) Western blot immunoanalysis of SP-A in rat lung surfactant. Surfactant, isolated from bronchoalveolar lavage fluid of adult rats by centrifugation, was loaded (10 μg of protein) and separated on 15% (wt/vol) polyacrylamide gel under reducing conditions. Protein was transferred to a nitrocellulose membrane and immunostained with either preimmune serum (*lane 3*) or anti-rat SP-A (*lane 4*); protein bands were detected at 40, 33, and 26 kD. Similar sized bands were detected in total protein extracts from whole lung (data not shown). *Lane 1* shows molecular weight markers and *lane 2* a Coomassie Blue staining. (*Right panel*) Immunolocalization of SP-A in adult rat lung. Epithelial type II cells show positive immunoreactivity to SP-A (*arrow*). *A* = alveolar space.

roform alone, the RNA was ethanol-precipitated, collected by centrifugation, lyophilized, and dissolved in water. RNA integrity was confirmed by fractionation on 1% (wt/vol) agarose-formaldehyde gels and staining the ribosomal RNA bands with ethidium bromide.

In slot blot analysis, aliquots of RNA (5.0–0.625 μg/sample) were serially diluted in a final 50 μL solution of 6.15 M formaldehyde and 10 × SSC (1.5 M sodium chloride, 0.15 M sodium citrate, pH 7.0). The RNA was denatured at 65°C for 15 min and immediately immobilized on a nylon membrane under low vacuum filtration. After the solution had filtered through, the wells were rinsed with 500 μL of 10 × SSC, and RNA was fixed by baking at 80°C for 2 h. The linearity of the slot blot assays was confirmed by serial dilution and the specificity by Northern blot analysis. For Northern blot analysis, 20 μg of total RNA were fractionated on 1.0% (wt/vol) agarose gels containing 6% (vol/vol) formaldehyde, transferred to a nylon membrane, and then fixed by baking at 80°C for 2 h. GR and β-actin cDNA were labeled with D-[α-^{32}P]CTP by random hexamer priming with the Klenow fragment of *Escherichia coli* DNA polymerase I. Prehybridization (1 h overnight) and hybridization (24–48 h) were performed in 50% (vol/vol) formamide, 750 mM NaCl, 75 mM sodium citrate, 5 × Denhardt's solution [0.4% (wt/vol) each of BSA, Ficoll, and polyvinylpyrrolidone], 10% (wt/vol) dextran sulfate, and 100 μg/mL denatured salmon sperm DNA at 42°C. Blots were washed in 5 × SSC, 0.1% (wt/vol) SDS at 42°C for 20 min, followed by 1 × SSC, 0.1% SDS at 42°C for 15 min, and then exposed to Kodak XAR-5 film using Dupont Cronex intensifying screens. The mRNA detected was quantified by an Ultrascan XL laser densitometer (LKB, Bromma, Sweden). Multiple exposure times were obtained to ensure that the autoradiographs were in the linear range for quantitative scanning.

Data presentation. Values are presented as the mean ± SE. Statistical significance ($p < 0.05$) was determined by an analysis of variance followed by assessment of differences using Dunnet's two-sided test (34), or Duncan's multiple range test (35).

