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REGULATION OF THE GLUCOCORTICOID RECEPTOR IN FETAL RAT LUNG DURING DEVELOPMENT

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Summary—The effect of the synthetic glucocorticoid betamethasone on the regulation of the glucocorticoid receptor mRNA and on receptor protein was studied in fetal rat lung during development. Using a glucocorticoid receptor cRNA probe, glucocorticoid receptor mRNA was examined by Northern blot hybridization and by solution hybridization. A monoclonal antibody against the glucocorticoid receptor was used to study regulation of the receptor protein by the Western immunoblotting technique. In fetal rat lungs, of 16–21 days of gestation, as well as in adult lungs, betamethasone treatment resulted in a significant decrease of glucocorticoid receptor mRNA to 50–65% of the control level. In contrast, betamethasone treatment did not down-regulate the receptor protein in rat lungs of 16–19 days of gestation, whereas a decrease of glucocorticoid receptor protein to 40–60% of control was seen in lungs of 21 days of gestation, in postnatal and adult lung. These results provide data for a change in regulation *in vivo* of the glucocorticoid receptor by its homologous ligand in fetal rat lung during development.

INTRODUCTION

Glucocorticoids, and steroid hormones in general, act mainly by altering the rates of transcription of specific genes both positively and negatively [1]. In addition, glucocorticoids exert effects on gene expression through post-transcriptional effects. This includes effects on mRNA as well as protein stability [2, 3].

One gene regulated by glucocorticoids is the glucocorticoid receptor (GR) gene itself [4]. The autoregulation of GR expression by its cognate ligand is complex involving both transcriptional and posttranslational processes [4, 5]. Glucocorticoids down-regulate the GR level by both reducing GR gene transcription rate and increasing GR protein turnover. Indirect evidence also suggests additional control levels [4].

Previous studies have indicated that glucocorticoids differentially regulated specific gene expression with a distinctive developmental and hormonally regulated expression pattern [6]. In the present study, we have analyzed the expression of the GR protein and mRNA in fetal rat lung during development and studied the autoregulation of the GR protein and mRNA in fetal rats after maternal treatment with the synthetic glucocorticoid, betamethasone.

MATERIALS AND METHODS

Animals

Pregnant Sprague–Dawley rats were injected i.m. with 0.2 mg/kg betamethasone (Celestona Soluspan, Schering Corp., Kenilworth, NJ, U.S.A.; containing equal amounts of betamethasone acetate and betamethasone phosphate) or saline (controls) 12 and 24 h prior to sacrifice. The time of conception was known within 8 h. Betamethasone was used because this glucocorticoid has a prolonged biological activity in target tissues and no mineralocorticoid effects. The rats were killed by cervical dislocation and the fetuses delivered by cesarean section.

RNA isolation and quantitation

For the measurement of mRNA, complementary RNA (cRNA) probes were synthesized *in vitro* and radiolabeled with [^{32}P]UTP for Northern blot hybridization and with [^{35}S]UTP for solution hybridization (Amersham, Bucks., U.K.), using reagents supplied by Promega Biotech (Madison, WI, U.S.A.) [7, 8]. Total cellular RNA was prepared from fetal rat lungs of different gestational age by homogenization of

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the tissue in 4 M guanidinium thiocyanate and centrifugation through cesium chloride [9]. The RNA was analyzed by Northern blot hybridization using a cRNA probe for the rat GR exactly as described previously [10]. A cDNA probe for β-actin cloned into the pGEM 3 vector (Promega Biotech.) was kindly provided by Cleveland *et al.* [11]. For quantitative analysis, mRNA was measured by solution hybridization. The hybridization analysis of RNA in solution was performed as described previously [12], using the same cRNA probe as for Northern blot hybridization. The relative expression of GR mRNA in glucocorticoid treated and nontreated rat lung was related to the expression of β-actin mRNA and expressed as a percentage of the control values. Data are presented as mean values $\pm$ SD, and the paired t-test was used for statistical analysis.

