REGULATION OF HUMAN SKIN COLLAGENASE ACTIVITY BY HYDROCORTISONE AND DEXAMETHASONE IN ORGAN CULTURE

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SUMMARY Hydrocortisone and dexamethasone (9α-fluoro, 16α-methyl prednisolone) prevent the appearance of collagenase in cultures of normal human skin, human rheumatoid synovium and rat uterus. Hydrocortisone is maximally inhibiting at 10^{-7}M and dexamethasone at 10^{-8}M in culture medium. Neither steroid is an inhibitor of enzyme activity. The loss of collagenase activity in cultured tissue is not accompanied by detectable inhibition of protein synthesis. Reduction of enzyme activity in culture medium is concomitant with a parallel cessation of tissue collagen degradation, indicating that the tissue fails to produce active collagenase in the presence of physiologic levels of glucocorticoids.

INTRODUCTION Human skin in organ culture, produces a specific collagenase which is required for the initiation of collagen catabolism (1). Normal physiologic control of collagenase synthesis and thus the control of mammalian collagen catabolism is only poorly understood, and is currently under intensive investigation (2-7).

Corticosteroids have been reported to exert major effects on the metabolism of protein in mammals in a number of systems (8). In view of the profound effects of these hormones *in vivo*, it was of interest to determine whether steroids could affect *in vitro* collagen metabolism in human skin. We report here the effects of two glucocorticoids, hydrocortisone and its potent synthetic analogue, 9 alpha-fluoro, 16 alpha-methyl prednisolone (dexamethasone), on the *in vitro* production of human skin collagenase. Both steroids prevent the appearance of active collagenase and concomitantly inhibit the *in vitro* degradation of the endogenous collagen of cultured skin.

MATERIALS AND METHODS Normal human skin was obtained at surgery and was immediately prepared for organ culture as previously described (1).

Briefly, tissue was cultured in Dulbecco's Modified Eagles Medium-High glucose (Grand Island Biological Company), containing 200 units/ml Penicillin and Streptomycin. The tissue was incubated at 37°C in an atmosphere of 95% O_2 and 5% CO_2. The medium was changed daily and each day's medium was collected and buffered with Tris-HCl, pH 7.4 to 0.05 M. Steroids were added in absolute ethanol (10 ul/100 ml) from appropriate stock solutions to obtain the desired concentrations. Cycloheximide was added directly to the medium. Collagenase activity in crude culture medium was assayed on reconstituted, native, ^{14}C-glycine labeled collagen fibrils as previously described (9). Tissue collagen degradation was quantitated by determining the level of hydroxyproline-containing peptides in culture medium (4,5). Hydroxyproline was measured by the method of Bergmann and Loxley (10). Protein synthesis was determined by measuring the amount of ^{3}H-leucine incorporated into CCl_3COOO insoluble protein.

RESULTS AND DISCUSSION The effect of hydrocortisone and dexamethasone on cultures of human skin explants is compared to that of cycloheximide in Tables 1 and 2.

Both dexamethasone, at 10^{-8}M, and hydrocortisone, at 10^{-7}M, reduce enzyme

Table 1

Effect of Hydrocortisone on Collagenase Activity and Collagen Degradation in Human Skin Cultures

Culture Conditions	Medium Collagenase *	% Inhibition	Medium Hydroxyproline†	% Inhibition
Control	4.05		115	
Hydrocortisone, 10^{-7}M	0.39	90	38	68
" 10^{-8}M	1.58	61	85	26
" 10^{-9}M	4.02	0	120	0

Organ cultures of human skin were prepared as described in Methods. Aliquots (150 ul) of crude culture medium on the third day of culture were incubated for 18 hrs. with 200 ug native ^{14}C-glycine labeled collagen fibrils (3600 cpm). *Results are expressed as ug collagen degraded per mg medium protein. †Medium hydroxyproline represents the total imino acid in the medium from two culture flasks (30 ml).

Table 2

Culture Conditions	Medium Collagenase*	% Inhibition	Medium Hydroxyproline+	% Inhibition
Control	10.49		290	
Dexamethasone, 10^{-8}M	1.51	86	76	74
" 10^{-9}M	2.30	78	96	67
" 10^{-10}M	4.89	54	222	23
" 10^{-11}M	6.26	41	179	38
" 10^{-12}M	9.10	14	298	0
" 10^{-13}M	11.20	0	319	0
Cycloheximide, 40 ug/ml	0.53	95	-	-

Cultures and assays were performed as described in Methods and in Table 1. *Collagenase activity is expressed as ug collagen degraded per mg medium protein. +Medium hydroxyproline represents the total imino acid in the medium from two culture flasks (30 ml).

activity by 80-90%. Cycloximide at a concentration of 40 ug/ml, as expected, inhibits protein synthesis in the cultured skin by 95%-100% (Table 3). Cycloheximide also prevents the appearance of collagenase in the cultures (Table 2), and the reduction in enzyme activity is a direct result of the inhibition of overall protein synthesis in this as in other mammalian tissues (1,4,5). However, neither of the steroids affects overall protein synthesis (Table 3), hence dexamethasone at 10^{-8}M and hydrocortisone at 10^{-7}M in culture medium, appear to selectively inhibit the production of active collagenase.

