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Glucocorticoids Decrease the Synthesis of Type I Procollagen mRNAs†

Debra Cockayne, Kenneth M. Sterling, Jr.,‡ Susan Shull, Keith P. Mintz, Sharon Illeyne, and Kenneth R. Cutroneo*

Department of Biochemistry, College of Medicine, University of Vermont, Burlington, Vermont 05405

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ABSTRACT: Glucocorticoids selectively decrease procollagen synthesis in animal and human skin fibroblasts. β-Actin content and β-actin mRNA are not affected by glucocorticoid treatment of chick skin fibroblasts. The inhibitory effect of glucocorticoids on procollagen synthesis is associated with a decrease in total cellular type I procollagen mRNAs in chick skin fibroblasts. These effects of dexamethasone are receptor mediated as determined by pretreatment with the glucocorticoid antagonists progesterone and RU-486 and with the agonist β-dihydrocortisol. Dexamethasone has a small but significant inhibitory effect on cell growth of chick skin fibroblasts. The ability of this corticosteroid to decrease the steady-state levels of type I procollagen mRNAs in nuclei, cytoplasm, and polysomes varies. The largest decrease of type I procollagen mRNAs is observed in the nuclear and cytoplasmic subcellular fractions 24 h after dexamethasone treatment. Type I procollagen hnRNAs are also decreased as determined by Northern blot analysis of total nuclear RNA. The synthesis of total cellular type I procollagen mRNAs is reversibly decreased by dexamethasone treatment. In addition the synthesis of total nuclear type I procollagen mRNA sequences is decreased at 2, 4, and 24 h following the addition of radioactive nucleoside and dexamethasone to cell cultures. Although the synthesis of pro α1(I) and pro α2(I) mRNAs is decreased in dexmethasone-treated chick skin fibroblasts, the degradation of the total cellular procollagen mRNAs is not altered while the degradation of total cellular RNA is stabilized. These data indicate that the dexamethasone-mediated decrease of procollagen synthesis in embryonic chick skin fibroblasts results from the regulation of procollagen gene expression.

Collagen metabolism is markedly altered by glucocorticoids (Cutroneo et al., 1986). Both natural and synthetic glucocorticoids decrease collagen synthesis in various connective tissues including skin (Smith & Allison, 1965; Uitto et al., 1972; Newman & Cutroneo, 1978; Robey, 1979), granuloma (Nakagawa et al., 1971; Wehr et al., 1976; Kruse et al., 1978), and bone (Uitto & Mustakallio, 1971; Rokowski et al., 1981; Oikarinen & Ryhanyen, 1981; Canalis, 1983).

Topical application of high doses of synthetic glucocorticoids to skin results in skin atrophy. Glucocorticoid treatment decreases collagen content of skin which in turn results in a

† Supported by NIH Grants AM 19808 and HL 14212.
* Author to whom correspondence and reprint requests should be addressed.
‡ Present address: Departments of Pediatrics and Biochemistry, Mount Sinai School of Medicine, New York, NY 10029.

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marked decrease in skin thickness and an increased susceptibility to rupturing forces (Oxlund et al., 1982). These antianabolic effects of glucocorticoids on skin collagen metabolism result from a selective decrease of collagen synthesis (Newman & Cutroneo, 1978). Glucocorticoids coordinately decrease skin type I and type III procollagen synthesis (Shull & Cutroneo, 1983) while fibronectin synthesis is increased (Cutroneo et al., 1986). The inhibitory effect of glucocorticoids on skin collagen synthesis does not result from a steroid-induced change in the specific activity of the precursor prolyl-tRNA pool. Skin polysomes (McNelis & Cutroneo, 1978) and polysomal poly(A) mRNA (Rokowski et al., 1981) isolated from glucocorticoid-treated rats and translated in an in vitro protein synthesis lysate system synthesize less collagen.

Glucocorticoid treatment of normal (Ponec et al., 1977; McCoy et al., 1980; Russell et al., 1981) and keloid-derived human skin fibroblasts (McCoy et al., 1980) and embryonic chick skin fibroblasts (Sterling et al., 1983a) causes a selective decrease of procollagen synthesis. In embryonic chick skin fibroblasts glucocorticoids coordinately decrease procollagen synthesis and the total cellular concentrations of type I procollagen mRNAs (Sterling et al., 1983a). Glucocorticoids also block the bleomycin-induced increase of procollagen synthesis and polysomal type I procollagen mRNAs in embryonic chick skin fibroblasts (Sterling et al., 1983b). It has also been shown that hydrocortisone decreases translatable type I procollagen mRNAs in human skin fibroblasts (Oikarinen et al., 1983). We now report that dexamethasone decreases the synthesis of total cellular as well as nuclear type I procollagen mRNA sequences.

Experimental Procedures

Egg Maintenance. Fertilized white leghorn chicken eggs were obtained from Oliver Merrill and Sons, Londonderry, NH, and were maintained as previously described (Sterling et al., 1983a). Primary chick skin fibroblasts were prepared from 12–13-day-old embryos as described (Hunter, 1979). Cells were cultured and maintained for up to 5 days with the daily addition of 2×10^{-4} M ascorbate.

Cellular Collagen Synthesis and β-Actin Content. Late log phase embryonic chick skin fibroblasts were incubated in the presence or absence of 2.5×10^{-5} M dexamethasone for 24 h. [5-^{3}H]Proline (10 μCi/mL of medium) was added to all cell cultures 2 h prior to collection. In addition the cell culture medium was supplemented with 2×10^{-4} M ascorbate at the time of labeling. The cells were harvested and washed with phosphate-buffered saline (Ca^{2+} and Mg^{2+} free). Cellular homogenates were prepared and assayed for cell layer collagen synthesis and noncollagen protein synthesis by the collagenase digestion assay as described (Newman & Cutroneo, 1978).