Preparation of cellular extract and Western immunoblotting

Lung tissue was homogenized in ETG buffer [20 mM Tris–HCl, 1 mM EDTA, 2 mM dithiothreitol and 10% (w/v) glycerol, pH 7.0] containing 0.4 M NaCl as described previously [4]. Following ammonium-sulfate precipitation the pellet was dissolved in the above buffer without salt and the protein concentration determined according to Lowry *et al.* [13]. Protein (100 μg) from each lung was separated on a 12% sodium dodecyl sulfate polyacrylamide gel and electroblotted onto nitrocellulose filters (Bio-Rad protocol, Richmond, CA, U.S.A.). Protein content on the nitrocellulose filters was also visualized with Pancean-red (Bio-Rad) and scanned (see below) to ensure that an equal amount of total protein had been applied on the gel and evenly transferred to the nitrocellulose membrane. The protein bands were identified by the monoclonal anti-GR antibodies (No. 7 [14]) and visualized by the Proto Blot Immunoblotting system (Promega Biotech.). The signal intensity of the filters was determined by measuring reflections using a Shimadzu dual-wavelength TLC scanner CS-930 (Kyoto, Japan) densitometer.

RESULTS AND DISCUSSION

Glucocorticoid regulation of GR mRNA in fetal rat lungs

Northern blot analysis of total cellular RNA isolated from fetal rat lungs of different gestational age, showed hybridization of the GR cRNA probe to a major 7 kb transcript similar to what has been described for other organs and identical to GR mRNAs seen in adult lungs (Fig. 1) [4]. Both in gestational day 18 and 21 as well as in adult lung there was a down-regulation of the 7 kb GR transcript, seen following betamethasone treatment.

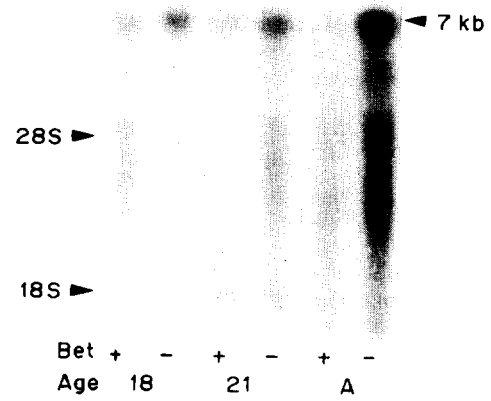

Fig. 1. Northern blot analysis of GR mRNA in fetal rat lung. 20 μg Of total RNA from fetal and adult lung was hybridized with a cRNA probe for the rat GR as described in Materials and Methods. Standards corresponding to RNA species of 28S and 18S are indicated. Bet—betamethasone.

These data were confirmed by a more quantitative solution hybridization assay. Values were compared to β-actin mRNA levels and expressed as the ratio between GR and β-actin mRNA. At all gestational ages investigated (day 16–21) GR mRNA levels were significantly decreased to 50–65% of control ($P < 0.05$) 24 h after betamethasone administration (Fig. 2). No major difference was found when comparing down-regulation of GR mRNA in fetal rat lungs with adult or postnatal lungs. Furthermore, fetal GR mRNA levels showed a continuous increase with gestational age and declined prior to term (Fig. 2). This decrease is most likely due to physiological down-regulation of GR mRNA by high levels of endogenous corticosteroids [15]. β-Actin mRNA in fetal rat lungs showed only minor changes due to hormone treatment.