RESULTS

Changes in lung GR during ontogeny. In whole fetal rat lung, steady-state GR mRNA levels were relatively low on d 18. On gestational d 19, there is a short-lived increase in GR mRNA, abruptly followed (d 20–22) by a return of the mRNA to levels present before d 19 (Fig. 3). Specific GR ligand binding increased progressively from d 19 to d 21 of gestation (Fig. 3). On Northern blot analysis of total RNA from fetal rat whole lung, rat GR cDNA hybridized to a 7-kb mRNA species (Fig. 3, *inset*), consistent with published data (10, 16). Scatchard analyses revealed that the apparent dissociation constant (k_d) of GR did not change significantly with advancing gestation (d 19: $k_d = 1.7 \pm 1$ nM; d 21: $k_d = 1.5 \pm 1$ nM, mean ± SEM, $n = 3$ independent experiments). The k_d value for [^{3}H]triamcinolone acetonide binding in whole fetal rat lung is similar to values published previously for [^{3}H]dexamethasone binding (18).
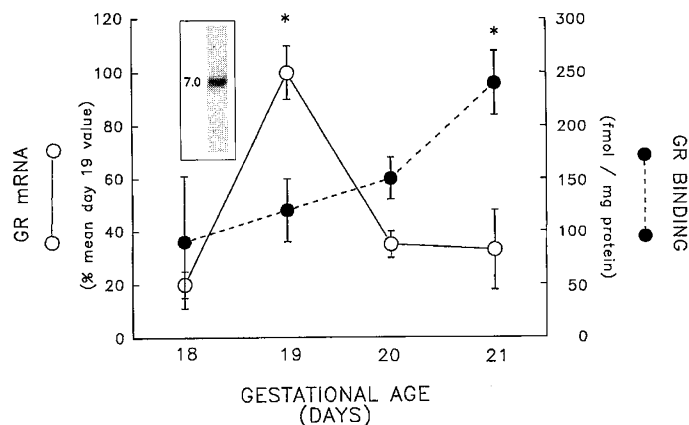

Figure 3. Developmental profiles of GR in fetal rat whole lung. GR mRNA levels (○). Hybridization of specific GR probe to Northern blots of total RNA from whole lungs of 18–21 d of gestation. Data normalized for β-actin mRNA levels to control for mRNA loading and transfer. Mean ± SE, $n = 3$ independent experiments for each data point (lungs from at least two litters were pooled in each experiment). *Inset*, GR mRNA, 7 kb. Glucocorticoid binding activity (●). Cytosolic glucocorticoid binding activity, 18–21 d of gestation. Mean ± SE, $n = 3$ independent experiments carried out in triplicate (lungs from at least two litters were pooled in each experiment); *significantly different ($p < 0.05$) from d 18 value.

In fibroblasts, GR mRNA levels were maximal at 19 d of gestation, accompanied by maximal GR binding activity on d 19 and 20 (Fig. 4A). In distal airway epithelial cells, GR mRNA levels and GR binding activity each peaked at 20 d of gestation (Fig. 4B). In both cell types, maximal GR binding activity represented substantial ($\approx$4-fold) increases over immature (d 18) activity. Furthermore, GR mRNA levels were highly variable on d 19 (epithelial cells) and 20 (fibroblasts).

Regulation of GR by glucocorticoid hormone. Whole lung GR mRNA levels were not changed by *in utero* exposure to glucocorticoids (Fig. 5A), but GR binding activity was increased. A single injection of 1 mg of dexamethasone/kg to the mother, 24 h before killing, increased d 20 fetal rat lung GR
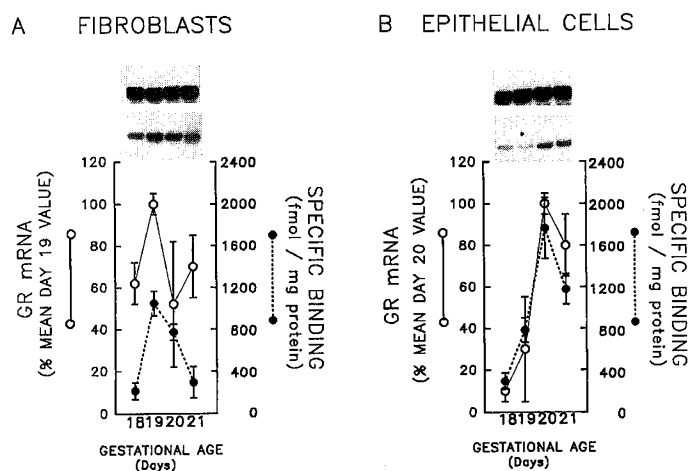

