Pretranslational Regulation of Type I Collagen, Fibronectin, and a 50-Kilodalton Noncollagenous Extracellular Protein by Dexamethasone in Rat Fibroblasts*

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The effect of dexamethasone on the synthesis of total cellular and extracellular proteins and specifically on the synthesis of type I procollagen chains, fibronectin, and a 50-kDa extracellular noncollagenous polypeptide was examined in cultured rat dermal fibroblasts. A slight but consistent inhibition of total protein synthesis by dexamethasone was dose and time dependent. Treatment of cells with 1 μM dexamethasone for 24 h while abolishing procollagen synthesis nearly completely (<95%) had the opposite effect (5–7-fold increase) on the synthesis of an extracellular noncollagenous 50-kDa polypeptide. Dexamethasone did not significantly affect the rates of synthesis of fibronectin.

Cell-free translation of mRNA from dexamethasone-treated cells revealed corresponding changes in the steady-state levels of functional mRNAs coding for procollagens, the 50-kDa polypeptide, and fibronectin. Northern blot hybridization using nick-translated cDNA plasmids coding for pro-α1(I), fibronectin, and cytoplasmic β-actin mRNA corroborated the data obtained from cell-free translation experiments. Run-off transcription assays using nuclei from cells treated with 1 μM dexamethasone for 24 h revealed that glucocorticoid treatment did not significantly affect the rate of transcription of type I collagen genes; similarly, the rate of transcription of fibronectin and cytoplasmic β-actin genes also remained unchanged under these conditions. An analysis of the kinetics of decay of radiolabeled mRNA coding for pro-α1(I), pro-α2(I), and fibronectin in dexamethasone-treated cells revealed that procollagen mRNAs were turned over at an accelerated rate in glucocorticoid-treated cells. These data suggest that dexamethasone regulates type I collagen gene expression by preferentially decreasing the stability of pro-α1(I) and pro-α2(I) mRNAs. Although dexamethasone increased the levels of translatable mRNAs coding for a 50-kDa polypeptide, the molecular mechanism(s) of how hormone exerts this effect remains unknown.

Glucocorticoids are a major class of steroids synthesized in the adrenal cortex and are important modulators of cellular metabolism; the multiple metabolic changes brought about by steroid hormones are mediated through their action on specific genes (1). In studies involving whole organisms as well as cultured cells, several specific gene products regulated by glucocorticoids have been identified (2). The effect of glucocorticoids on collagen biosynthesis has been studied by several investigators, both in the intact tissues of animals and in the cultured cells (3). Contrary to the earlier claims (4–8), more recent observations have convincingly demonstrated a preferential inhibition of collagenous polypeptides after glucocorticoid treatment (9). However, the question of selectivity of glucocorticoid action on collagen synthesis is not yet settled. For instance, in contrast to several studies showing glucocorticoid-mediated suppression of collagen synthesis in intact tissues and cultured cells (3), there were two recent reports demonstrating increased rates of collagen synthesis in bovine aortic smooth muscle cells (10, 11). Based on these observations, it was surmised that the outcome of glucocorticoid action may be critically dependent on the stage of differentiation of the target cell (11). Finally, the effect of glucocorticoids on different collagen types is also controversial. While preferential inhibition of type III collagen synthesis was reported in one study (12), another more recent report showed a coordinate down-regulation of both type I and III collagens (9).

Although a reduced amount of translatable procollagen-specific mRNAs in glucocorticoid-treated cells suggested a pretranslational regulation of these proteins by steroid hormones (13–15), the molecular mechanisms of how such an effect was exerted are poorly defined.

A detailed analysis of cellular protein synthesis in cultured hepatoma cells treated with dexamethasone revealed that the synthesis of 0.5–1% of cellular proteins (from a total of more than 1000 polypeptides resolved by two-dimensional gels) was affected (16). Among the gene constellations affected by glucocorticoid hormones, both positive and negative regulation were shown to occur (16). We reasoned therefore that the domains of hormone action (positive, negative, and neutral), so elegantly demonstrated by examining total cellular protein synthesis (16), may also be revealed if one observed the effect of glucocorticoids on the extracellularly released subset of cellular proteins. With this goal in mind, we investigated the regulation of extracellular protein biosynthesis by dexamethasone in cultured rat fibroblasts. We show that while dexamethasone has opposite effects on the synthesis of type I collagen (inhibition), and a 50-kDa noncollagenous protein (stimulation), the synthesis of fibronectin, like the majority of extracellular proteins, is only minimally affected; the effect

