Dexamethasone modulates the metabolism of type IV collagen and fibronectin in human basement-membrane-forming fibrosarcoma (HT-1080) cells

Aarne OIKARINEN,* Tuula SALO,† Leena ALA-KOKKO† and Karl TRYGGVASON‡

Collagen Research Unit, Biocenter and *Departments of Dermatology and Anatomy, †Medical Biochemistry and Clinical Chemistry, and ‡Department of Biochemistry, University of Oulu, SF-90220 Oulu, Finland

The effect of dexamethasone on the synthesis and degradation of type IV collagen was studied in human fibrosarcoma cells, HT-1080. A dexamethasone concentration as low as 0.1 μM markedly increased collagen synthesis in HT-1080 cells labelled with [^{14}C]proline. The increase in type IV collagen synthesis was not specific, since total protein synthesis was also increased. Further studies indicated that part of the increase was due to an increase in the specific radioactivity of the intracellular proline pool, after dexamethasone treatment. In fact, with dexamethasone concentrations of 0.1–10 μM the relative collagen synthesis was decreased, indicating that synthesis of other protein was increased more than that of type IV collagen. This was also confirmed by measuring the relative amount of type IV collagen RNA by using recombinant plasmid cDNA specific for the human procollagen $\text{pro}\alpha 1(\text{IV})$ RNA. The results indicated that relative collagen synthesis and the relative amount of type IV collagen messenger RNA was decreased similarly, indicating that dexamethasone affected type IV collagen synthesis at the pre-translational level. The dexamethasone-induced effect on total protein and collagen synthesis was maximal after 12–24 h. Dexamethasone induced a marked accumulation of collagen into the cell layer, leading to diminished deposition of soluble collagen into the medium. Since bacterial-collagenase treatment of the cell layer drastically decreased the collagen content of the dexamethasone-treated cells, this indicates that dexamethasone caused an accumulation of collagen into the extracellular matrix of the cell layer. In contrast, the amount of fibronectin was markedly increased in the medium. Dexamethasone decreased the type IV collagen-degrading activity in HT-1080 cells. The HT-1080 cells contained glucocorticoid receptors, as demonstrated by two different methods: by a whole-cell binding assay and by using a cytosol-gel-filtration method. The number of specific binding sites was similar to that in human skin fibroblasts. In conclusion, glucocorticoids affect the metabolism of type IV collagen and fibronectin in HT-1080 cells, and, since these cells contain specific glucocorticoid receptors, the effects are apparently receptor-mediated.

INTRODUCTION

Glucocorticoids affect various cellular functions such as energy metabolism, protein synthesis and proliferation of cells (for reviews, see Baxter & Forsham, 1972; Thompson & Lippman, 1974; Pratt, 1978). It is generally accepted that glucocorticoids affect cells through specific receptor molecules, and after binding to receptors in the cytosol they induce changes in gene expression (for reviews, see Baxter & Funder, 1979; Roy & Clark, 1983). It has been demonstrated that glucocorticoid-receptor molecules bind to specific regions of DNA and affect transcription (Groner *et al.*, 1984). Several studies indicate that the metabolism of connective-tissue components such as collagens is partially regulated by glucocorticosteroids. Animal and cell studies have shown that glucocorticoids inhibit the synthesis of interstitial collagens (for reviews, see Cutroneo *et al.*, 1981). Glucocorticoids also inhibit the production of interstitial collagenases (Koob *et al.*, 1974), which are able to degrade fibrillar collagen types I, II and III.

Hormonal regulation of the metabolism of basement membranes is currently poorly understood. In one study, insulin increased the synthesis of the basement-membrane-specific type IV collagen in mouse tissues (Pihlajaniemi *et al.*, 1982). However, it is not known if

glucocorticoids regulate the synthesis of type IV collagen. In the present study we have used human fibrosarcoma cells (HT-1080) to study the effect of glucocorticoids on the biosynthesis and degradation of type IV collagen and fibronectin. These cells are suitable for these kind of studies, since they synthesize type IV collagen (Alitalo *et al.*, 1980; Pihlajaniemi *et al.*, 1981).

MATERIALS AND METHODS

Cell cultures

Human fibrosarcoma cells, HT-1080 (Rasheed *et al.*, 1974), were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). Human skin fibroblast cultures were established from skin obtained from surgical procedures performed for cosmetic reasons, after obtaining informed consent. The cells were maintained at 37 °C in Dulbecco's modified Eagle's medium with 10% newborn-calf serum, 100 i.u. of penicillin/ml and 100 μg of streptomycin/ml.