Fibroblast cultures were treated with dexamethasone and assayed for total cellular protein (Lowry et al., 1951) and β-actin content. β-actin content was determined by disrupting the cell pellet in 0.05 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 6.8), 1% (w/v) sodium dodecyl sulfate, and 0.1% (v/v) β-mercaptoethanol by using a glass–teflon homogenizer. The cell homogenate was then incubated at 37 °C for 2 h and centrifuged for 1 min in a microcentrifuge (12800*g*). The supernatant was subjected to electrophoresis using a 1% (w/v) sodium dodecyl sulfate–7.5% separating gel as described by Laemmli and Favre (1973). Following electrophoresis the proteins were electroeluted onto nitrocellulose paper and immunostained as described by Towbin et al. (1979) except that the substrate-staining solution was 1.4×10^{-3} M diaminobenzidine in 0.05 M Tris-HCl (pH 7.5) containing 5 μL of 30% (w/v) H_2O_2. A mouse monoclonal antibody, obtained from Dr. James Lessard, Children's Hospital Medical Center, with specificity for chicken gizzard β-actin was used to detect β-actin on the nitrocellulose papers. Goat anti-mouse immunoglobulin G (heavy and light chain specific) conjugated to horseradish peroxidase (Calbiochem) was used to detect primary antibody binding. After being stained the immunoblots were scanned by using a Shimadzu dual wavelength thin-layer chromatoscanner (Model CS-930) and the area under the peaks integrated and normalized to protein content.

Isolation of Total Cellular RNA. Total cellular RNA was isolated from embryonic chick skin fibroblasts by a modification of the method of Meyers et al. (1981) as previously described (Sterling et al., 1983a).

Subcellular Fractionation and Isolation of RNA. Subcellular fractionation was done by a modification of the methods of Favaloro et al. (1980) and Maniatis et al. (1982) described by Sterling et al. (1983b). Nuclei were treated with citric acid, pelleted through a 0.88 M sucrose cushion (Busch, 1967), and nuclear RNA was isolated as described by Roop et al. (1978) except that the RNA obtained by this method was not passed through a Sephadex G-50 column. To isolate polysomes, the postnuclear cytoplasm was placed over a 2.0 M sucrose cushion and centrifuged at 136000*g* for 4.5 h at 4 °C. The resulting postpolysomal cytoplasmic supernatant was removed, and the polysomal pellet was suspended in an equivalent volume of 0.14 M NaCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.6), 0.5% (v/v) Nonidet P-40, and 1 mM aurintricarboxylic acid. An equal volume of 2 × proteinase K (Beckman) buffer [0.2 M Tris-HCl (pH 7.5), 25 mM ethylenediaminetetraacetic acid (EDTA), 0.3 M NaCl, and 2% (w/v) sodium dodecyl sulfate] was added to both suspensions. Proteinase K was added at 200 μg/mL and the mixture incubated at 37 °C for 30 min. The proteinase K treated mixtures were extracted with an equal volume of phenol–chloroform–isoamyl alcohol (25:25:1), the aqueous phases removed, and the nonaqueous PCI phases reextracted with 0.5 volume of 2 × proteinase K buffer. The aqueous phases from each subcellular fraction were pooled, adjusted to 0.2 M sodium acetate (pH 5.0), and precipitated with 2 volumes of ethanol. The resulting RNA was lyophilized and stored at −80 °C. RNA isolated by this procedure had A_{260}/A_{280} ratios of ~2.0.

Isolation of hnRNA. Embryonic chick skin fibroblasts were collected in phosphate-buffered saline (Ca^{2+} and Mg^{2+} free), and nuclei were isolated by the citric acid method of Busch (1967) as modified by Roop et al. (1978). Nuclear RNA was isolated as described above. The nuclear RNA (30 μg) was chromatographed on an 0.8% agarose–2.2 M formaldehyde denaturing gel as described (Maniatis et al., 1982). The RNAs were subjected to Northern blot analysis (Alwine et al., 1977) as described in detail by Thomas et al., (1983).

Recombinant DNA. The recombinant plasmids containing type I procollagen cDNA inserts in pBR322 were obtained from Dr. Helga Boedtker, Department of Biochemistry and Molecular Biology, Harvard University, Boston, MA. These recombinant plasmids, pCg54 for pro α1(I) mRNA and pCg45 for pro α2(I) mRNA have been thoroughly characterized (Lehrach et al., 1978, 1979). DH1 *Escherichia coli* cells were transformed (Hanahan, 1983) and amplified, and the plasmids were isolated (Maniatis et al, 1982). Determination of host DNA contamination and the identification of recombinant DNA plasmids were carried out as described previously (Sterling et al., 1983a). All recombinant DNA techniques were done under P1 containment. The recombinant cDNA containing plasmids were used for hybridization to embryonic

chick skin fibroblast mRNA preparations. The plasmids containing procollagen cDNAs were nick-translated by the method of Rigby et al. (1977). The nick-translated cDNA containing plasmids were isolated by the spun minicolumn procedure (Maniatis et al., 1982). The specific activity of all recombinant probes used was greater than or equal to 1×10^8 cpm/μg.

The plasmid pA1 contains a full-length 2000 base pair (bp) chicken β-actin cDNA inserted at the *Pst*I site of pBR322 (Cleveland et al., 1980). This recombinant cDNA probe was obtained from Dr. Don W. Cleveland, Department of Biochemistry and Biophysics, University of California, San Francisco, CA. *Escherichia coli* 1776 cells containing pA1 were amplified, and the plasmids were isolated (Maniatis et al., 1982). The plasmid pA1 was identified by restriction digestion with *Pst*I and subsequent agarose gel electrophoresis (Cleveland et al., 1980).