Glucocorticoid regulation of GR protein levels in fetal rat lungs

GR protein content in fetal rat lung was studied by Western immunoblotting using a monoclonal anti-GR antibody. At all develop-

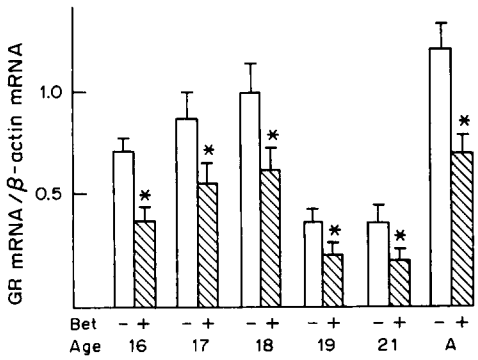

Fig. 2. GR mRNA levels in fetal rat lung determined by solution hybridization. RNA was prepared and solution hybridization carried out with a cRNA probe for rat GR and β-actin, as described in Materials and Methods. Each day represents the mean of 3 separate experiments ± SD [betamethasone (Bet +) and control (−) animals]. Each analyzed day represents between 10–15 fetal lungs obtained from 3 mothers. Results are presented as the ratio between GR mRNA and β-actin mRNA expression. A − adult lung. Significant differences between treated and untreated animals are indicated by *($P < 0.05$).

mental stages studied there was a 94 kDa protein detected (Fig. 3). In extracts from gestational day 21, postnatal day 5 and adult lung, a reduction to 40–60% of control in GR protein level following betamethasone administration was demonstrated. However, in contrast to GR mRNA, no down-regulation of GR protein was seen in fetal lung extracts from day 16–19 (Fig. 3).

Previous studies of fetal rat lung have shown that proteolysis of intact GR to a fragment containing only the DNA- and hormone-binding domain may occur [16]. This results in the loss of the amino-terminal domain recognized by the monoclonal antibodies. To exclude the possibility that our observations reflected changes in detectable amounts of GR, we utilized a rabbit antiserum which instead interacts with the DNA binding domain of the rat GR. Only minor traces and no differences between treated and nontreated rat lungs, with regard to the concentration of the receptor fragment containing the DNA- and hormone-binding domain of GR, was seen during development (data not shown). These results indicate that no degradation of the receptor to a smaller form containing the hormone- and DNA-binding domain occurs under the experimental conditions utilized.

As shown in Fig. 3, the GR protein level increases during development but does not show the same decrease prior to term as compared to GR mRNA levels. However, postnatal GR protein levels are lower than fetal GR protein levels, day 18–21. The discrepancy between GR mRNA and protein can be explained by the fact that GR protein half-life is longer than GR mRNA half-life, differing by at least a factor of 2.5–5 [4].