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of the hormone on the target genes appears to be exerted at the pretranslational level.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dexamethasone, triamcinolone, ascorbate, and ß-aminopropionitrile were purchased from Sigma. [³H]Methionine and [³H]proline were from Amersham Corp. [¹⁰⁰³H]uridine, [¹⁰¹⁰³H]cytidine, and [²⁵³²P]-labeled nucleoside triphosphates were purchased from New England Nuclear. Methionine-free media were prepared from a RPMI kit from Gibco, New York. Collagenase was obtained from Advanced Biotechnology, New York. Restriction endonucleases and other enzymes were purchased from New England Biolabs and Bethesda Research Laboratories.

**Cell Culture**—A continuous line of rat skin fibroblasts obtained from American Type Tissue Culture Collection (CRL 1213) was grown in Dulbecco's minimal essential medium (DMEM) with 10% fetal calf serum in 100-mm dishes to a 90-95% confluency. Cells were obtained at passage 9 and were maintained by subculturing at weekly intervals. All experiments were done on cells grown between 10 and 30 passages, since the rates of collagen and fibronectin synthesis diminish substantially in these cells after 40 passages. A clone of rat hepatoma (Clone M1.54; 17) that contains 10 copies of mouse mammary tumor virus (MMTV) genome stably integrated in its genome was similarly grown in DMEM. All serum used in these experiments, except for routine subculturing to maintain cell stocks, was depleted of endogenous steroids by treatment with activated charcoal (18). Methionine-free media were used for experiments involving [³H]methionine labeling.

**Northern Treatment of Cells, Radioactive Labeling, and Quantitation of Protein Synthesis**—Varying amounts of dexamethasone and triamcinolone were added in fresh 3.0 ml of steroid-free DMEM and incubated for 24 h; cells were washed with 2.0 ml of phosphate-buffered saline between media changes. Cells were then incubated with either 50 μCi of [³H]proline or 25 μCi of [³H]methionine in 2.0 ml of media and labeled for various lengths of time as indicated in individual experiments according to protocols outlined in detail previously (19). Ascorbate and ß-aminopropionitrile (50 μg/ml) were added to each culture; after incubation for appropriate duration, cells were washed two times with 10 ml of DMEM supplemented with cold uridine (10 μM), cytidine (10 μM), and actinomycin D (5 μg/ml). Following a 4-6 h additional incubation in DMEM containing actinomycin D (5 μg/ml), cells were incubated in normal DMEM, and poly(A)⁺ mRNA was extracted at 5, 6, 12, 24, and 36 h after the radioactive pulse. Ten μg of linearized cDNA plasmids containing pro-α1 collagen and pro-α2 collagen, and ß-actin immobilized on nitrocellulose filters were subjected to hybridizations under previously described conditions (19). The radioactivity (counts/min) specifically bound to filters was calculated after subtracting the machine background of 15-20 cpm. Non specific binding to bacteriophage λ and ph12522 DNA represented between 20-25 cpm. A plot of counts/min bound to filters versus time after dexamethasone addition was obtained and the average half-lives of mRNA with standard deviation were calculated from three separate determinations.

