Dexamethasone Decreases the Amounts of Type I Procollagen mRNAs in Vivo and in Fibroblast Cell Cultures*

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Dexamethasone treatment of neonatal chicks resulted in a time- and dose-dependent selective decrease of skin collagen synthesis. Total RNA of chick skin that was isolated and hybridized to the cloned cDNAs pCg54 for pro-α1(I) mRNA and pCg45 for pro-α2(I) mRNA, RNA isolated from the skin of chicks receiving various doses of dexamethasone had dose-related decreases of pro-α1(I) and pro-α2(I) mRNAs. The decrease of type I procollagen mRNAs for various doses of dexamethasone were similar to the decreases observed for collagen synthesis in vivo.

Dexamethasone treatment of chick skin and chick lung fibroblasts resulted in a selective decrease of procollagen synthesis. A dose-related decrease of procollagen synthesis was observed with chick skin fibroblasts. Dexamethasone-treated chick skin and chick lung fibroblasts had decreased levels of pro-α1(I) and pro-α2(I) mRNAs as determined by solid support hybridization with pCg54 and pCg45. The dexamethasone-mediated decreases of type I procollagen mRNAs in skin fibroblasts and lung fibroblasts were similar to the decreases observed in procollagen synthesis.

Glucocorticoids have an anti-anabolic effect on collagen metabolism and not a catabolic one since urinary hydroxyproline is decreased after steroid treatment (1). In isotopic labeling studies of steroid-treated animals, collagen synthesis is decreased to the same extent as noncollagen protein synthesis in connective tissues (2-4). However, recently glucocorticoids have been shown to selectively decrease collagen synthesis in tissues (5-7) and in fibroblast cell cultures (8-15). Although procollagen synthesis is selectively decreased when compared to noncollagen protein synthesis, both type I and type III procollagen synthesis are decreased to the same extent in skin (16).

Glucocorticoids regulate RNA synthesis in palates (17) as a result of changes in chromatin template activity (18). RNA synthesis is also regulated by glucocorticoids (19) in fibroblasts. Decreased RNA synthesis was also observed in nuclei isolated from glucocorticoid-treated fibroblasts (20). In addition, Peck et al. (21) demonstrated decreased RNA content in glucocorticoid-treated bone cells.

Several studies suggest that glucocorticoids regulate procollagen synthesis by controlling procollagen gene expression. Polysomes isolated from the dermis of glucocorticoid-treated neonatal rats incorporate less proline into procollagen as compared to noncollagen protein (22). In another study, polysomal poly(A) mRNA was isolated and was used as template for protein synthesis in the nuclelease-treated reticulocyte lysate system (23). Noncollagen protein synthesis was decreased to a lesser extent than procollagen synthesis while prolyl hydroxylase synthesis was not affected by glucocorticoid treatment. In an independent study, cortisol was shown to selectively decrease total translatabl type I procollagen mRNA species in chick embryonic calvaria (24). These latter findings strongly suggest that glucocorticoids are selective effectors of procollagen synthesis.

The present study was undertaken to determine the effect of glucocorticoid treatment in vivo and in primary fibroblast cell culture on the amounts of type I procollagen mRNAs by the use of cloned recombinant cDNA probes. Our results demonstrate that in chick skin in vivo and chick lung and skin fibroblasts, glucocorticoids decrease the total cellular type I procollagen α1(I) mRNA and procollagen α2(I) mRNA.

EXPERIMENTAL PROCEDURES

Data on the kinetics of hybridization with varying amounts of total chick skin RNA are in the Miniprint following this paper. The Miniprint also contains the response of cell layer collagen and noncollagen protein synthesis of chick skin fibroblasts to various doses of dexamethasone.

Collagen and Noncollagen Synthesis in Chick Skin—Intra-peritoneal injection of the synthetic glucocorticoid dexamethasone caused a dose-related decrease of both collagen and noncollagen synthesis in the skin of neonatal chicks (data not shown). Collagen synthesis was selectively decreased as compared to noncollagen protein synthesis at all doses of dexamethasone. The maximum decrease of collagen synthesis observed was 78% at a dose of 1.6 mg/kg. A temporal response of collagen and noncollagen protein synthesis to dexamethasone was also observed (data not shown). Maximum inhibition of collagen synthesis, 72%, was observed 18 h after a single injection of dexamethasone.