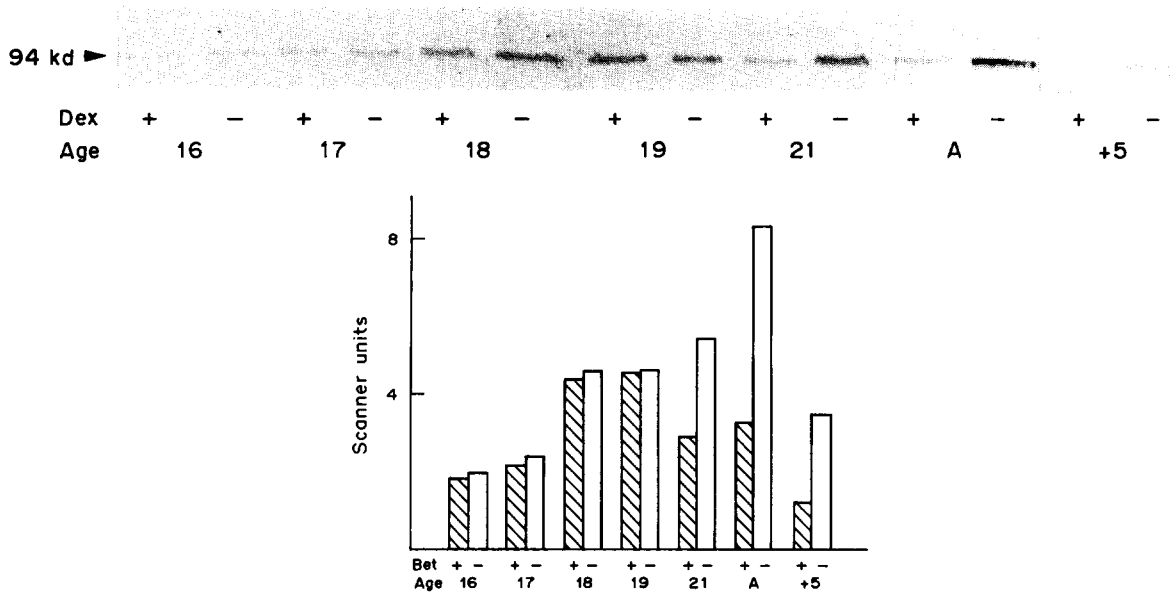

Fig. 3. Western blot analysis of GR levels in fetal rat lung. Cellular lung extracts were prepared and subjected to Western blot analysis as described in Materials and Methods. Each lane contains 100 μg total cellular protein. The apparent sizes of the signals were estimated from the electrophoretic mobilities of standard proteins. The lower part of the figure summarizes the results from densitometric scans of 2 separate experiments. No variations were observed between the 2 experiments. Each analyzed day consists of between 10–15 fetal lungs obtained from 3 mothers. The data are presented as scanner units from densitometric peak areas.

The regulation of GR is an important aspect of physiological control of glucocorticoid responsiveness since most data support a relationship between sensitivity of target cells to glucocorticoids and concentration of GR [17]. A general conclusion from a number of studies is that receptor concentration is a major factor determining hormone sensitivity in a given cell type [18–20].

A number of factors regulate GR expression, e.g. its cognate ligand. This autoregulation of GR concentration by its cognate ligand as determined by ligand-binding, immunochemical and mRNA transcriptional assay is well documented for a number of cell systems and tissues in different species [4, 21, 22]. Several studies have indicated that the autoregulation of GR is complex and involves a number of regulatory levels including transcriptional as well as post-translational mechanisms [4, 5]. These findings become even more complicated by the fact that GR autoregulation seems to be cell and tissue specific [22, 23]. In contrast to most other cells which show a down-regulation of GR expression following treatment with glucocorticoids, human leukemic T-cells (CEM C7) show an up-regulation of GR expression [23]. An up-regulation of GR following cortisol treatment has also been described in rat prostate [24]. In addition, rat hepatoma H4IIE cells show an up-regulation prior to a down-regulation [4, 10]. The reasons for these discrepancies are not known.

It is known that biochemical and morphological maturation of fetal tissues such as lung and chick retina are influenced by glucocorticoids [25–27]. This effect on differentiation is time specific and there seem to exist periods of hormone responsiveness and unresponsiveness during development, e.g. (1) the induction of glutamine synthetase in embryonic chick retina, (2) surfactant synthesis in fetal lung and (3) tyrosine aminotransferase in rat liver [28, 29]. During development, periods of unresponsiveness may also occur with regard to GR autoregulation. This report describes resistance to autoregulation of GR during fetal lung development and in the postnatal rat brain it has previously been shown that GR expression is refractory to autoregulation [30].

In the current study we observed changes in GR mRNA and protein expression in fetal rat lung during development by glucocorticoids. In contrast to the GR mRNA levels, no down-regulation of GR protein could be seen in fetal rat lung, day 16–19. It is unclear why changes in the GR mRNA level are not reflected in changes at the GR protein level. One possible explanation could be that the time curve for down-regulation differs during early and late periods of fetal lung development. A refractory posttranslational mechanism could be due to the fact that proteases necessary for degradation of GR are absent in early developmental stages.

Finally, other still unknown factors may account for differences in GR autoregulation during fetal development, e.g. differential promoter utilization. It is also known that ACTH levels vary in late gestation and may influence the expression of GR [15]. Whether either these or other factors are involved in fetal GR autoregulation is unknown and thus further investigation is required.

The inability of glucocorticoids to down-regulate GR protein, day 16–19, in fetal rat lung may also be of physiological importance. In rats, this fetal period is critical for the final pulmonary maturation process involving a number of biochemical events important for adaptation to postnatal respiratory functions [31]. These processes are known to be influenced by glucocorticoids and may require high levels of GR.

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