**Run-off Transcription**—Previously published techniques (19, 29) with minor modifications were used for the isolation of nuclei, in vitro transcription, and subsequent determinations of the rates of transcription by hybridization. Nuclei from control or dexamethasone-treated cells were either used immediately after isolation or stored at −70°C in 25% glycerol, 15 mM Tris-HCl, pH 7.5. To start transcription, nuclei (100-150 μg of DNA) were incubated in a 100-μl reaction mix that contained 150 mM KCl, 50 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, 1 mM each ATP, GTP, and CTP, and 250 μCi of [α-³²P]UTP at 25°C for 30 min. Under these conditions, the [α-³²P]UTP showed a linear incorporation for approximately 25-30 min following which, in some cases, there was an actual decline in the trichloroacetic acid-precipitable radioactivity. An identical aliquot of nuclei was also incubated with α-amanitin (90 μg/ml) to determine the specificity of RNA polymerase II-mediated transcription. Radiolabeled RNA was extracted and hybridized to DNA immobilized on nitrocellulose filters. In each case, 5 μg of linearized, alkali-denatured, plasmid DNA was immobilized on nitrocellulose filter discs (0.5 cm diameter). For determining nonspecific background, bacteriophage λ DNA immobilized on nitrocellulose filters were separately hybridized with radiolabeled run-off transcripts. Nitrocellulose filters were baked as described above, prehybridized, and hybridized at 42°C for 24 and 48 h, respectively. These hybridizations were done under conditions of DNA excess; hybridization for longer than 24 h did not result in increased signal intensity, and therefore we believe that maximum hybridization was achieved under these conditions. To measure the efficiency of hybridization, [³H]cRNA was synthesized from HF677 as described (29) and hybridized to immobilized HF677 DNA under identical conditions. For some experiments, equal amounts of RF nuclei were mixed with nuclei isolated from Clone ML.54 clone of the hepatoma cell line 

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1 The abbreviations used are: DMEM, Dulbecco’s minimal essential medium; MTV, mouse mammary tumor virus; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; RF, rat fibroblast(s).

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**Glucocorticoids and Extracellular Protein Synthesis**

1. **Experimental Procedures**
   - **Materials**
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   - **Cell Culture**
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   - **Northern Treatment of Cells, Radioactive Labeling, and Quantitation of Protein Synthesis**
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nearly complete genome of MTV (17) was used to determine the transcriptional activation of MTV-specific sequences in response to dexamethasone. Dr. Keith Yamamoto, University of California, San Francisco, kindly provided pMTV1 to us. Large scale cultures of Escherichia coli strains harboring appropriate plasmids were grown, and supercoiled plasmid DNA was purified (32). All plasmid preparations were tested for the characteristics of the inserted DNA by digestion with appropriate restriction endonucleases and gel electrophoresis. For Northern and dot blot analyses, intact plasmid DNA was nick-translated and served as a probe; in some experiments, the inserted cDNA was excised from the vector and nick-translated to increase the specific activity of the probe.

**RESULTS**

**Glucocorticoids Affect Cellular and Extracellular Protein Synthesis in a Dose-dependent Manner**—We systematically investigated the effects of dexamethasone and triamcinolone on the synthesis of extracellular proteins. Since both synthetic glucocorticoids gave identical results, we present experiments utilizing dexamethasone only. To determine the optimum effective concentration of glucocorticoid, we incubated fibroblast cultures with varying concentrations of dexamethasone for 24 h. Cells were further incubated with either [3H]proline or [35S]methionine for 1 h, and trichloroacetic acid-precipitable incorporation of radioactive amino acids into cellular and extracellular polypeptides was determined. Although the synthesis of both cellular and extracellular proteins was inhibited by dexamethasone treatment, there were apparent differences in the extent of inhibition of protein synthesis depending on the radiolabeled precursor used (Fig. 1). In contrast to a quantitatively similar decline in [35S]methionine incorporation into cellular and extracellular polypeptides in dexamethasone treated cells, the incorporation of [3H]proline showed a much greater decline in the extracellular proteins (Fig. 1). An examination of radiolabeled extracellular poly-

peptides by SDS-PAGE revealed part of the reason for this discrepancy (see below). Although analysis of trichloroacetic acid-precipitable incorporation showed 0.25 μM to be maximally effective (Fig. 1), we treated cells with 1 μM dexamethasone in all subsequent experiments to analyze various parameters of glucocorticoid action. After a 24-h treatment with 1 μM dexamethasone, cells were labeled with either [3H]proline or [35S]methionine, and extracellular polypeptides were analyzed by SDS-PAGE followed by fluorography. The pattern of polypeptides labeled with [3H]proline was much simpler compared to the pattern obtained from [35S]methionine-labeled cells (Fig. 2). Polypeptide bands representing procollagen chains were the most heavily labeled, accounting for more than 60% of [3H]proline incorporation (data not shown). Consistent with earlier observations, dexamethasone treatment of fibroblasts almost completely abolished procollagen synthesis (Fig. 2A). A visual inspection of the pattern of [35S]methionine-labeled extracellular polypeptides clearly showed that out of more than 30 radiolabeled polypeptide species released extracellularly, only three polypeptide bands were significantly affected by dexamethasone treatment (Fig. 2B). While the synthesis of extracellularly released procollagens was greatly reduced in dexamethasone-treated cells, synthesis and/or release of a 50-kDa polypeptide was markedly elevated (Fig. 2B). Synthesis of fibronectin, another major extracellular polypeptide synthesized by fibroblasts, was not affected by dexamethasone treatment (Fig. 2, A and B). The effects of dexamethasone treat-