Figure 4. Developmental profiles of GR in fetal rat lung cells. (A) Fibroblasts; (B) epithelial cells from distal airway. *Upper inset*, autoradiograph of β-actin mRNA; *lower inset*, GR mRNA, same representative blot. GR mRNA levels (○): Quantitation using laser densitometry, normalized for β-actin. Mean ± SEM. For each data point, $n = 3$ independent experiments. Glucocorticoid binding activity (●): Mean ± SEM. For each data point, $n = 16$ fetuses, from four litters, in two independent experiments.
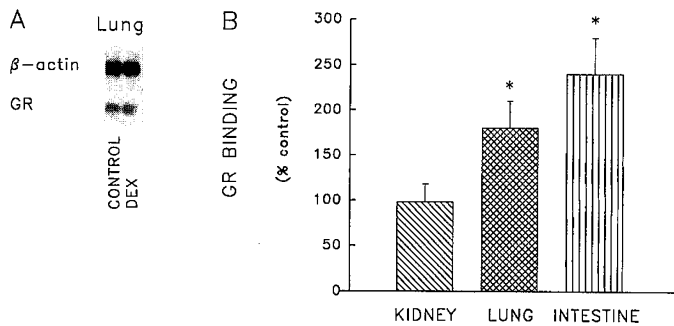
Figure 5. (*A*) Lack of effect of glucocorticoid on GR mRNA levels in fetal whole lung. GR mRNA levels were unaltered by maternal administration of dexamethasone. *DEX* = dexamethasone 1 mg/kg in 200 μL of DMSO intraperitoneally to pregnant rat 24 h before sacrifice; control = 200 μL of DMSO only; *n* = 3. (*B*) Tissue dependence of effect of glucocorticoid on GR binding activity. Foregut-derived tissues (lung or intestine) increased GR binding activity in response to dex, whereas urogenital ridge-derived tissue (kidney) did not. *Significantly different (*p* < 0.05) from control value; *n* = 3.

binding activity (175 ± 20% of control, mean ± SD, *n* = 5, *p* < 0.05, dexamethasone *versus* DMSO control) (Fig. 5*B*). Two consecutive daily injections of dexamethasone (d 18 and 19) before sacrifice (d 20) further increased GR binding activity to 240 ± 40% of control (not shown). The stimulatory effect of dexamethasone on GR binding activity was of limited duration. A single dose of dexamethasone on d 18 increased GR binding activity in fetal lungs assayed on d 19 (226% of control, *n* = 2) or 20 (154% of control, *n* = 2), but not on d 21 (103% of control, *n* = 2). Neither the protein:DNA ratio (97 ± 6% of control, *n* = 3), the total protein per g of tissue (103 ± 5% of control, *n* = 3), nor the mean total lung weight (102 ± 4% of control, *n* = 3) was altered by dexamethasone treatment.

The organ specificity of the response to glucocorticoid stimulation *in utero* was tested. Like fetal lung, fetal intestine also demonstrated augmented GR binding activity subsequent to dexamethasone administration *in utero*, whereas fetal kidney did not show any change (Fig. 5*B*). Consistent with previous studies of the effect of glucocorticoid on postnatal lung (10), nonpregnant adult female rat lungs showed decreased GR binding activity (63–66% of control, *n* = 2) after dexamethasone exposure.

Fibroblasts from fetal lungs of 20 d gestation responded to 24 h of incubation in 10^{-7} M cortisol with increases in GR binding activity (50%, Fig. 6) and GR protein content (40%, Fig. 7), even though there was no change in steady-state GR mRNA levels (Fig. 8*A*). At cortisol concentrations of ≤10^{-9} M, GR binding activity was not significantly different from control, but a significant increase occurred with cortisol at ≥10^{-8} M (Fig. 6*A*). Similar increases in GR binding activity also occurred in fibroblasts of 19 or 21 d gestation (data not shown). Exposure to 10^{-7} M cortisol for 24 h increased GR binding, but incubations of ≤16 h did not (Fig. 6*B*).