Collagen-synthesis studies

HT-1080 cells were preincubated with steroids as indicated below, in Dulbecco's modified Eagle's medium containing 5% fetal-calf serum and ascorbic acid

(50 µg/ml). After preincubation, the cells were labelled with [¹⁴C]proline (2 µCi/ml) for 12–24 h. In order to study the secretion of proteins and collagen, cells were pulsed with [¹⁴C]proline and incubation was stopped after a 0–25 h chase period. After the labelling period, the medium was collected and proteinase inhibitors were added to give final concentrations of 25 mM-Na₂EDTA, 10 mM-*N*-ethylmaleimide, 1 mM-phenylmethanesulphonyl fluoride, and 1 mM-*p*-aminobenzamide. The medium proteins were then precipitated by adding (NH₄)₂SO₄ (390 mg/ml) and, after stirring overnight at 4 °C, the proteins were collected by centrifugation for 60 min at 10000 *g*. The precipitate was then dissolved in, and dialysed for 48 h against, 0.4 M-NaCl/0.1 M-Tris/HCl, pH 7.4, and a part of the sample was used for assay of total radioactivity and hydroxyproline (Juva & Prockop, 1966).

Cell fractions were scraped into a buffer containing 0.1% Triton X-100, proteinase inhibitors were added as described above, and the cells were then homogenized and centrifuged, and the supernatant was precipitated by (NH₄)₂SO₄, collected by centrifugation, dialysed and used for assays of total radioactivity and [¹⁴C]hydroxyproline (Juva & Prockop, 1966).

Assay of steady-state amount of type IV collagen mRNA

Confluent cultures of human fibrosarcoma cells HT-1080 were incubated with 0.1 µM-dexamethasone for 12, 24 and 48 h. After incubation, total cellular RNA was purified as described previously (Oikarinen & Ryhänen, 1981). The total RNA samples (three dilutions, ranging from 1 to 4 µg) were denatured with formamide and dotted on to a nitrocellulose paper by using a dot-blot apparatus (Minifold II; Schleicher & Schuell). The papers were then baked at 78 °C for 2 h in a vacuum oven to bind the RNAs covalently to them.

The recombinant plasmid HT-21 containing cDNA for the human procollagen proα1(IV) RNA, as characterized in detail previously (Pihlajaniemi *et al.*, 1985), was purified and labelled radioactively with [α-³²P]dATP, [α-³²P]dCTP, [α-³²P]dGTP and [α-³²P]dTTP (3000 Ci/mmol; Amersham International) by nick-translation (Maniatis *et al.*, 1982).

DNA and RNA hybridization was carried out (Thomas, 1980), and the amount of recombinant plasmids hybridized to the mRNA quantified by autoradiography by using Kodak X-OMAT films and cassettes with intensifying screens. The films were scanned with a Kontes K 49500 densitometer connected to a Spectra-Physics SP4100 computing integrator.

Glucocorticoid-receptor assays

Two different methods were utilized for receptor assay. Firstly, a whole-cell binding assay, which has been extensively utilized for steroid-receptor determinations in other cell systems (Lippman & Barr, 1977; Werb *et al.*, 1978; Kontula *et al.*, 1980; Eil *et al.*, 1980), was adapted for glucocorticoid-receptor determinations in fibroblasts. Confluent human fibrosarcoma cells and skin fibroblasts were harvested by brief trypsin treatment in modified Krebs medium (Dehm & Prockop, 1971). Trypsin was inhibited by the addition of soya-bean trypsin inhibitor, and cells were washed twice by centrifugation, and finally suspended in Krebs medium. In a standard assay, 0.8 ml portions of the cell suspension were pipetted into