![Fig. 1. Dexamethasone inhibits cellular and extracellular protein synthesis in a dose-dependent manner.](image)

![Fig. 2. Dexamethasone selectively affects the synthesis of procollagens and a 50-kDa noncollagenous extracellular polypeptide.](image)
ment on the kinetics of regulation of procollagens and 50-kDa polypeptides were investigated in more detail.

Dexamethasone Action on Cellular and Extracellular Protein Synthesis Is Time-dependent—To investigate the kinetics of temporal response of glucocorticoid action, fibroblasts treated with 1 μM dexamethasone for increasing lengths of time were radiolabeled with [35S]methionine for 1 h, and the cellular and extracellular protein fractions were subjected to SDS-PAGE followed by fluorography. A visual inspection of extracellular and cellular polypeptides in the fluorograms (Fig. 3) reveals the following features: First, consistent with the data on trichloroacetic acid-precipitable incorporation, there appeared only a small decline in the overall polypeptide synthesis; 10–15% decline was calculated by densitometric analyses (data not shown). Second, the inhibition of procollagen synthesis by 1 μM dexamethasone was virtually complete between 9 and 12 h; the inhibitory action of dexamethasone on procollagens can be unambiguously seen in the profile of extracellular polypeptides (Fig. 3). A quantitative comparison of how dexamethasone influenced the synthetic rates of procollagens, fibronectin, and 50-kDa polypeptide is shown in Fig. 4. Compared to its effect on the inhibition of procollagens, the stimulatory effect of dexamethasone on the synthesis of 50-kDa polypeptide was much more rapid; the maximum stimulation was evident between 3 and 6 h (Figs. 3 and 4). Finally, the synthesis of both cell-associated and extracellular fibronectin was only minimally affected by dexamethasone (Figs. 3 and 4).

Glucocorticoid-responsive Extracellular 50-kDa Polypeptide Is a Noncollagenous Protein—Since the untreated rat fibroblasts synthesized detectable amounts of 50-kDa polypeptide, it was important to investigate whether the 50-kDa polypeptides in the untreated and glucocorticoid-treated cells were the same. A comparison of the 50-kDa polypeptide from control and dexamethasone-treated cells by one-dimensional peptide mapping following digestion with Staphylococcus aureus V8 protease revealed these to be identical (data not shown). We concluded, therefore, that the glucocorticoid-responsive 50-kDa polypeptide was constitutively synthesized by fibroblasts under normal conditions. Lack of measurable incorporation of [3H]proline into the 50-kDa polypeptide (Fig. 2; compare A and B) and its resistance to degradation by highly purified bacterial collagenase under conditions that completely degraded procollagen chains suggested that the 50-kDa polypeptide was a noncollagenous extracellular protein.2 Since we were unable to demonstrate elevation of 50-kDa polypeptide in the cellular fraction even after very short labeling times, we believe that the 50-kDa polypeptide exits the cellular compartment very quickly (see below).

Dexamethasone Regulates the Steady-state Levels of mRNAs Coding for the Glucocorticoid-responsive Genes—A number of eukaryotic genes have been shown to be regulated by glucocorticoids; both transcriptional and post-transcriptional regulatory mechanisms have been demonstrated (1). We investigated whether the dexamethasone-mediated changes in the rates of synthesis of various extracellular polypeptides were reflected in the amounts of their corresponding messenger RNAs. Reticulocyte extracts were programmed with equal amounts of poly(A+) mRNA from control and dexamethasone-treated cells and radiolabeled polypeptide products were analyzed by SDS-PAGE. A comparative densitometric scan of fluorograms representing cell-free polypeptides clearly shows a reduction in procollagens and an increased synthesis of 50-kDa polypeptide (Fig. 5). A quantitative analysis of cell-free products by direct determination of radioactivity in the various polypeptide bands showed more than 85% reduction