Epithelial cells from lung of 20 d gestation, in contrast, displayed a decrease (40%) in GR protein content in response to incubation for 24 h with 10^{-7} M cortisol (Fig. 7). However, cortisol did not alter the steady-state GR mRNA content of the epithelial cells, except for a decrease on gestational d 21 (Fig. 8).
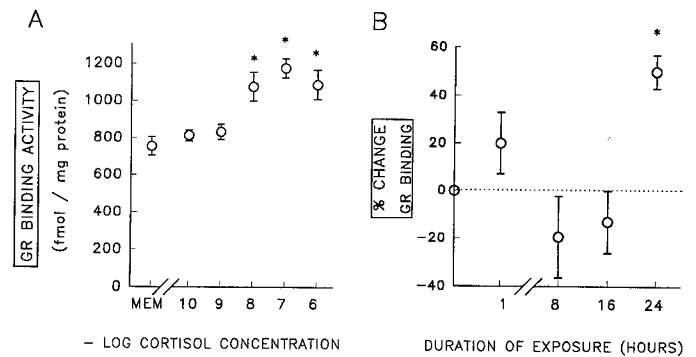

Figure 6. Glucocorticoid stimulation of d 20 fibroblast GR binding activity. (*A*) Dose effect. Stimulation occurs at cortisol concentrations of 10^{-8} M to 10^{-6} M. Mean ± SEM. For each data point, *n* = 12 fetuses, from four litters, in two independent experiments. *p* < 0.05 by analysis of variance. (*B*) Effect of duration of exposure. Stimulation occurs after 24 h of exposure to 10^{-7} M cortisol. Mean ± SEM. For each data point, *n* = 4 independent experiments. *p* < 0.05 by analysis of variance.
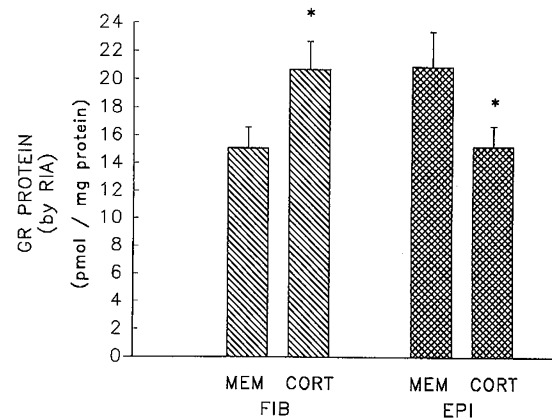

Figure 7. Effect of glucocorticoid on cellular GR immunoreactive protein. In response to cortisol exposure, GR protein content as measure by RIA was increased in d 20 fibroblasts, but decreased in d 20 epithelial cells. *Different (*p* < 0.05) from control value; *n* = 3.

To test whether the glucocorticoid treatment used in these studies stimulates lung maturation, we determined its effect on the amount of SP-A protein, a marker of lung maturation known to be responsive to glucocorticoid in rat lung (26–28). The SP-A protein content of d 20 lungs, determined by ELISA, increased after dexamethasone treatment on d 19 (260 ± 31 ng/mg total protein (1 mg dexamethasone/kg) *versus* 149 ± 26 ng/mg (DMSO control), mean ± SEM, *n* = 3–5 independent samples, *p* < 0.05 dexamethasone *versus* DMSO control).