Dot Blot. The dot-blot hybridization assay of Thomas (1980) was used. Steady-state mRNA levels were determined by hybridization analysis using ^{32}P-nick-translated recombinant plasmids. Radioactive [5,6-^{3}H]uridine- (ICN, Irvine, CA) labeled RNA was quantified by spotting unlabeled recombinant plasmids onto nitrocellulose. The amount of specific radioactive mRNA sequences was determined by hybridization analysis as described by Lyons and Schwarz (1984).

Quick Blot. Embryonic chick skin fibroblasts [(4–6) $\times 10^6$ cells] were collected in phosphate-buffered saline (Ca^{2+} and Mg^{2+} free) and suspended in 2 mL of 1.5 mM $MgCl_2$, 1 mM aurintricarboxylic acid, and 50 μg/mL cycloheximide. This sample was prepared and spotted onto nitrocellulose filters for hybridization as described by Bresser et al. (1983). Two hundred microliter samples of serial dilutions of the cellular homogenate were spotted. Hybridization with ^{32}P-nick-translated recombinant plasmids was carried out by a modification (Sterling et al., 1983a) of the method of Thomas (1980).

Specific Activities of the [5,6-^{3}H]UTP and [5,6-^{3}H]CTP Pools. Primary embryonic chick skin fibroblasts were incubated with [5,6-^{3}H]uridine for various times. The cells were collected, washed once with phosphate-buffered saline (Ca^{2+} and Mg^{2+} free), and suspended in H_2O. The cells were homogenized by using a Polytron ST system, precipitated with 60% (v/v) methanol (Donofrio et al., 1978) overnight at −20 °C, and centrifuged at 10000*g* for 30 min. The supernatant fraction was evaported to dryness, and the residue was taken up in H_2O. The sample was made 1 N HCl, hydrolyzed at 100 °C for 7 min, and neutralized with KOH. The sample was then applied to a 0.6 × 13 cm column of AGl-X8 (200–400 mesh) Dowex-formate (Bio-Rad, Richmond, CA) as described by Busch et al. (1952). The column was washed extensively with H_2O until there was no radioactivity in the effluent. The nucleotides were eluted by using a linear gradient from 0 to 4 N formic acid. The column was calibrated by hydrolyzing unlabeled uridine 5′-triphosphate and cytidine 5′-triphosphate (Sigma, St. Louis, MO) and monitoring the column at 260 nm. The A_{260}-containing fractions were then subjected to paper chromatography as described by Markham and Smith (1951). The solvent system used was 7.5 M butanol and 0.8 N HCl in H_2O. The positions of the pyrimidine monophosphate nucleotides were determined by using uridine 5′-monophosphate and cytidine 5′-monophosphate (Sigma, St. Louis, MO).

RESULTS

Glucocorticoid Regulation of Fibroblast Growth and Procollagen Synthesis. Glucocorticoids have been shown to markedly decrease DNA synthesis in connective tissues in vivo and in fibroblast cell cultures (Cutroneo et al., 1986). As seen in Figure 1 dexamethasone only slightly inhibited the growth of embryonic chick skin fibroblasts during stationary and late log phase of growth. The use of this cell type to determine the molecular effects of glucocorticoids on procollagen synthesis is therefore not totally complicated by marked inhibitory effects on cell growth. The growth inhibitory effect is not as dramatic as the selective effect of dexamethasone on procollagen synthesis during late log phase growth (Figure 2). The dexamethasone-mediated selective decrease of procollagen synthesis is correlated with a coordinate decrease of total cellular pro α1(I) and pro α2(I) mRNAs. Total cellular noncollagen protein synthesis is decreased to half the extent compared to the inhibition of procollagen synthesis. Dexamethasone treatment of embryonic chick skin fibroblasts did not decrease the total cellular content of either β-actin or β-actin mRNA. This lack of effect of glucocorticoids on β-actin was surprising since the synthesis of the total pool of noncollagen proteins in chick skin fibroblasts is slightly decreased by dexamethasone (Figure 2).

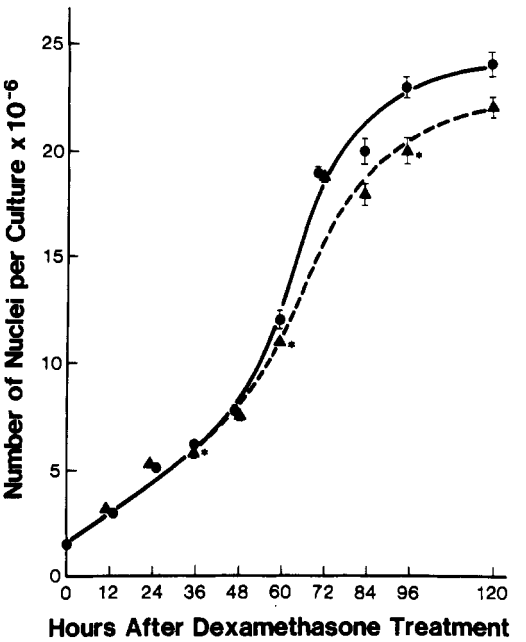

FIGURE 1: Growth curves of control and dexamethasone-treated chick skin fibroblasts. Chick skin fibroblasts were plated at 2.5×10^6 cells. At the start of the growth curve fresh medium was added, and the medium of half the cultures was supplemented with dexamethasone at 2.5×10^{-5} M. The medium of all cell cultures was supplemented with ascorbate at 2×10^{-4} M daily. At the time intervals indicated three control and three glucocorticoid-treated cultures were stained with crystal violet, and nuclei were counted by using a hemocytometer. Control (●); dexamethasone treated (▲).