2 R. Raghow, D. Gossage, and A. H. Kang, unpublished observations.
FIG. 5. Dexamethasone regulates the levels of translatable mRNAs of glucocorticoid-responsive genes. Initiation-competent reticulocyte extracts were programmed with equal amounts of poly(A$^+$) mRNA from control and dexamethasone-treated (1 $\mu M$ for 24 h) cultured fibroblasts. Cell-free translation products radiolabeled with [$^{35}$S]methionine were analyzed in 7.5% PAGE and fluorographed. Densitometric scans of fluorograms depicting cell-free translation products from untreated (top panel) and dexamethasone-treated (bottom panel) samples are shown. The polypeptide bands that were quantitated by densitometry as well as by direct measurements of incorporated [$^{35}$S]methionine using a scintillation counter are denoted. The origin of the gel is marked by O.

### TABLE I

**Effect of dexamethasone on translatable mRNAs coding for procollagens, fibronectin, and a 50-kDa polypeptide**

Initiation-competent reticulocyte extracts were programmed with equal amounts of poly(A$^+$) mRNA from control or dexamethasone-treated (1 $\mu M$, 24 h) (Dex) rat fibroblasts. Cell-free translation products labeled with [$^{35}$S]methionine were fractionated in 7.5% SDS-PAGE and autoradiographed. Areas of the gel representing denoted polypeptide bands were excised, and radioactivity was determined in a Beckman Scintillation Spectrometer; 58 cpm of machine background was subtracted from each value.

| Polypeptide | Control | Dex | Dex/control |
|-------------|---------|-----|-------------|
| Pro-$\alpha$1(I) | 1483 | 278 | 0.14 |
| Pro-$\alpha$2(I) | 871 | 82 | 0.13 |
| Fibronectin | 988 | 926 | 0.93 |
| 50-kDa | 243 | 1369 | 5.6 |

*Under electrophoretic conditions used here, pro-$\alpha$1(I) and pro-$\alpha$1(III) co-migrate and, therefore, these values represent combined incorporation into these two polypeptide chains.

in the procollagen chains, while the amount of mRNA coding for 50-kDa increased 5–6-fold (Table I). These observations were consistent with the idea that dexamethasone regulated the steady-state levels of functional mRNAs of the target genes. Alternatively, changes in the relative efficiency of translation of the mRNAs as affected by dexamethasone could also result in similar effect.

To further corroborate the cell-free translation experiments, we analyzed the amounts of three representatives of cellular mRNAs by Northern blot hybridization. Using radiolabeled recombinant DNA probes, we measured the steady-state levels of pro-$\alpha$1(I), fibronectin, and cytoplasmic $\beta$-actin (representing intracellular protein ubiquitously expressed in eukaryotic cells) from control and dexamethasone-treated cells. As shown in Fig. 6, while there was a substantial reduction in the amount of pro-$\alpha$1(I) mRNA in the dexamethasone-treated cells, the amounts of mRNAs coding for fibronectin and $\beta$-actin were not significantly affected. Quantitation by densitometry revealed more than 80% reduction in the pro-$\alpha$1(I) mRNA in dexamethasone-treated cells (data not shown). Since we do not have a cDNA clone representing mRNA coding for 50-kDa polypeptide yet, we could not do similar measurements on the amount of 50-kDa-specific mRNA directly.

Conceivably, dexamethasone could alter the steady-state levels of mRNAs by preferentially altering the rate of transcriptions of the target genes. Alternatively, glucocorticoid...
treatment could affect the stability of procollagen mRNAs resulting in a net decline in the steady-state levels. Evidence for both types of control by glucocorticoids has been presented in different eukaryotic systems (1). We measured the rate of transcription of various genes, in glucocorticoid-treated cells by run-off transcription assay (18). The nuclei isolated from plasmids immobilized on nitrocellulose, under conditions of cally by dexamethasone (17). We investigated the effect of altered under these conditions (Table 11).

The nuclei isolated from untreated (control) DNA from untreated (control) or dexamethasone-treated (1 μM, 24 h; DEX) cells were electrophoresed in 2.2 M formaldehyde. Filters were then sequentially hybridized to 32P-radiolabeled cDNA probes representing pro-al(I) (lanes a and b), fibronectin (lanes c and d), and β-actin (lanes e and f).

