Glucocorticoids Affect the Synthesis of Pulmonary Fibroblast-Pneumonocyte Factor at a Pretranslational Level*

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Glucocorticoids accelerate fetal lung maturation by acting on the fetal lung fibroblast to induce the synthesis of fibroblast-pneumonocyte factor which in turn stimulates pulmonary surfactant synthesis by the alveolar type II cell. We have studied the site of glucocorticoid regulation of fibroblast-pneumonocyte factor synthesis in primary cultures of fetal rat lung fibroblasts. Conditioned media from fetal rat lung fibroblasts exposed to cortisol stimulate [Me-3H]choline incorporation into saturated phosphatidylcholine by primary cultures of fetal rat lung alveolar type II cells. This effect is blocked by the presence of actinomycin D during the first, but not the second, 24 h of incubation of the fibroblasts with cortisol. Cycloheximide blocks this effect if present during either the first or second 24 h of incubation. We fractionated mRNA from fetal rat lung fibroblasts incubated in the presence or absence of dexamethasone and observed that cell-free translation products from a fraction of ~500 bases possess biological activity in the bioassay. Such activity is only present in cell-free translation products of mRNA isolated from fibroblasts treated with dexamethasone. These results suggest that glucocorticoids act at a pretranslational level to induce production of fibroblast-pneumonocyte factor and that the primary translation products are biologically active.

In the present study, we have examined the site of regulation of fibroblast-pneumonocyte factor synthesis in primary cultures of fetal rat lung fibroblasts with the use of metabolic inhibitors and of cell-free translation products from such cells incubated in the presence or absence of dexamethasone.

MATERIALS AND METHODS

Isolation of Fetal Lung Fibroblasts and Type II Cells—The cell isolation procedure involved trypsin dissociation of lung tissue from 18 days gestation rat fetuses into individual cells (9). The cells were incubated for 65 min in plastic culture flasks to allow adherence of fibroblasts (9, 13). The nonadherent cells were aspirated, and the attached fibroblasts were grown to confluence in Eagle's minimal essential medium with 10% charcoal-stripped calf serum (9, 13). After a further 60-min incubation as a pellet, the nonadherent cells were inoculated onto Gel foam sponges and incubated for 5 days in minimal essential medium containing 2% charcoal-stripped calf serum (9, 14). At the end of this incubation period, the Gel foam sponges were digested with collagenase (9, 15). The resulting alveolar-like structures, primarily composed of alveolar type II cells, were recovered and propagated in primary culture in plastic multivell plates in minimal essential medium with 10% charcoal-stripped calf serum (9). Purity and viability of the fetal type II cells were determined by trypan blue exclusion.

Effect of Inhibitors of RNA and Protein Synthesis on Fibroblast-Pneumonocyte Factor Production by Fetal Lung Fibroblasts—At confluence, the fibroblasts were incubated in serum-free minimal essential medium with or without 10^-7 M cortisol. Inhibitors of RNA synthesis (actinomycin D, 0.1 μg/ml) or protein synthesis (cycloheximide, 1 μg/ml) were added for either the first or second 24-h portion of this 48-h incubation. These agents did not affect the viability of the fibroblasts as judged by trypan blue exclusion. After the incubation, the media were collected, and their effect on saturated phosphatidylcholine formation by alveolar type II cells was tested. The specificity of observed responses was checked utilizing monoclonal antibodies raised against fibroblast-pneumonocyte factor (12).

Fetal lung type II cells were incubated for 6 h in the conditioned media from fetal rat fibroblasts (above) mixed 1:1 with fresh serum-free minimal essential medium. The incubation medium was supplemented with 1 μCi/ml [Me-3H]choline. At the end of this incubation, the cells were recovered by trypsinization, and lipids were extracted from the cell suspension (9, 10). Saturated phosphatidylcholine was isolated from the lipid extracts by the modified method of Mason et al. (16) utilizing osmication (16) and thin-layer chromatography (17). After detection with Bromthymol blue, the saturated phosphatidylcholine spots were transferred to scintillation vials and radioactivity was measured.