silicone-treated glass tubes, followed by 0.2 ml of Krebs medium containing [6,7-³H]dexamethasone (sp. radioactivity 38 Ci/mmol; New England Nuclear), the final concentration of the steroid ranging from 1.2 to 100 nM. For the estimation of non-specific binding, parallel sets of tubes containing a 200-fold excess of unlabelled dexamethasone were included in each assay. The tubes were incubated for 45 min at 37 °C with intermittent shaking. Then 2 ml of cold Krebs medium (4 °C) was added to each tube, and the tubes were centrifuged for 5 min at 1000 *g* at 22 °C. The supernatants were discarded and the pellets were resuspended in 3 ml of Krebs medium. The tubes were further incubated for 10 min at 22 °C, and the cells were again collected by centrifugation. The washing procedure was repeated once, and the cell pellets were resuspended in 0.5 ml of Krebs medium plus 1 ml of ethanol. Samples were taken for liquid-scintillation counting of the radioactivity, and the remainder of the cells was used for assay of DNA (Brunk *et al.*, 1979) or protein (Bradford, 1976), or for cell counting by haemocytometer. The specific binding of [³H]dexamethasone was calculated by subtracting the non-specific binding from the total binding. The maximum binding was estimated from Scatchard (1949) plots.

Secondly, a cytosol binding assay was utilized. Cells, after harvest by brief trypsin treatment, were centrifuged at 1000 *g* for 5 min, and the pellet was homogenized on ice with a Teflon/glass homogenizer in 20 mM-Tris/HCl, pH 7.5, containing 2 mM-CaCl₂, 2 mM-MgCl₂, 2 mM-dithiothreitol, 10 mM-sodium molybdate and 10% (v/v) glycerol. The homogenates were centrifuged at 100000 *g* for 60 min at 4 °C. The supernatants were then used for receptor-binding assay and for protein determination. For receptor assay, cytosol preparations were incubated with 50 nM-[³H]dexamethasone for 20 h at 4 °C. For estimation of non-specific binding, parallel samples were incubated for [³H]dexamethasone in the presence of 200-fold excess of unlabelled dexamethasone.

After incubation, the unbound [³H]dexamethasone was removed by gel filtration. After incubation with [³H]dexamethasone, the cytosol preparations were chromatographed on a Sephadex G-100 column (1.0 cm × 20.0 cm), equilibrated and eluted with 20 mM-Tris/HCl, pH 7.5, containing 2 mM-CaCl₂ and 2 mM-MgCl₂. Fractions (1.0 ml) were collected and counted for ³H radioactivity.

Assay of type IV collagen degradation

For enzyme-activity determinations, cell cultures were washed with phosphate-buffered saline (13 mM-Na₂HPO₄·2H₂O/14 mM-KH₂PO₄/0.15 M-NaCl, pH 7.4) to remove traces of serum, and medium was replaced with serum-free Dulbecco's modified Eagle's medium containing 0.25% bovine serum albumin, 0.1% transferrin, and various concentrations of dexamethasone. Dexamethasone was dissolved in ethanol, and the final concentration of ethanol was adjusted to the same percentage (0.1%, v/v) in each flask. After incubation for up to 24 h, the cells were counted and the cell culture medium was collected after centrifugation for 10 min at 600 *g* to remove cells and debris, and the supernatant was stored frozen.

The type IV collagen-degrading activity from cell-culture medium proteins was assayed as described previously (Salo *et al.*, 1982) by using soluble [³H]proline-labelled type IV procollagen as a substrate (Tryggvason

et al., 1980). Briefly, enzyme samples were activated with trypsin (10 $\mu\text{g/ml}$), followed by the addition of soya-bean trypsin inhibitor (50 $\mu\text{g/ml}$), *N*-ethylmaleimide (4 mM), aprotinin (1000 IU/ml) and substrate (3000 c.p.m.). The reaction was carried out for 18 h at 35 °C and was terminated by adding 20 μl of bovine serum albumin (1 mg/ml) and 100 μl of a solution containing 10% (w/v) trichloroacetic acid and 5% (w/v) tannic acid. The mixture was incubated on ice, and the undigested material was precipitated and removed by centrifugation at 5000 *g* for 15 min. Radioactivity in the supernatant was measured in a scintillation counter.

Other assays

The proteins from the $(\text{NH}_4)_2\text{SO}_4$ precipitate of the culture medium were also analysed by SDS/6%-polyacrylamide-slab-gel electrophoresis (Laemmli, 1970). The ^{14}C -labelled polypeptides were detected by fluorography (Bonner & Laskey, 1974), and bands representing fibronectin and type IV collagen were quantified by densitometry.