The inhibition of collagenase activity is dependent on steroid concentration (Tables 1 and 2) in the culture medium, with consistently detectable inhibition occurring at 10^{-12}M dexamethasone, and 10^{-8}M hydrocortisone.

Concomitant with the inhibition by these steroids of collagenase activity is a reduction in the level of hydroxyproline-containing peptides in the culture medium (Tables 1 and 2). It has been shown that the level of these

Table 3

Effect of Glucocorticoids on Protein Synthesis in Human Skin Cultures

	cpm ^{3}H-leucine incorporated	% Inhibition
Control	10,390	-
Dexamethasone 10^{-8}M	11,160	0
Cycloheximide	510	95
Control	11,450	0
Hydrocortisone 10^{-7}M	10,850	5

Cultures were initiated and maintained as described in Methods. On the third day of culture, ^{3}H-leucine was added to culture medium (0.2 uc/ml) for 6 hrs. The tissue was harvested, blotted, weighed and homogenized in 5 volumes of 10% TCA and dissolved in 10 ml of Soluene (Nuclear-Chicago) at 60°C, and radioactivity determined by liquid scintillation spectrometry. Results are expressed as ^{3}H-leucine cpm per g wet weight.

peptides parallels the collagenase activity in cultures of rat uterus (4,5), resorbing tadpole tailfin (11), and recent studies in this laboratory (manuscript in preparation) indicate that this relationship is true in human skin cultures as well. Thus, any reduction in collagenase production results in a reduced level of hydroxyproline-containing peptides.

At steroid concentrations which maximally inhibit the appearance of active collagenase, the release of these peptides into the culture medium is abolished. Thus, by inhibiting the production of active enzyme, dexamethasone and hydrocortisone inhibit endogenous collagen degradation of human skin in vitro. This inhibition of collagen catabolism is dependent upon steroid concentration (Tables 1 and 2) and is parallel with the inhibition of collagenase activity.

By two criteria then, direct assay of collagenase in the medium and measurement of collagen degradation, it appears that glucocorticoids can inhibit collagen degradation by preventing the production of active collagenase in human skin explants. The effect appears to be highly selective, in that

Table 4

Effect of Corticosteroids on Collagenase Activity in Cultures of Human Rheumatoid Synovium and Involuting rat uterus

Tissue		Medium Collagenase*	% Inhibition
Rat Uterus	Control	87	-
	Dexamethasone 10^{-7}M	15	83
Human Rheumatoid Synovium	Control	63	-
	Dexamethasone 10^{-8}M	3	95

Cultures were prepared and assays performed as described in Methods and in Table I. *Collagenase activity is expressed as ug collagen degraded per mg medium protein.

collagenase activity is totally abolished by concentrations of steroid which exhibit no detectable effect on tissue protein synthesis.

An indication that the effect of these two steroids on collagenase and collagen degradation may be of general significance in the regulation of collagen metabolism is the observation that in two tissues other than human skin, human rheumatoid synovium, and rat uterus, dexamethasone similarly inhibits the in vitro production of active collagenase (Table 4). In rheumatoid synovial cultures a concentration of 10^{-8}M is sufficient to abolish enzyme activity 95-100%, while in cultures of rat uterus, maximal inhibition ($\geq$ 80%) is reached at 10^{-7}M.

The mechanism by which corticosteroids inhibit collagenase production is presently unknown. Indeed, very little is known about the primary role of corticosteroids in the regulation of any eukaryotic metabolic process. Numerous specific enzymes produced by cultured cells have shown that glucocorticoids can induce the production of enzymes such as tyrosine amino transferase and alkaline phosphatase (12). A recent report suggests that corneal fibroblast collagenase is induced by high concentrations of dexamethasone phosphate (13). In the systems described here hydrocortisone and dexamethasone are added to culture medium at concentrations well below that of the

concentration of hydrocortisone in human plasma. In no case has a stimulation of collagenase been observed. Rather, inhibition is consistently observed even at very low steroid concentrations and cannot be attributed to an overall effect on protein synthesis. Thus, the effect of corticosteroids on collagenase and collagen catabolism that we observe *in vitro* may be indicative of a physiologic regulatory role of corticosteroids on *in vivo* collagen metabolism in human skin and possibly other animal tissues as well.

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