Preparation of RNA from Fetal Rat Lung Fibroblasts—Confluent fetal rat lung fibroblast cultures were incubated in serum-free minimal essential medium in the presence or absence of dexamethasone (5 × 10^-7 M) for 7 h or 22 h. RNA was then prepared from the fibroblasts in the presence of 4 mM guanidium thiocyanate (18). The DNA was removed by passing the lysate through a 22-gauge needle, and the resulting material was centrifuged over a cushion of 5.7 M cesium chloride (19). The resulting total RNA was then passed through an oligo(dT) column (20) and the poly(A) RNA was size-fractionated on a 1.4% methyl mercury-agarose gel (21). The resulting gel was cut into 1-mm slices, and neighboring triplicates of gel slices were pooled and transferred into 1.5-ml microcentrifuge tubes for extraction of RNA (22).
Translation of RNA Fractions and Effect of the Translation Products on Saturated Phosphatidylcholine Synthesis by Fetal Rat Lung Type II Cells—The various mRNA fractions from the methyl mercury gel were translated in an mRNA-dependent cell-free reticulocyte lysate system (23). The bioactivity of the translation products from the various fractions was examined by incubating them for 24 h with fetal rat lung alveolar type II cells and studying their effects on choline incorporation into saturated phosphatidylcholine, as described above. Each assay well involved incubation of the cells with translation products resulting from the size fractionation of 3.75 μg of oligo(dT)-selected RNA. This represents product from the equivalent of approximately 10^7 cells, as compared to fibroblast-conditioned medium, representing fibroblast-pneumocyte factor produced by about 10^6 cells.

RESULTS AND DISCUSSION

Fig. 1 demonstrates that conditioned media from fibroblasts exposed to cortisol have a significant stimulatory effect on saturated phosphatidylcholine formation by alveolar type II cells. In previous studies (5–12), it has been shown that this effect is mediated by fibroblast-pneumocyte factor. It can also be seen from Fig. 1 that, compared to control media, no effect on saturated phosphatidylcholine synthesis was noted when the type II cells were incubated with conditioned media from fibroblasts incubated in the presence of actinomycin D or cycloheximide alone. The stimulatory effect of cortisol-conditioned medium on saturated phosphatidylcholine synthesis by type II cells was, however, completely abolished by actinomycin D if the drug was present during the first 24 h of the 48-h incubation period of the fibroblasts with cortisol. No inhibition was observed if the actinomycin D was present only during the second 24-h period of incubation. Conversely, cycloheximide blocks the stimulatory effect of cortisol-conditioned medium on saturated phosphatidylcholine synthesis by type II cells whether it is present during the first or second 24 h of the 48-h incubation period of cortisol with the fibroblasts.

In 1973, Farrell and Zachman (24) studied the effects of glucocorticoid administration on the maturation of the rabbit fetal lung in vivo. They observed enhanced synthesis of phosphatidylcholine by fetal lung fibroblasts showed no activity in the assay used (data not shown), indicating that the mRNA species of interest is likely to be present in very low abundance, as is the putative translation product, fibroblast-pneumocyte factor (26).

These authors to conclude that the steroid effect was exerted at a translational level. More recently, Gross et al. (25), studying organ cultures of rat fetal lungs, observed inhibition of dexamethasone stimulation of saturated phosphatidylcholine accumulation with both cycloheximide and actinomycin D. They concluded that both RNA and protein synthesis are required for the glucocorticoid effect (25). Our observations confirm and extend those of Gross et al. (25). These results suggest that cortisol-induced fibroblast-pneumocyte factor production by fetal lung fibroblasts involves de novo synthesis of fibroblast-pneumocyte factor mRNA with subsequent synthesis of fibroblast-pneumocyte factor protein.

Translation products from total or unfraccionated poly(A) RNA from control or dexamethasone-treated fetal lung fibroblasts showed no activity in the assay used (data not shown), indicating that the mRNA species of interest is likely to be present in very low abundance, as is the putative translation product, fibroblast-pneumocyte factor (26).

**Table I**

<table>
<thead>
<tr>
<th>Addition</th>
<th>n</th>
<th>SPC formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>100.0 ± 4.0</td>
</tr>
<tr>
<td>F + Actinomycin (0–24 hr)</td>
<td>6</td>
<td>158.3 ± 3.4^a</td>
</tr>
<tr>
<td>F + Actinomycin (24–48 hr)</td>
<td>11</td>
<td>130.0 ± 6.9^p</td>
</tr>
<tr>
<td>F + Cycloheximide (0–24 hr)</td>
<td>23</td>
<td>96.6 ± 4.3 (range: 54.7–121.8)</td>
</tr>
<tr>
<td>F + Cycloheximide (24–48 hr)</td>
<td>11</td>
<td>104.6 ± 7.2</td>
</tr>
<tr>
<td>Other fractions from dex-treated fibroblasts</td>
<td>23</td>
<td>96.1 ± 5.6 (range: 71.8–114.4)</td>
</tr>
</tbody>
</table>

^a As derived from conditioned medium from fibroblasts exposed to cortisol, as in Fig. 1.

^p < 0.001 versus control.

^p < 0.005 versus control.
In vitro translation products produced in mRNA-dependent reticulocyte lysate systems are essentially devoid of post-translational modifications. That the products under study here demonstrate biological activity indicates that any such modifications which might occur in the intact cells are not qualitatively necessary for this biological activity.

In summary, these observations indicate that glucocorticoid regulation of proteins required for stimulation of fetal alveolar type II cell surfactant synthesis occurs in the fetal lung fibroblast and at a pretranslational level.

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REFERENCES