RNA Content of Chick Skin—The RNA isolated from the skins of chicks receiving various doses of dexamethasone for 18 h was bound to nitrocellulose and hybridized to nick translated pCg54 and pCg45. Multiple hybridization experi-

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ments were done on different total RNA preparations (Table I). Different doses of dexamethasone resulted in decreased concentrations of pro-α(1) and pro-α(2) mRNAs. The percentage decreases of both the α(I) and α(II) procollagen mRNAs in Table I were similar within experimental error to the steroid-mediated decreases of skin collagen synthesis described above. At a dose of 1.6 μg/kg, collagen synthesis was decreased by 78% while pro-α(1) mRNA was decreased by 68% and pro-α(2) mRNA was decreased by 70% (Table I).

**Fibroblast Collagen and Noncollagen Synthesis**—Primary fibroblast cultures of chick lung and skin were treated with 2.5 × 10⁻⁶ M dexamethasone for 24 h. Dexamethasone treatment resulted in a selective decrease of collagen synthesis in both cell types (Table II).

**RNA Content of Fibroblast Cultures**—Chick lung and skin fibroblast cultures were treated with dexamethasone at 2.5 × 10⁻⁶ M for 24 h. The total chick skin and chick lung cellular RNA was isolated as described under “Experimental Procedures” and bound to nitrocellulose in equal amounts for control and dexamethasone-treated fibroblasts. The nitrocellulose fixed RNA was then hybridized to nick-translated plasmids containing either pro-α(1) or pro-α(2) cDNAs. The amounts of either probe hybridized per μg of total RNA bound to nitrocellulose was significantly decreased for the dexamethasone-treated cell cultures (Table III). Higher amounts of pro-α(1) and pro-α(2) mRNA were observed in chick skin and lung fibroblasts as compared to total RNA of skin. The

**Table I**

Relative amounts of pro-α(1) and pro-α(2) chick skin mRNAs in response to dexamethasone treatment

<table>
<thead>
<tr>
<th>Dexamethasone dose</th>
<th>Pro-α(1) mRNA</th>
<th>Pro-α(2) mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td>cpm hybridized/μg RNA</td>
<td>cpm hybridized/μg RNA</td>
</tr>
<tr>
<td>0</td>
<td>438 ± 16.7</td>
<td>408 ± 28.0</td>
</tr>
<tr>
<td>0.4</td>
<td>217 ± 29.7*  (50)</td>
<td>186 ± 17.2* (54)</td>
</tr>
<tr>
<td>0.8</td>
<td>167 ± 24.3* (62)</td>
<td>162 ± 18.8* (60)</td>
</tr>
<tr>
<td>1.6</td>
<td>182 ± 19.0* (64)</td>
<td>150 ± 16.0* (63)</td>
</tr>
<tr>
<td>2.4</td>
<td>138 ± 10.7* (68)</td>
<td>121 ± 10.3* (70)</td>
</tr>
</tbody>
</table>

* Significantly different from control at p ≤ 0.05.

**Table II**

The response of cell layer procollagen synthesis in chick lung and chick skin fibroblasts to glucocorticoid treatment

The fibroblasts were grown to late log phase. The media was replaced with serum-free media plus 10⁻⁴ M ascorbate and 2 × 10⁻⁵ M dexamethasone. After 6 h, fetal calf serum was added to the medium. The cells were incubated for another 16 h at which time ascorbate (10⁻⁴ M) and 10 μCi/ml [5-³H]proline were added to the medium and the cultures incubated for more h. Cellular collagen synthesis was determined by the collagenase assay as described in the text. The numbers in parentheses indicate the per cent decrease from control values.