For immunoprecipitation, medium proteins after $(\text{NH}_4)_2\text{SO}_4$ precipitation were precipitated with either affinity-purified rabbit anti-fibronectin or affinity-purified rabbit anti-(type IV collagen) antibodies for 2 h at 22 °C. The immune complexes were then precipitated by goat anti-rabbit IgG overnight at +4 °C and collected by centrifugation. The pellets were then solubilized and used for SDS/6%-polyacrylamide-slab-gel electrophoresis.

For measurement of the intracellular specific radioactivity of ^{14}C proline, the cells were preincubated in the presence and absence of 0.1 μM -dexamethasone for 48 h, and labelled for 4 h at 37 °C with ^{14}C proline. After labelling, the cells were rinsed with phosphate-buffered saline, pH 7.4, and scraped into 20% (w/v) sulphosalicylic acid, and the free proline was separated on a JEOL-AH amino acid analyser. Radioactivity was determined by liquid-scintillation counting, and the quantity of proline in the corresponding peak was determined by the ninhydrin reaction.

For the assay of prolyl hydroxylase activity, the cells were preincubated with and without dexamethasone, and the cells were harvested and stored at -70 °C before the assay. Prolyl hydroxylase activity was then assayed as described previously, with ^{14}C proline-labelled type I procollagen as the substrate (Kivirikko & Myllylä, 1982).

RESULTS

Glucocorticoid receptors

The presence of specific glucocorticoid receptors was demonstrated in the human fibrosarcoma cells by two different methods. Firstly a whole-cell binding assay was used. As shown in Fig. 1, HT-1080 cells contained saturable amounts of glucocorticoid-binding sites. Scatchard-plot analysis gave approx. 9.7 fmol of dexamethasone-binding sites/ μg of DNA, and the dissociation constant, K_d , was 10.5×10^{-9} M (results not shown). In comparison, human skin fibroblasts contained 5.8 ± 1.7 fmol/ μg of DNA (mean $\pm$ S.D., $n = 5$), and the K_d was $11.8 (\pm 7.0) \times 10^{-9}$ M (mean $\pm$ S.D.). Secondly, a cytosol gel-filtration assay was used. The specific binding protein was eluted in the void volume in Sephadex G-100

column chromatography (results not shown). The cytosol assay gave approx. 0.23 fmol of dexamethasone-binding sites/ μg of cytosol protein.

Collagen-synthesis studies

Dexamethasone increased incorporation of ^{14}C proline and synthesis of ^{14}C hydroxyproline (cells plus medium fractions) in HT-1080 cells (Figs. 2a and 2b). The increase was dose-dependent. The increase in total collagen synthesis was not specific, since the ratio of the hydroxyproline/total radioactivity was not increased in the presence of dexamethasone. In fact, the relative collagen synthesis in cells plus medium was decreased with dexamethasone. The time course of the dexamethasone effect on HT-1080 cells was studied by preincubating the cells for various times (0–48 h) in the presence of 0.1 μM -dexamethasone. There was slight stimulation of the total content of radioactivity and ^{14}C hydroxyproline after 2 h preincubation, but the most marked increase was noted between 12 and 24 h preincubation (Fig. 3). Other non-glucocorticoid steroids tested (progesterone, oestradiol, testosterone) did not significantly affect total protein or collagen synthesis in HT-1080 cells (results not shown).

Dexamethasone increased markedly the accumulation of collagen in the cell layer (Fig. 2c). The pulse-label experiments indicated that dexamethasone decreased the secretion of collagen into the medium and induced the accumulation of collagen into the cell layer (Fig. 4b). The secretion of other proteins was slightly stimulated by dexamethasone (Fig. 4a).

Treatment of the cell layer with bacterial collagenase after labelling with ^{14}C proline decreased the ^{14}C hydroxyproline content by about 50% in control cells and by about 73% in dexamethasone-treated cells,

Fig. 1. Typical binding curve of $[^3\text{H}]$ dexamethasone to glucocorticoid receptors in HT-1080 cells

For determination of total binding, the cells were incubated for 45 min at 37 °C with various concentrations of $[^3\text{H}]$ dexamethasone. For determination of non-specific binding, a parallel set of tubes was incubated in the presence of a 200-fold excess of unlabelled dexamethasone. Specific binding was calculated by deducting the non-specific binding from the total binding. Maximal binding was calculated from the Scatchard plot (insert).