Fig. 6. Northern blot hybridizations to determine the amounts of mRNAs coding for pro-al(I), fibronectin, and β-actin in dexamethasone-treated rat fibroblasts. Ten μg of total RNA from untreated (control) or dexamethasone-treated (1 μM, 24 h; DEX) cells were electrophoresed in 2.2 M formaldehyde, 1.0% agarose gels and blotted to nitrocellulose. Filters were then sequentially hybridized to 32P-radiolabeled cDNA probes representing pro-al(I) (lanes a and b), fibronectin (lanes c and d), and β-actin (lanes e and f).

Since we failed to detect significant changes in the rates of transcription of Type I collagen and fibronectin genes in dexamethasone-treated RF cells, it was important to determine the sensitivity of our run-off transcription assay using a well-studied, glucocorticoid-responsive system. MTV-specific transcription has been shown to be increased dramatically by dexamethasone (17). We investigated the effect of dexamethasone on the rate of MTV-specific transcription in a clone of rat hepatoma cell that contains 10 stably integrated copies of MTV genome (17). As shown in Table III, we could detect a nearly 30-fold increase in the rate of MTV-specific transcription in dexamethasone-treated cells. When a 50:50 mixture of nuclei from M1.54 and RF cells was subjected to run-off transcription assays (Table III), the relative rates of MTV transcription remained unchanged. The relative rates of transcription of collagen, fibronectin, and β-actin genes also remained unchanged when the run-off transcription was performed in the mixture of M1.54 and RF nuclei, further substantiating the specificity of the glucocorticoid effect.

Finally, we measured the relative rates of mRNA turnover in dexamethasone-treated cells. As shown in Table II, the relative rates of decay of pro-al(I), and pro-al(II) increased significantly. The τ0 of the pro-al(I) and pro-al(III) declined substantially in the dexamethasone-treated cells (from approximately 12 h versus 6–8 h; Table IV). The effect of dexamethasone on the stability of procollagen mRNAs appeared preferential since the apparent half-lives of fibronectin and β-actin mRNAs remained unchanged after dexamethasone treatment (Table IV). These experiments strongly suggest that glucocorticoids selectively reduce the stability of Type I procollagen genes. Whether the transcripts for 50-kDa polypeptide are also regulated by a similar mechanism remains to be investigated.

### Table II

| DNA probe | α-Amanitin Control | Dex | Dex/control |
|-----------|--------------------|-----|-------------|
| Pro-al(I) | 31.2               | 37.5| 1.02        |
| Pro-al(II)| 38.5               | 31.6| 0.98        |
| Fibronectin| 51.2              | 62.7| 1.22        |
| β-Actin   | 176.8              | 184.9| 1.10       |
| Charon 4A | 2.6                | 1.9 | 2.2         |

### Table III

| DNA probe | α-Amanitin Control | Dex | Dex/control |
|-----------|--------------------|-----|-------------|
| MTV⁺⁺      | 81.1               | 2359.2| 29.01      |
| Pro-al(I)⁻⁻ | 3.6              | 2.8 | 1.13       |
| Pro-al(II)⁻⁻| 2.3               | 3.1 | 1.03       |
| β-Actin⁻⁻   | 142.4              | 156.3| 1.09       |

"Run-off transcripts isolated from M1.54 nuclei were hybridized.

"Run-off transcripts from a 50:50 mixture of nuclei of RF and M1.54 cells were hybridized.
TABLE IV
Rate of mRNA turnover in dexamethasone-treated cells

| mRNA        | Apparent half-life (h) |
|-------------|------------------------|
| Control     | Dexamethasone          |
| Pro-α(1)    | 11.8 ± 2.4             |
| Pro-α(2)    | 10.2 ± 3.1             |
| Fibronectin | 12.7 ± 2.3             |

DISCUSSION

The experimental evidence presented here shows that glucocorticoids selectively regulate the biosynthesis of three different extracellular polypeptides in a time- and dose-dependent manner. While the overall cellular protein synthesis was only marginally affected, the biosynthesis of procollagens (both type I and III) and a 50-kDa noncollagenous protein was dramatically influenced by dexamethasone. The selectivity of hormone action was further shown by the opposite effects of dexamethasone treatment on the 50-kDa polypeptide (several-fold stimulation) and procollagens (more than 95% inhibition); the biosynthetic pattern of fibronectin changed little following dexamethasone treatment. Our results of the selective inhibition of procollagen by dexamethasone are similar to those reported (3, 9, 15).