Glucocorticoid Receptors and Dexamethasone-Mediated Inhibition of Procollagen Synthesis. Classically the modulation of protein synthesis by steroid hormones is mediated through a steroid-receptor complex that binds to sensitive genes and affects their rate of transcription. To determine if the glucocorticoid-mediated decreases of procollagen synthesis and total cellular type I procollagen mRNAs are receptor mediated, the glucocorticoid antagonists progesterone and RU486 (Jung-Testas & Baulieu, 1983; Bourgeois et al., 1984) and the agonist 5-β-dihydrocortisol (Weinstein et al., 1983) were used. Pretreatment of embryonic chick skin fibroblasts with either antagonist for 24 h blocked the dexamethasone-mediated decrease of procollagen synthesis. Pretreatment of cell cultures with the agonist 5-β-dihydrocortisol enhanced the inhibitory

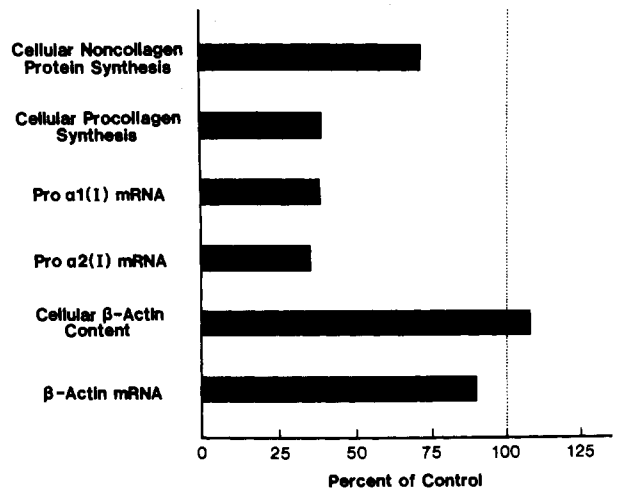

FIGURE 2: Selective effect of glucocorticoids on procollagen synthesis, total noncollagen protein synthesis, and cellular β-actin content. Embryonic chick skin fibroblasts were treated for 24 h with 2.5×10^{-5} M dexamethasone. Prior to cell harvest, some cultures were fed [5-^{3}H]proline for 2 h. Proline incorporation into cellular noncollagen and collagen proteins was determined by the collagenase digestion assay as described in the text. Cellular β-actin content was also determined as described. The steady-state levels of total cellular procollagen and β-actin mRNAs were determined by hybridization analysis as described.

Table I: Effects of a Glucocorticoid Agonist and Antagonists on the Dexamethasone-Mediated Decrease of Cellular Collagen Synthesis[a]

treatment	dpm/10^6 cells × 10^{-4} collagen	noncollagen
control	5.1 ± 0.1	10.7 ± 1.9
dexamethasone	2.5 ± 0.1[b]	10.1 ± 1.3
progesterone	4.5 ± 0.2	9.6 ± 0.5
RU-486	6.0 ± 0.3	13.1 ± 1.1
5-β-dihydrocortisol	4.3 ± 1.0	9.8 ± 2.5
progesterone + dexamethasone	4.9 ± 0.4	10.7 ± 0.6
RU-486 + dexamethasone	5.4 ± 0.2	13.7 ± 0.6
5-β-dihydrocortisol + dexamethasone	1.5 + 0.1[b]	8.9 ± 1.4

[a] Late log phase embryonic chick skin fibroblasts in fresh medium were treated for 24 h with either 10^{-6} M progesterone, RU-486, or 5-β-dihydrocortisol. The cells were then treated with 10^{-6} M dexamethasone for another 22 h. Another set of fibroblasts were treated with dexamethasone alone. All cells were labeled for 2 h with [5-^{3}H]-proline, homogenates were prepared, and proline incorporation into collagen and noncollagen protein was determined by the collagenase digestion assay as described in the text. The values represent the mean ± SE of three or four cell cultures. [b] Significantly different from control at $p \leq 0.05$.

effect of this corticosteroid on procollagen synthesis (Table I). Both the antagonists and the agonist alone did not significantly affect the synthesis of total cellular collagen and noncollagen protein synthesis. The antagonist RU486 almost totally blocked the dexamethasone-mediated decrease of total cellular pro α1(I) mRNA and completely blocked the glucocorticoid-mediated decrease of pro α2(I) mRNA (Table II). The antagonist RU486 was able to reverse the effect of dexamethasone on both pro α1(I) and pro α2(I) mRNAs. With respect to control values, RU486 treatment resulted in a 23% decrease in pro α(I) mRNA and a 16% decrease in pro α2(I) mRNA as compared to a 56% and 48% decrease, respectively, in the presence of dexamethasone alone. In the case of the pro α2(I) mRNA there was no statistically significant difference between control and RU486 values while in the case of the proα1(I) mRNA a significant though slight decrease was observed from the control value. However, none of the above treatments significantly altered the steady-state level of cellular β-actin mRNA.

Table II: Dexamethasone Effect on the Total Cellular Concentrations of Pro α1(I), Pro α2(I) mRNA in the Presence and Absence of Glucocorticoid Antagonists and an Agonist[a]

	dpm hybridized/10^6 cells		
	pro α1(I) mRNA	pro α2(I) mRNA	β-actin mRNA
control	463 ± 25	278 ± 40	1151 ± 207
dexamethasone	205 ± 24[b]	144 ± 6[b]	1018 ± 107
RU-486	486 ± 9.2	234 ± 24	1196 ± 255
5-β-dihydrocortisol	482 ± 43	265 ± 15	1615 ± 238
RU-486 + dexamethasone	355 ± 29[b]	233 ± 41	1123 ± 87
5-β-dihydrocortisol + dexamethasone	172 ± 30	117 ± 9[b]	1435 ± 30

[a] Late log phase embryonic chick skin fibroblasts were treated as described in footnote *a* of Table I. The cells were collected and subjected to hybridization analysis using the quick-blot procedure described in the text. The values represent the mean ± SE of three or four cell cultures. [b] Significantly different from control at $p \leq 0.05$.