Fig. 2. Effect of dexamethasone on total protein synthesis, collagen synthesis and the secretion of collagen in HT-1080 cells

Sub-confluent HT-1080 cells were preincubated in the presence of various concentrations of dexamethasone. Dexamethasone was dissolved in ethanol, and the ethanol concentration was adjusted to be the same ($< 0.1\%$) in control cells and dexamethasone-treated cells. After preincubation for 48 h, the cells were labelled with [^{14}H]proline for 12 h, and medium and cell fractions were used for the assay of total radioactivity (a) and collagen synthesis as determined by hydroxyproline incorporation (b). In (c) the accumulation of collagen in the cells is illustrated by expressing the ratio of hydroxyproline in the cells to total hydroxyproline in the cells plus medium. The values are the means of duplicate samples.

indicating that most of the hydroxyproline in the cell layer was outside the cells and was sensitive to collagenase (results not shown). After bacterial-collagenase treatment of the cell layer, the hydroxyproline that was recovered in the cells was the same in control and dexamethasone-treated cells.

The mechanisms of dexamethasone-induced increase in total protein synthesis were studied by measuring the specific radioactivity of [^{14}C]proline in the cell layer. Dexamethasone ($0.1 \mu\text{M}$) increased the specific radioactivity of [^{14}C]proline from $354 (\pm 27) \times 10^3$ to $472 (\pm 66) \times 10^3$ d.p.m./ μmol of proline (means $\pm$ S.D.). Further studies indicated that dexamethasone did not affect the activity of prolyl hydroxylase in HT-1080 cells.

The [^{14}C]proline-labelled proteins secreted into the medium by HT-1080 cells were analysed by SDS/6%-polyacrylamide-gel electrophoresis. The major component observed in control media was a 220 kDa band (Fig. 5, lane 1), which contained fibronectin, as

Fig. 3. Time course of the dexamethasone effect on total protein and collagen synthesis in HT-1080 cells

HT-1080 cells were preincubated for 0, 2, 4, 12, 24 or 48 h in the presence of $0.1 \mu\text{M}$ -dexamethasone. After preincubation, the cells were labelled for 4 h in the presence of dexamethasone with [^{14}C]proline. Control cells were labelled in the presence of ethanol for 4 h (shaded column). Total radioactivity and [^{14}C]hydroxyproline were then assayed as described above. The values are the means of duplicate samples. (a) Total radioactivity in cells plus medium. (b) Hydroxyproline in cells plus medium.

demonstrated by immunoprecipitation (not shown), and type IV collagen $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$ chains, 185 and 170 kDa respectively. Additionally two prominent unidentified polypeptides, of about 110 and 76 kDa, were seen. The addition of dexamethasone to the medium caused a marked increase in fibronectin synthesis, whereas the amount of type IV collagen did not increase (Fig. 5, lanes 2–4), and the amount of type IV collagen in the soluble medium fraction was relatively decreased when analysed by immunoprecipitation (results not shown). These changes were most prominent at $10 \mu\text{M}$ -dexamethasone. Under the same conditions a new band of about 150 kDa had also appeared (Fig. 5).

Effect of dexamethasone on the steady-state amount of type IV collagen mRNA

To study this in HT-1080 cells, the cells were incubated for 12–48 h in the presence of $0.1 \mu\text{M}$ -dexamethasone and used for the measurement of collagen synthesis and type IV collagen mRNA as described in the Materials and methods section. Dexamethasone decreased the relative amounts of type IV collagen mRNA in cells; the largest decrease (over 40%) was noted after 48 h incubation with dexamethasone (Table 1). The relative synthesis of type IV collagen was decreased similarly (Table 1).

Fig. 4. Effect of dexamethasone on the total secretion of protein and collagen

HT-1080 cells were preincubated for 48 h in the presence and absence of $0.1 \mu\text{M}$ -dexamethasone. After preincubation the cells were pulsed with $[^{14}\text{C}]\text{proline}$, and the pulse was stopped after 0–25 h chase periods, and medium and cell fractions were used for the assay of total radioactivity and hydroxyproline as described above. The values are the means of duplicate samples. (a) Total radioactivity; (b) hydroxyproline: $\blacktriangle$, control cells; $\bullet$, control medium; $\triangle$, dexamethasone-treated, cells; $\circ$, dexamethasone-treated, medium.

Type IV collagen degradation

Dexamethasone decreased the activity of type IV collagen-degrading enzyme(s) in HT-1080 cells (Table 2). However, the inhibition was variable from one experiment to another. It should also be noted that, when marked inhibition was found, $0.1 \mu\text{M}$ -dexamethasone caused maximal inhibition, and larger concentrations were not more inhibitory.