To delineate the mechanism of glucocorticoid action on the affected genes, we programmed cell-free translation extracts with mRNAs from dexamethasone-treated cells; steady-state levels of mRNAs of procollagens and fibronectin, and cytoplasmic β-actin were also measured directly, by Northern blot hybridization to radiolabeled recombinant DNA probes. These experiments strongly suggested that dexamethasone regulated the steady-state levels of mRNAs of the target genes. Our measurements of procollagen mRNA levels in dexamethasone-treated cultures were similar to those of Sterling et al. (15) who elegantly demonstrated a decline in the steady-state levels of procollagen mRNAs in glucocorticoid-treated tissues and cultured cells. We have extended these studies to determine an important pretranslational step of dexamethasone action on the procollagen genes and show that the hormone treatment accelerated the rate of mRNA turnover without affecting the rates of transcription of procollagen genes. The specificity of glucocorticoid action on stability of procollagen mRNA by dexamethasone was clearly shown by a lack of a similar effect on the rate of turnover of fibronectin and cytoplasmic β-actin mRNAs. Recently, Hamulainen et al. (33) showed that the stability of procollagen mRNAs in cultured human fibroblasts treated with cortisol was decreased, resulting in a net decline in the steady-state levels of mRNAs. Although the precise molecular mechanism(s) of such actions remains elusive, it was speculated that glucocorticoids either induce or activate an RNA degrading enzyme(s) that specifically break down some mRNAs (33). How do we reconcile the apparent discrepancy between the rate of mRNA turnover (about 50% reduction) and the amount of Type I procollagen chains (less than 15%) synthesized in the glucocorticoid-treated cells? It is reasonable to assume that the mRNA molecules will be rendered nonfunctional with a single nick(s) and yet could potentially hybridize very efficiently to recombinant plasmid DNAs. We believe that our mRNA turnover experiments are overestimating the amount of functional mRNA. Direct experimental evidence for this explanation, however, is yet to be obtained. However, based on the experimental evidence presented here, we cannot rule out the possibility that an additional effect of glucocorticoids is exerted at step(s) subsequent to mRNA degradation. The gene for 50-kDa polypeptide resembles several other eukaryotic genes whose synthesis is stimulated by glucocorticoids; some of these have been shown to be regulated at the transcriptional level (1). However, due to the lack of a suitable cDNA probe, the rates of transcription as well as mRNA turnover could not be determined for the 50-kDa polypeptide gene. Therefore, the mechanism of how mRNA for 50-kDa polypeptide is regulated is still unclear.

Whether the stimulation of 50-kDa polypeptide and inhibition of procollagens are casually related was not tested experimentally. Based on very different kinetics of the two effects, we speculate that these two events were independent of each other. Clearly, more data are needed before this question can be answered unambiguously. Another unsettled issue is the identity of the 50-kDa polypeptide. Its constitutive synthesis in untreated cells in culture and sensitivity to regulation by dexamethasone combined with its short intracellular transit time (minutes) make it an interesting molecule for further study. Experiments are in progress to investigate if the 50-kDa polypeptide is analogous to a class of hormonesensitive, extracellularly released polypeptides described under the names of "renocortin," "lipomodulin," and "macrocortin"; these polypeptides have been shown to be capable of inhibiting phospholipase A, thus modulating prostaglandin metabolism (34-47).

Finally, as is well known, in a variety of virally transformed cells, procollagens and fibronectin are coordinately down-regulated; this regulation has been shown to be mediated by reduced transcription of collagen and fibronectin genes by RNA polymerase II (38-42). It was also shown that, in a variety of malignant cells, dexamethasone could reverse the transformed phenotype as well as restore fibronectin synthesis (41). Therefore, it is significant that pro-α1(I) and pro-α2(I) collagen genes are regulated by dexamethasone by changing their stability, while there is no effect of the hormone on the stability of fibronectin mRNA. These results point to the multiple regulatory pathways involved in the transcriptional and post-transcriptional controls of target genes in glucocorticoid-treated and transformed cells.

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