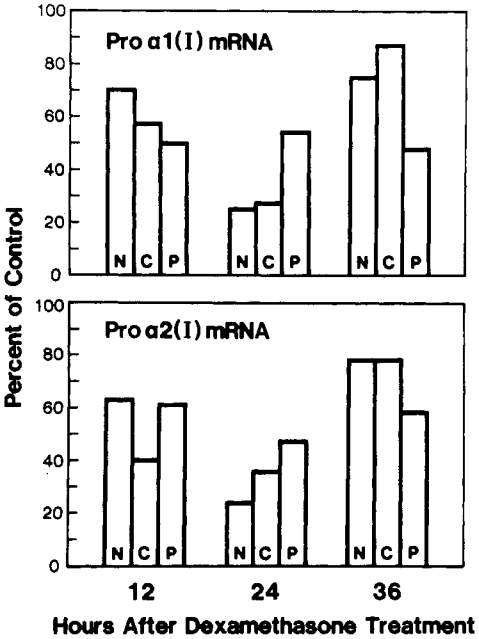

FIGURE 3: Dexamethasone-mediated decrease of pro α1(I) and pro α2(I) mRNAs in various subcellular fractions. Late log phase embryonic chick skin fibroblasts were incubated in fresh medium with or without 2.5×10^{-5} M dexamethasone for time periods indicated. Following incubation, the cells were collected and fractionated into nuclei, polysomes, and postpolysomal cytoplasm as described in the text. The RNA was isolated from each subcellular fraction and submitted to dot-blot hybridization to determine the concentrations of type I procollagen mRNAs. The values represent the type I procollagen mRNA concentrations as percent of the values for nontreated cultures. The mean of data from two samples each containing cells from 18 cultures were used to calculate percent control.

Glucocorticoid-Mediated Decrease of Type I Procollagen mRNA Sequences in Various Subcellular Fractions. Glucocorticoids decrease the total cellular content of type I procollagen mRNAs. The temporal effect of dexamethasone on the steady-state levels of type I procollagen mRNAs in nuclei, polysomes, and the post polysomal cytoplasm is shown in Figure 3. The greatest effect of this corticosteroid in decreasing type I procollagen mRNAs in all the subcellular fractions is seen 24 h after steroid treatment. After 24 h of dexmethasone treatment, pro α1(I) mRNA is decreased 75% in nuclei and 73% in the cytoplasm while the content of pro α1(I) mRNA is decreased by 47% in polysomes. The level of pro α2(I) mRNA is decreased by 76%, 65%, and 53% in the nuclei, cytoplasm, and polysomes, respectively. The maximum inhibitory effect of glucocorticoids on the steady-

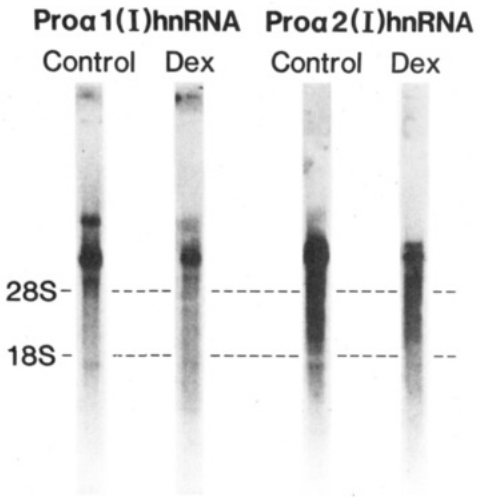

FIGURE 4: Dexamethasone-mediated decrease of pro α1(I) and pro α2(I) hnRNAs. Late log phase embryonic chick skin fibroblasts were treated with 2.5×10^{-5} M dexamethasone for 24 h. Nuclei were prepared by using the citric acid method, and nuclear RNA was isolated from 20 pooled cell cultures as described in the text. The RNA (30 μg/lane) was chromatographed on an 0.8% agarose denaturing formaldehyde gel as described in the text. The RNA species were transferred to nitrocellulose and hybridized with either ^{32}P-labeled pCg45 or pCg54 recombinant chick type I procollagen cDNA probes followed by autoradiography.

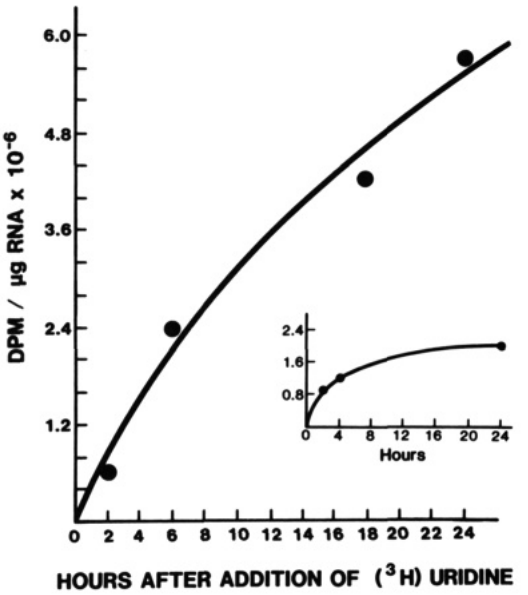

FIGURE 5: Uridine incorporation into total cellular RNA. Late log phase embryonic chick skin fibroblasts were incubated in fresh medium in the presence of [^{3}H]uridine (125 μCi/mL of medium). At the times indicated the fibroblasts from four cell cultures were collected and pooled. Total cellular RNA was isolated and the specific activity determined as described in the text. The insert represents [^{3}H]uridine incorporation into total nuclear RNA. At the times indicated the fibroblasts from 20 cell cultures were collected and pooled. Total nuclear RNA was isolated and the specific activity determined.