DISCUSSION

The results of the present study indicated that glucocorticoids regulate the deposition of basement-

Fig. 5. Effect of dexamethasone on the fibronectin/type IV collagen ratio in HT-1080 cells

HT-1080 cells were preincubated for 48 h in the presence of various concentrations of dexamethasone. The cells were then labelled with $[^{14}\text{C}]\text{proline}$ for 12 h. Fractions were precipitated from the medium with $(\text{NH}_4)_2\text{SO}_4$, and precipitates were analysed by SDS/polyacrylamide-gel electrophoresis. The $[^{14}\text{C}]\text{polypeptides}$ were detected by fluorography, and bands corresponding to fibronectin and type IV collagen polypeptide chains (thin arrows) are indicated. Also the positions of three other bands (A, B and C) are shown, corresponding to molecular masses of 150, 110 and 76 kDa respectively. Lane 1, control; lane 2, dexamethasone $10 \mu\text{M}$; lane 3, dexamethasone $0.1 \mu\text{M}$; lane 4, dexamethasone 1 nM .

membrane components in HT-1080 cells. Since these cells contain specific glucocorticoid receptors, it is plausible that the effects of glucocorticoids are mediated through receptors.

Dexamethasone, but not other non-glucocorticoid steroids tested, increased total protein synthesis of HT-1080 cells. The increase in the radioactivity of the intracellular proline pool after dexamethasone treatment could partially explain the increase in total protein synthesis. Dexamethasone at $0.1 \mu\text{M}$ increased the specific radioactivity of proline by 33%. This is in agreement with the results of previous studies with human skin fibroblasts, in which cortisol increased the uptake of proline by about 41% (Russell *et al.*, 1982).

The relative synthesis of type IV collagen was

Table 1. Effect of dexamethasone on the relative rate of type IV collagen synthesis and amount of type IV collagen RNA in HT-1080 cells

The conditions and pretreatments were the same as in Fig. 3. After preincubations with 0.1 M-dexamethasone for 12, 24 or 48 h, cells were used for the assay of collagen synthesis or for the measurement of type IV collagen mRNA as described in the Materials and methods section. The relative collagen synthesis was determined by measuring [^{14}C]hydroxyproline and total radioactivity in the cells plus medium. The ratio [^{14}C]hydroxyproline/total ^{14}C expresses the relative synthesis of type IV collagen. The values are means $\pm$ S.D. for five parallel samples. For the measurement of type IV collagen RNA, cellular RNA was dotted on nitrocellulose paper and hybridized with recombinant plasmid HT-21 containing cDNA for the human procollagen pro α 1(IV) RNA. The samples were then detected by autoradiography and quantified by scanning the bands with a densitometer. The values are expressed as densitometric units calculated from the surface areas of densitometric scans of the bands. The values are means $\pm$ S.D. for three parallel samples. Significances: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.025$.

Treatment of samples	Relative rate of type IV collagen synthesis		Relative amount of type IV collagen mRNA	
	($10^2 \times [\text{C}^{14}]$ Hydroxyproline/ total ^{14}C)	(% of control)	(Densitometric units)	(% of control)
Control	1.73 $\pm$ 0.20	100	18.45 $\pm$ 3.46	100
Dexamethasone 12 h	1.47 $\pm$ 0.11*	85 $\pm$ 6.4	19.44 $\pm$ 3.35	105 $\pm$ 18
Dexamethasone 24 h	1.40 $\pm$ 0.14**	81 $\pm$ 8.1	15.58 $\pm$ 2.51	84 $\pm$ 14
Dexamethasone 48 h	1.14 $\pm$ 0.10***	66 $\pm$ 5.8	9.92 $\pm$ 1.70**	54 $\pm$ 9

Table 2. Effect of dexamethasone on type IV collagen-degrading activity in the culture media of HT-1080 cells

In all these experiments HT-1080 cells were preincubated with various concentrations of dexamethasone for 48 h before medium was collected for type IV collagen-degradation assay. The values are the means of duplicate determinations.