DISCUSSION

The results of the present study indicate that the ontogeny of GR mRNA and GR binding activity in fetal rat lung is cell-specific. Both GR binding activity and steady-state levels of GR mRNA were maximal in lung fibroblasts (and also in whole lung) at 19 d of gestation, 24 h before the maximal levels in distal airway epithelial cells. In whole lung, GR functional (hormone binding) activity increased progressively during late gestation, consistent with previously published data (17, 18). However, unlike the results of Brönnegård and Okret
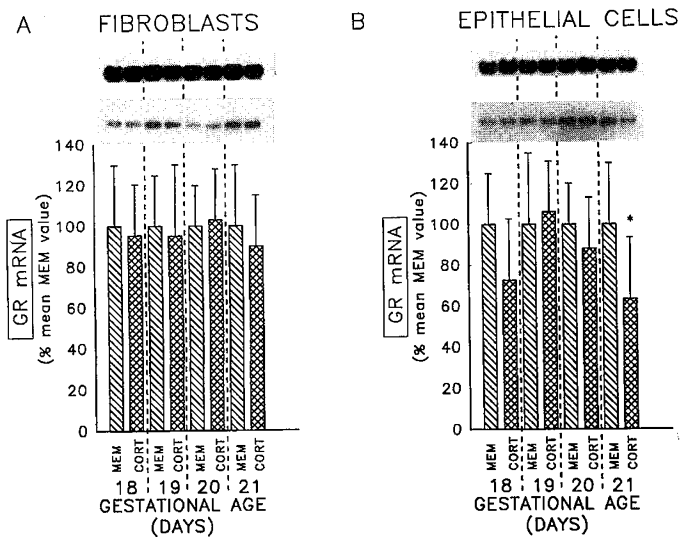
Figure 8. Effect of glucocorticoid on cellular GR mRNA levels during development. (*A*) Fibroblasts; (*B*) epithelial cells. *Upper insets*, autoradiograph of β-actin mRNA; *lower insets*, GR mRNA, same representative blot. Incubation in 10^{-7} M cortisol (*CORT*) did not change steady-state levels of cellular GR mRNA compared with control cells incubated in serum- and hormone-free medium (*MEM*), except for d 21 epithelial cells. *Different ($p < 0.05$) from control value; $n = 3$.

(36), no progressive increase in the steady-state GR mRNA levels was noted.

Previously, Ballard *et al.* (18) reported specific binding of glucocorticoid to fetal rat lung fibroblasts and epithelial cells. However, the fetal lung epithelial cells they studied were obtained from organotypic cultures incubated for 2–3 wk. Furthermore, lung fibroblastic cells, derived from a 16-wk-old human fetus, were studied from passage 16 to 50 (18). Thus, our present finding of GR mRNA, protein, and specific binding activity in primary cultures of fetal rat lung fibroblasts and epithelial cells, within 1–2 d of killing, may more directly reflect the expression of GR by these cells *in vivo*. The observed discrepancy between the developmental profiles of GR binding in whole lung compared with those of the individual cell types is most likely due to the thinning of mesenchymal tissue (fibroblasts), flattening of epithelium, and invasion of capillaries which characterize the later stages of fetal lung development.

In the present study, we found that maternal administration of a high dose of dexamethasone (1 mg/kg) increased whole fetal rat lung GR binding activity, without alteration in GR mRNA levels. In contrast, Brönnegård and Okret (37) observed a 35–50% decrease in fetal rat whole lung GR mRNA levels after administration of much lower doses (0.2 mg/kg) of betamethasone. This suggests that a high level of glucocorticoid is required to induce increased GR levels. Because the GR of fetal intestine (like lung, a foregut derivative) was also increased in response to dexamethasone treatment, whereas GR from fetal kidney (of urogenital ridge origin) was not affected, the response of fetal GR levels to glucocorticoid stimulation is tissue-specific. Our data on fetal rat lung growth after antenatal glucocorticoid treatment are in contrast to two previous reports of growth inhibition (38, 39). In one study, the dose of dexamethasone was twice the dose used in the present investigation

(38). In the other, the dexamethasone was administered earlier in gestation in three divided doses, and it was given as the sodium phosphate salt in saline s.c. (39).