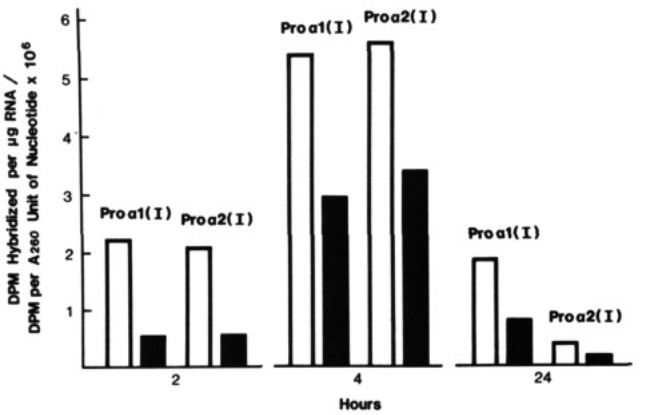

FIGURE 6: Incorporation of [^{3}H]uridine into type I procollagen nuclear RNA sequences. Chick skin fibroblasts were labeled with [^{3}H]uridine (125 μCi/mL) in the presence (■) or absence (□) of 2.5×10^{-5} M dexamethasone for various time periods. Total nuclear RNA was isolated from 20 control and 20 dexamethasone-treated cultures as described in the text. Total nuclear RNA was hybridized to unlabeled plasmid cDNA probes for pro α1(I) and pro α2(I). All hybridization data were corrected for the respective lengths of the cDNA probes and for the total precursor pool specific radioactivities of CTP and UTP as described in the text.

state levels of type I procollagen mRNAs occurred in nuclei and the cytoplasm. The cellular levels of type I procollagen mRNAs in polysomes remained relatively constant at 12, 24, and 36 h following dexamethasone treatment. Partial recovery of the steady-state levels of type I procollagen mRNAs was observed in the nuclear and cytoplasmic fractions after 36 h following glucocorticoid treatment.

Northern blot analysis was employed for determination of the dexamethasone-induced alteration of type I procollagen hnRNAs in embryonic chick skin fibroblasts. As observed in Figure 4 several type I procollagen hnRNA species of pro α1(I) and pro α2(I) α chains were resolved on denaturing agarose gels. Treatment of embryonic chick skin fibroblasts with dexamethasone significantly decreased the amount of these hnRNA species. These data reflect the decreased steady-state levels of nuclear procollagen specific RNA observed in Figure 3.

Glucocorticoid Regulation of Type I Procollagen mRNA Synthesis. To determine whether the glucocorticoid-mediated decrease of the steady-state levels of type I procollagen mRNAs in the total cell and in nuclei were reflective of alterations in the synthesis of procollagen mRNA species, embryonic chick skin fibroblasts were labeled with [^{3}H]uridine (Figure 5). Embryonic chick skin fibroblasts linearly incorporated [^{3}H]uridine into total cellular RNA. Furthermore, this linearity of incorporation of radioactive ribonucleoside into nuclear RNA was observed for 4 h. These properties allowed for synthetic and degradation experiments to be carried out with highly labeled type I procollagen mRNAs for hybridization analysis.

Embryonic chick skin fibroblasts were labeled for the latter 24 h of a 48-h treatment in the presence or absence of dexamethasone, and the total cellular radioactive type I procollagen mRNA sequences were determined. When the data were normalized on the basis of the total specific activity of the intracellular CTP and UTP pools, pro α1(I) and pro α2(I) mRNAs were reversibly decreased by 46% and 41%, respectively (data not shown). However, these data reflect the total cellular accumulation of pro α1(I) and pro α2(I) mRNAs since the cells were labeled for a relatively long period of time.

The effect of dexamethasone on pro α1(I) and pro α2(I) mRNA synthesis in embryonic chick skin fibroblasts was determined by treating and labeling the chick skin fibroblasts for 2, 4, and 24 h and quantifying nuclear pro α1(I) and pro α2(I) mRNA sequences (Figure 6). When the hybridization data was normalized on the basis of the total specific activity of the intracellular nucleotide precursor pools, the greatest inhibition of pro α1(I) and pro α2(I) mRNA synthesis was observed after 2 h of labeling with [^{3}H]uridine and simultaneously treating with dexamethasone. The synthesis of pro α1(I) and pro α2(I) mRNAs was decreased by 77% and 73%, respectively, following 2 h of labeling in the presence of the

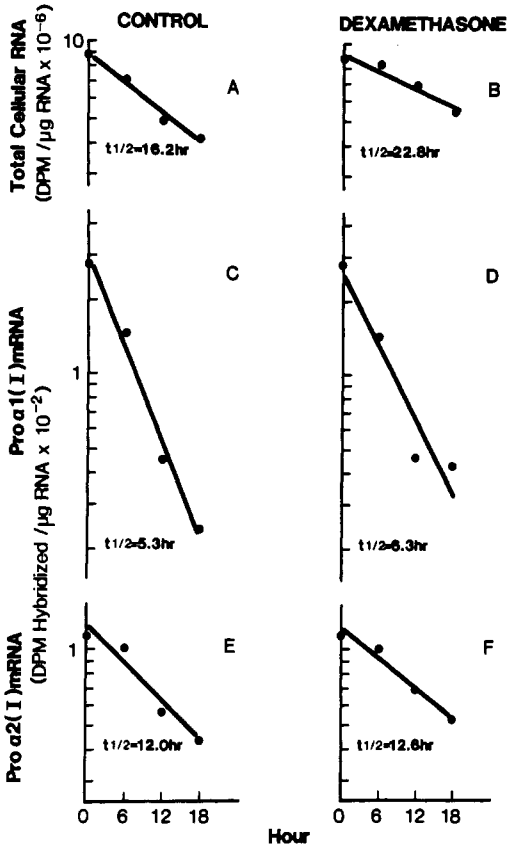

FIGURE 7: Decay of radioactive total cellular RNA and type I procollagen mRNAs. Chick skin fibroblasts were labeled with [^{3}H]uridine (125 μCi/mL) for 24 h and subsequently chased with 3 mM cytidine and 5 mM uridine either in the presence or in the absence of 2.5 × 10^{-5} M dexamethasone. At various times, total cellular RNA was isolated from 20 control and 20 dexamethasone-treated cultures as described in the text. The specific radioactivity of total cellular RNA was determined when chased in the absence (A) or presence (B) of dexamethasone. Total cellular RNA was hybridized to cold plasmid cDNA probes for pro α1(I) and pro α2(I) mRNAs, and the decay of radioactive procollagen mRNAs was determined when chased in the absence (C, E) or presence (D, F) of dexamethasone. All hybridization data were corrected for the respective lengths of the cDNA probes. Decay lines were fitted by using semilog regression analysis.