Expt. no.	[Dexamethasone] (μM)	Type IV collagen degradation	
		(c.p.m./ 10^6 cells)	(%)
1	0	236	100
	0.1	40	16
	10	37	15
2	0	197	100
	0.1	43	21
	10	70	35
3	0	904	100
	0.1	516	57
	10	634	70

decreased with dexamethasone. The decrease was accompanied by a similar decrease in the abundance of type IV collagen mRNA, indicating that type IV collagen synthesis was affected at the pre-translational level. Similarly, glucocorticoids have been shown to decrease the amounts of type I procollagen mRNA (Sterling *et al.*, 1983; Oikarinen *et al.*, 1984) and type II procollagen mRNA (Oikarinen *et al.*, 1985) in fibroblasts and chick embryos.

In the present study, a certain time period (12–24 h) was needed before the dexamethasone effect was maximal. The reasons for this are unknown, but it is possible that glucocorticoids induce the synthesis of some regulatory proteins which can then affect amino acid transport and protein synthesis.

Dexamethasone decreased the secretion of collagen into the medium in HT-1080 cells, thus causing an

accumulation of collagen in the cell layer. More detailed studies demonstrated, however, that the collagen which accumulated in the cell layer in the presence of dexamethasone was not inside the cells, since treatment of the cell layer by bacterial collagenase drastically decreased the collagen in the cell layer. In fact, the residual collagen content, which reflects the intracellular collagen, was the same in control cells and dexamethasone-treated cells. This clearly demonstrated that dexamethasone caused accumulation of type IV collagen in the close proximity of the cell, and collagen was not released into the medium as efficiently as in control cells. The explanation of the decreased secretion of type IV collagen is not clear. It is possible that dexamethasone affects the activity of enzymes involved in the cross-linking of basement-membrane collagen, leading to decreased release of type IV collagen into the medium.

The secretion of fibronectin, another major protein synthesized by HT-1080 cells (Alitalo *et al.*, 1980), was not inhibited. In fact, a marked increase in the secretion of fibronectin in the medium was seen in the presence of steroid. The results are in agreement with findings that in virus-transformed human skin fibroblasts, treatment of the cells with 10 nM–10 μM -dexamethasone for 24 h induced the deposition of an extensive fibronectin matrix (Furcht *et al.*, 1979); those authors suggested that corticoids might prevent the degradation of fibronectin. Another possibility could be that corticoids stimulate the synthesis of fibronectin. Dexamethasone was also shown to stimulate the synthesis of fibronectin in HT-1080 cells and normal human skin fibroblasts (Oliver *et al.*, 1983). The stimulation of fibronectin synthesis in HT-1080 cells was, however, much larger than that observed in normal fibroblasts (Oliver *et al.*, 1983).

Laminin, another basement-membrane component, was not seen when the cells were labelled with [^{14}C]proline (Alitalo *et al.*, 1980). However, a minor band was seen in the position corresponding to laminin when the cells were labelled with [^{35}S]methionine (results not shown). Interestingly, a new band of 150 kDa was seen on gels when the cells were incubated with dexamethasone.

The activity of type IV collagen-degrading enzyme(s) was inhibited by dexamethasone in HT-1080 cells. However, the inhibition was variable, and in some experiments only a slight effect was noted. The reason for the variable collagenolytic activity in the presence of glucocorticoid is unknown. However, it does not appear to be associated with a lack of steroid receptors, these being found in all experiments. Even though detailed characterization of the glucocorticoid receptors was not carried out, the binding parameters, such as the K_d , indicated that the receptor was quite similar to that which has been found in other tissues (Ponec *et al.*, 1980; Epstein & Munderloh, 1981; Leiferman *et al.*, 1983) or in HT-1080 cells in a previous study (Oliver *et al.*, 1983). Thus it is highly unlikely that the variable response of collagenase activity in the presence of dexamethasone was due to alterations in the receptor molecule. In rat mammary epithelium, dexamethasone has been reported to decrease the activity of type IV collagenase, inducing the accumulation of type IV collagen (Salomon *et al.*, 1981). In contrast, another growth factor, epidermal growth factor, stimulated collagen synthesis without affecting collagen degradation. More recently, cortisol was found to decrease the type IV collagenolytic activity of human skin fibroblasts, both in normally dividing cells and in cells after administration of a tumour promoter (Salo & Oikarinen, 1985). It has also been shown that type V collagenase activity is decreased by glucocorticoid (Mainardi, 1984). Thus it seems that glucocorticoids are mainly inhibitory to mammalian and basement-membrane collagenases.

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