Within the lung, the response of GR to glucocorticoid stimulation is also cell-specific. Exposure of primary cultures of d 20 fetal rat lung fibroblasts to cortisol increased GR binding activity and GR protein content compared with control cells incubated in serum- and hormone-free medium. The effect on GR binding was dose- and time-dependent. However, identically treated primary cultures of distal airway epithelial cells derived from the same lungs displayed a decrease in cellular GR protein content. Interestingly, on gestational d 20 neither cell type responded to glucocorticoid treatment with any change in GR mRNA levels. These findings suggest a differential posttranscriptional effect of glucocorticoids on GR protein content in fetal lung cells. It is not yet clear whether this is due to altered rates of GR protein synthesis, degradation, or both. The two cell types studied differ in that fetal rat lung fibroblasts reduce inactive intracellular cortisone to active cortisol, whereas the epithelial cells do not (40). Resulting differences in intracellular levels of active glucocorticoid might account for the observed differential effect of the hormone on GR protein levels.

The analysis of glucocorticoid binding activity after *in vivo* glucocorticoid treatment (1 mg dexamethasone/kg) may be complicated by translocation of the receptor-ligand complex to the nucleus, and also by possible residual tissue dexamethasone interfering with the binding assay *in vitro*. Even though we have not addressed these issues experimentally in this study, both of these effects would be expected to decrease the apparent binding activity in the *in vitro* assay, and hence to underestimate the extent to which glucocorticoid treatment up-regulates GR in fetal whole lung or in fetal lung fibroblasts. Our finding of increased GR binding activity in fetal lung fibroblasts was further confirmed by a parallel increase in GR protein. The RIA used to assess cellular GR protein levels has been shown to be unaffected by residual tissue dexamethasone (11). However, because GR protein levels were only measured in the cytosolic component, the epithelial cell GR protein levels may be underestimated due to translocation of the receptor-ligand complex to the nucleus.

In late gestation, glucocorticoids induce production of a differentiation factor, FPF, which stimulates surfactant phospholipid formation in the fetal lung (1, 2, 19, 21). Maximal FPF production by fetal rat lung fibroblasts in response to glucocorticoid stimulation has been reported on d 19 (1, 21), coincident with maximal cellular levels of GR mRNA and binding activity. Fibroblast cellular sensitivity to glucocorticoid stimulation should thus be optimal when FPF activity is greatest. Furthermore, contrary to what might have been anticipated based on studies of postnatal lung (10, 16), in the fetal lung fibroblast GR function (as assessed by glucocorticoid binding activity) and GR protein content (as assessed by RIA) are enhanced by hormonal exposure. This enhancement, in the context of substantial physiologic increases in circulating glucocorticoid concentrations, may serve to achieve adequate levels of fibroblast GR for the normal production of FPF.

The maximum observed levels of specific binding of ligand per mg of cellular protein were greater in the epithelial cells (on d 20) than in the fibroblasts (on d 19). This is consistent with direct actions of glucocorticoids on the epithelial cells, independent of mediation by FPF, such as have been suggested with respect to surfactant proteins (26–28). Although mesenchymal enrichment with GR relative to epithelium has been reported (40), our data would suggest that the relative GR content of mesenchyme compared with epithelium will vary as a function of gestational age. Furthermore, in previous studies we have demonstrated relative enrichment in GR in the subpopulation of adjacent fibroblasts compared with peripheral fibroblasts (22). Direct comparison of the GR content of the epithelial cells with that of adjacent fibroblasts is not possible based on the results of the present study of fibroblast cultures containing a mixture of subpopulations.

In summary, we report that the autoregulation exerted on GR by its own hormone during late gestation depends upon multiple factors (including the tissue and cell type, developmental period, dose, and duration of exposure), and occurs principally at a posttranscriptional level. Although glucocorticoid exposure was associated with a decrease in fetal lung epithelial cell GR protein content, it was followed by an increase in fibroblast GR, a finding of potential physiologic importance for the biochemical maturation of the antenatal mammalian lung.

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