glucocorticoid. Significant inhibition of nuclear type I procollagen mRNA synthesis was also apparent at 4 and 24 h of labeling with [^{3}H]uridine.

Glucocorticoid Regulation of Total Cellular Type I Procollagen mRNA Degradation. The degradation of total cellular RNA and total cellular type I procollagen mRNAs was determined by prelabeling embryonic chick skin fibroblasts with [^{3}H]uridine and chasing the radiolabeled cultures with "cold" 3 mM cytidine and 5 mM uridine (Figure 7). When dexamethasone was added during the chase, the specific activity of the total cellular RNA decayed with a half-life of 22.8 h compared to 16.2 h for control cultures (Figure 7). This apparent increase in the stability of total cellular RNA is more pronounced if the data are normalized on the basis of the specific activity of the CTP and UTP nucleotide precursor pools at 6 and 12 h into the chase period (Figure 8). The specific activity of the total nucleotide precursor pools of glucocorticoid-treated cultures during the chase period was decreased by 20% at 6 h and 30% at 12 h (Figure 8). Following 24 h of labeling with [^{3}H]uridine the specific radioactivity of the combined UTP and CTP precursor pools was 1.1 × 10^8 dpm/A_{260} unit of nucleotide × 10^{-8}. At 6 and 12 h after the chase the precursor pool specific radioactivity was 7.1 × 10^7 and 4.2 × 10^7, respectively, in the presence of

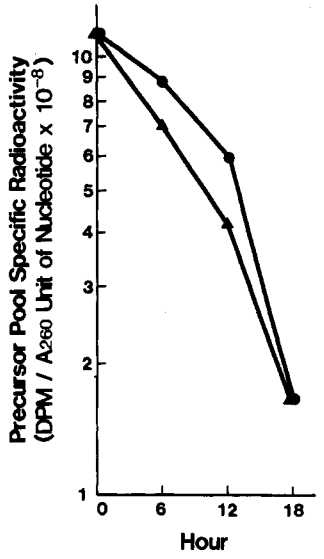

FIGURE 8: Decay of the specific radioactivity of the intracellular 5′-ribonucleotide precursor pools. Chick skin fibroblasts were labeled with [^{3}H]uridine (125 μCi/mL) for 24 h and subsequently chased with 3 mM cytidine and 5 mM uridine either in the presence (▲) or absence (●) of 2.5 × 10^{-5} M dexamethasone. At various times thereafter CTP and UTP precursor pools were isolated, and the total specific activities of these precursor pools were determined as described in the text.

dexamethasone as compared to 8.7 × 10^7 and 5.9 × 10^7 for control cells.

The degradation of the pro α1(I) and pro α2(I) mRNAs was determined by hybridization analysis of total cellular radioactive RNA isolated after chasing for various times in the presence or absence of dexamethasone. The presence of this corticosteroid in the chase media did not significantly alter the rate of degradation of either the pro α1(I) or the pro α2(I) mRNAs. The half-lifes of pro α1(I) mRNA in control and dexamethasone-treated cultures were 5.3 and 6.3 h, respectively. The half-lifes of pro α2(I) mRNA were 12.0 and 12.6 h, respectively. At the zero time point of degradation the pro α1(I) and pro α2(I) mRNAs of type I procollagen were in a ratio of 2.4. Interestingly, at all times during the chase period pro α2(I) mRNAs were more resistant to degradation than pro α1(I) mRNAs in either the presence or absence of 2.5 × 10^{-5} M dexamethasone.

Discussion

The extracellular matrix of skin is a defined structural entity which acts as a scaffold to which cells attach. This connective tissue network in turn influences the biochemical functions of associated cells. Since collagen is the major protein constituent of the extracellular matrix, hormones such as glucocorticoids which alter collagen metabolism can cause significant changes in the composition of the extracellular matrix. The present data provide a molecular basis for the selective inhibitory effect of glucocorticoids on procollagen synthesis as compared to cellular noncollagen protein synthesis.

Glucocorticoids decrease collagen deposition in skin. The effect of glucocorticoids on collagen metabolism in skin and in skin fibroblasts may be attributed to a selective decrease in procollagen synthesis which is associated with coordinate decreases of total cellular type I procollagen mRNAs. Glucocorticoids do not decrease β-actin content or β-actin mRNA.

The exact mechanism by which glucocorticoids decrease the steady-state levels of type I procollagen mRNAs has yet to be fully defined. Glucocorticoids have been shown to increase some proteins and decrease others in certain tissues (Cutroneo

et al., 1981). β-Actin content and β-actin mRNA are not affected by dexamethasone in the present study. Cellular noncollagen protein synthesis is either not significantly decreased or slightly decreased as compared to procollagen synthesis.