**Table III**

Dexamethasone-mediated decrease of type I procollagen mRNAs in chick skin and lung fibroblasts

Chick skin and lung fibroblasts received 2.5 × 10⁻⁴ M dexamethasone at late log phase of growth. After 24 h, the total RNA was isolated as described in the text. This RNA was bound to nitrocellulose paper and hybridized with the appropriate nick-translated cDNA cloned probes (pCG54 or pCG45). The hybridized dots were excised and counted. The values represent the mean ± S.E. of values from at least three separate experiments. The numbers in parentheses indicate the per cent decrease from control value.

**Discussion**

Glucocorticoids selectively decrease procollagen synthesis in *vivo* (5–7) and in fibroblast cell culture (8–14). This selective effect does not result from increased degradation of collagen. The decrease in the synthesis of nascent chains is equal to the per cent decrease of total tissue collagen synthesis in skin of glucocorticoid-treated rats (7). Furthermore, Koob et al. (8) demonstrated that dexamethasone prevents the appearance of collagenase in the media and inhibits collagen degradation in vivo.

The mechanism of the inhibitory effect of glucocorticoids on procollagen synthesis is not clearly understood. Only recently have studies been done which show that the in *vivo* administration of glucocorticoids alters the amount of translationally active procollagen messenger ribonucleic acid in rat skin and lung (23) and in chick embryo calvaria (24). We now have extended these observations by determining the total cellular content of type I procollagen mRNAs by hybridization analysis using pBR322 plasmids containing either pro-α(1) or pro-α(2) cDNA inserts. Our results suggest that the observed decline in procollagen synthesis in cells or tissues treated with glucocorticoids may be a reflection of the decrease in the total cellular content of procollagen specific mRNA sequences and not just the functional activity of those mRNAs. As seen by the data, the per cent decrease in procollagen pro-α(1) and pro-α(2) mRNAs closely resembles that in collagen protein synthesis as determined by the collagenase assay, both in *vivo* and in fibroblast cell culture. Glucocorticoids may regulate procollagen production primarily at the level of the mRNA. While these results cannot rule out effects on mRNA processing or degradation, they are consistent with a hypothesis that glucocorticoids decrease type I procollagen production by decreasing procollagen gene expression.
Acknowledgments—We thank Dr. Helga Boedtker and her colleagues for E. coli DH1 and the pCg45 pro-α2(I) and pCg54 pro-α1(I) cloned cDNA.

REFERENCES
Dexamethasone and Type I Procollagen mRNAs

EXPERIMENTAL PROCEDURES

Materials. Fertilized white leghorn eggs were obtained from Strain, N.C. and were incubated at 37°C. For injection, eggs were prepared for 8 to 12 days incubation by removing the air sac at 14 to 16 days incubation. The media components are also described in this reference. (6, 7).

Preparation of Cell Cultures. Two-day-old embryonic chicken fibroblasts were prepared for injection into embryos by trypsinization (0.1% trypsin) at 37°C. The media components are also described in this reference. (6, 7).

Preparation of Fibroblasts. A single 195 mm culture dish was used for each time point. The dishes were prepared for fibroblast injection by incubation at 37°C for 2 to 3 days. For injection, the dishes were incubated at 37°C and 5% CO2, and the cells were then washed with saline and injected into the embryos. The media components are also described in this reference. (6, 7).

Preparation of Collagenase. Collagenase was prepared for injection by incubation at 37°C for 2 to 3 days. For injection, the dishes were incubated at 37°C and 5% CO2, and the cells were then washed with saline and injected into the embryos. The media components are also described in this reference. (6, 7).

Sequential isolation of total RNA. Total RNA was isolated from cells by trypsinization (0.1% trypsin) at 37°C. The media components are also described in this reference. (6, 7).

Preparation of Dexamethasone. Dexamethasone was prepared for injection by incubation at 37°C for 2 to 3 days. For injection, the dishes were incubated at 37°C and 5% CO2, and the cells were then washed with saline and injected into the embryos. The media components are also described in this reference. (6, 7).

RESULTS

Figure 1. Dexamethasone and Type I Procollagen mRNAs

Figure 2. The response of collagen and noncollagen protein synthesis in chicken fibroblasts

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The decreases observed for collagen and noncollagen protein synthesis in chicken fibroblasts were dose related. Maximum inhibition of collagen synthesis was observed at 3.7 x 10^-6 M dexamethasone.
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