The steady-state amounts of functional messenger RNA species can be regulated at multiple levels in eucaryotic cells. Transcriptional, posttranscriptional, and translational levels of regulation have been suggested for several biological systems (Darnell, 1982). The present study was undertaken to determine possible regulatory mechanism(s) by which glucocorticoids mediate a selective decrease in total cellular type I procollagen mRNAs. Our studies indicate that the glucocorticoid-mediated decrease of procollagen synthesis and type I procollagen mRNAs in embryonic chick skin fibroblasts is a receptor-mediated process. Through the use of the glucocorticoid antagonists progesterone and RU486 we have demonstrated a reversal of the dexamethasone-mediated decrease in procollagen synthesis. Hybridization analysis of total cellular mRNA demonstrate that RU486 is able to block or partially block the dexamethasone-mediated decrease in the steady-state levels of type I procollagen mRNAs.

We have extended our observation that the levels of total cellular type I procollagen mRNAs are decreased by glucocorticoids in embryonic chick skin fibroblasts. At various times after dexamethasone treatment the steady-state levels of pro α1(I) and pro α2(I) mRNAs in the nucleus, polysomes, and postpolysomal cytoplasm are markedly decreased. It is significant to note that in all subcellular fractions dexamethasone resulted in a temporal decrease of pro α1(I) and pro α2(I) mRNA species. The steady-state levels of type I procollagen mRNA sequences were decreased to a greater extent in the nucleus and cytoplasm compared to polysomes. Also, pro α1(I) and pro α2(I) hnRNAs as determined by Northern analysis of nuclear RNA were decreased following glucocorticoid treatment. The decrease of type I procollagen mRNA species in the nucleus and cytoplasm appear to be reversible, while the decrease of polysomal type I procollagen mRNAs remains constant for at least 36 h following glucocorticoid treatment.

The effect of dexamethasone on pro α1(I) and pro α2(I) mRNA synthesis in embryonic chick skin fibroblasts was analyzed by pulse labeling late log phase chick skin fibroblasts with [^{3}H]uridine. Following a 2-, 4-, and 24-h pulse total nuclear pro α1(I) and pro α2(I) mRNA sequences were estimated, and the specific radioactivity of the intracellular UTP and CTP pools was determined. When 2.5×10^{-5} M dexamethasone was added, total nuclear type I procollagen mRNA sequences were significantly reduced at all time points. The greatest inhibition of type I procollagen mRNA synthesis was observed after 2 h of labeling with [^{3}H]uridine. Dexamethasone resulted in a 77% and 73% decrease in pro α1(I) and pro α2(I) mRNA sequences, respectively. Our data indicate that nuclear pro α1(I) and pro α2(I) mRNA sequences exist in equal ratios for 2 and 4 h after pulsing with [^{3}H]-uridine. The data also indicate that there is a coordinate decrease in the synthesis of both type I procollagen mRNAs.

The degradation of total cellular RNA and total cellular type I procollagen mRNAs was determined by prelabeling embryonic chick skin fibroblasts with [^{3}H]uridine and chasing the radiolabeled cultures in the presence of cold cytidine and uridine. At various time points thereafter total cellular RNA was isolated, and the decay of the pro α1(I) and pro α2(I) mRNAs was determined. The degradation of type I procollagen mRNAs is not altered by dexamethasone. Cortisol has been shown to increase the degradation of procollagen mRNAs in human skin fibroblasts (Hamalanien et al., 1985). Glucocorticoid treatment may stabilize type I procollagen mRNA degradation if the data are normalized on the basis of the total specific activity of the intracellular CTP and UTP precursor pools at 6 and 12 h into the chase period. In the presence of dexamethasone the total specific activity of the intracellular CTP and UTP precursor pools is less at 6 and 12 h compared to control values. Another interesting observation is that pro α2(I) mRNA exhibits an approximate 2-fold greater half-life as compared to pro α1(I) mRNAs in both control and glucocorticoid-treated cell cultures.

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Free Energy Contributions of G·U and Other Terminal Mismatches to Helix Stability†

Susan M. Freier,‡ Ryszard Kierzek,§ Marvin H. Caruthers,‖ Thomas Neilson,⊥ and Douglas H. Turner*,‡

Department of Chemistry, University of Rochester, Rochester, New York 14627, Institute of Bioorganic Chemistry, Polish Academy of Science, 60-704 Poznan, Noskowskiego 12/14, Poland, Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309, and Department of Biochemistry, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

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ABSTRACT: Thermodynamic parameters of helix formation were measured spectroscopically for seven hexaribonucleotides containing a GC tetramer core and G·U or other terminal mismatches. The free energies of helix formation are compared with those for the tetramer core alone and with those for the hexamer with six Watson–Crick base pairs. In 1 M NaCl, at 37 °C, the free energy of a terminal G·U mismatch is about equal to that of the corresponding A·U pair. Although other terminal mismatches studied add between −1.0 and −1.6 kcal/mol to ΔG°_{37} for helix formation, all are less stable than the corresponding Watson–Crick pairs. Comparisons of the stability increments for terminal G·U mismatches and G·C pairs suggest when stacking is weak the additional hydrogen bond in the G·C pair adds roughly −1 kcal/mol to the favorable free energy of duplex formation.

The wobble hypothesis includes a special stability of terminal G·U mismatches among the eight possible non-Watson–Crick mismatches of the four standard ribonucleotides (Crick, 1966). G·U mismatches are observed in the stems of tRNA cloverleaf structures (Sussman & Kim, 1976; Johnston & Redfield, 1981; Sprinzl et al., 1985) as well as in proposed double-helical regions in 5S rRNA (Fox & Woese, 1975; Kime & Moore, 1983; Noller, 1984; Erdmann et al., 1985), 16S RNA (Woese et al., 1983), viroids (Steger et al., 1984), and the excised intervening sequence from the rRNA of *Tetrahymena thermophila* (Cech et al., 1983). Frequently, these G·U mismatches are at the ends of proposed helical regions (Ninio, 1973; Clark, 1978; Mizuno & Sundaralingam, 1978). Al-

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‡ University of Rochester.
§ Polish Academy of Science.
‖ University of Colorado.
⊥ McMaster University.

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