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RECEPTOR MEDIATED GLUCOCORTICOID INHIBITION OF PROTEIN SYNTHESIS IN ISOLATED BONE CELLS

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SUMMARY

Dexamethasone at concentrations of 1.5×10^{-8} M and higher inhibited the incorporation of [^{14}C]-proline into proline and hydroxyproline-containing protein fractions of freshly-isolated bone cells. Amino acid incorporation was only partially blocked by 1.5×10^{-5} M dexamethasone after a 5 h incubation, although incorporation could be completely blocked with puromycin. Parathyroid hormone enhanced [^{14}C]-proline incorporation into total protein after a 3 h incubation, an effect which was abolished by simultaneous treatment with dexamethasone. Other steroids and vitamin A were tested alone and in combination with dexamethasone to determine whether the effects of these agents on bone cell protein synthesis reflected their affinities for bone cytosol binding sites previously demonstrated. At the concentrations tested, progesterone, the spirolactone SC-26304 and vitamin A all inhibited incorporation; cortexolone and estradiol did not. Cortexolone, progesterone and SC-26304 reversed the inhibitory effects of dexamethasone. These results are consistent with the earlier binding studies, and suggest that the effects of glucocorticoids on bone cell protein synthesis are mediated by cytosol receptors.

INTRODUCTION

The mechanism of action of glucocorticoids, as for all steroid hormones, is postulated to require initial binding of the hormone to specific receptors in the cytoplasm of target tissue [1]. We have previously demonstrated the presence of glucocorticoid binding proteins in bone cells prepared from fetal rat calvaria [2]. These binding sites have now been further characterized as receptors for hormone action, based upon the observation that they mediate transfer of the hormone to the nucleus [3]. Such isolated bone cells have previously proven useful in studies of bone metabolism, and provide a system in which glucocorticoid induced physiological functions can be correlated with glucocorticoid receptor binding. In the present study, employing freshly isolated bone cells and dexamethasone† as the glucocorticoid, we confirm the earlier results of Peck, *et al.* which demonstrated that glucocorticoids inhibit total protein and collagen synthesis in cultured fetal bone cells [4].

In addition, using inhibition of protein synthesis as a marker of glucocorticoid activity, we have examined the effects of a series of other steroids on this system in an attempt to relate receptor binding to biological activity. According to the postulated model of steroid action, those steroids capable of displacing dexamethasone from its receptor binding sites should exhibit biological activity. This binding activity may be functionally expressed as pure antagonism, that is reversal of the dexamethasone effect. Alternatively, binding activity may be manifested by agonist activity, that is a dexamethasone-like effect. Steroids that do not compete for the dexamethasone receptors would be expected to exhibit neither agonist nor antagonist activity in the system. The results of the present study are consistent with this postulated model of steroid–receptor interaction and support the contention that glucocorticoid inhibition of protein synthesis in isolated bone cells is a receptor mediated event.

Glucocorticoids have also been shown to inhibit parathyroid hormone (PTH) and vitamin A induced bone resorption as measured by ^{45}Ca release from fetal rat bone *in vitro* [5–7]. The continued effect of these two agents on bone resorption requires new protein synthesis [8–9]. Therefore, one possible mechanism of the glucocorticoid antagonism is via inhibition of the synthesis of new protein. Our current results are not consistent with such a simple explanation, since the effects of vitamin A and PTH and their

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† The following abbreviations have been used: dexamethasone: 9-α-fluoro-11β,17,21-trihydroxy-16α-methyl-pregna-1,4-diene-3, 20-dione; cortexolone: 17,21-dihydroxy-4-pregnene-3,20-dione; SC-26304: 3-(3-oxo-7α-carboxyisopropyl-17β-hydroxy-4-androstene-17α-yl) propionic acid γ-lactone; HEPES: hydroxylethylpiperazone N[1]-2-ethanesulfonic acid; TCA: trichloroacetic acid; PPO: 2,5-diphenyloxazole; dimethyl-POPOP: 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene; PTH: parathyroid hormone.

interactions with glucocorticoids were quite dissimilar.

EXPERIMENTAL

Isolation of bone cells. Bone cells were isolated from fetal rat calvaria by a modification [10, 11] of the method of Peck [12]. Fetuses were obtained from 20–21 day pregnant Holtzman Sprague–Dawley rats. The calvaria were scraped gently to remove attached connective tissue, rinsed with 0.9% saline and then incubated for 1.5 h in a HEPES-buffered (pH 7.4) medium [11] containing 2 mg/ml crude collagenase (Worthington Type II). At the end of the incubation, the digest was filtered through nylon mesh (35 μ) and 5 ml of incubation medium was added to the undigested bone fragments to wash off adherent cells, which were then pooled with the filtrate. The combined cells were washed 3 times and centrifuged at 1300 rev./min for 7 min. Cells were counted with a hemocytometer.

Studies of [^{14}C]-proline uptake and incorporation into protein. Cells were preincubated with hormones at 37°C for 15 min to 1 h prior to the addition of [^{14}C]-proline. The incubation medium, unless otherwise indicated, was the same as that used above without the collagenase and with 50–100 U/ml penicillin added. The cell concentration was 1–2 × 10^6/ml. Drugs were added in incubation medium or 95% ethanol. The final ethanol concentration was 0.2% or less. Control cultures received an equivalent volume of solvent. At the end of preincubation, each flask received the same amount of radioactivity and 0.1 ml was taken for liquid scintillation counting. At the end of the incubation, an aliquot was taken for protein analysis [13] and a volume of ice-cold non-radioactive medium equal to the volume of the incubation medium was added to each incubation vessel. The cells were washed and centrifuged 5 times with ice-cold medium and the washings discarded. The cellular free amino acid plus the soluble peptide pool (referred to as the free amino acid pool) was determined by extracting the cells for 3 h in ice-cold 5% TCA. An aliquot of the extract was taken for liquid scintillation counting. The cells were then washed 3 times with cold 5% TCA. For determination of [^{14}C]-proline incorporation into protein, the washed cell pellet was suspended in water and sonicated for 3 s with a Branson Sonic Power Model S73 Sonifier. The sonicate was transferred to a 2 ml glass ampoule, an equal volume of concentrated HCl was added and the sealed ampoule was incubated for 18 h at 120°C. The hydrolysates were transferred to ampoules and evaporated to dryness. Water was added, and aliquots of the hydrolysate taken for assay of radioactivity in total-TCA precipitable proteins and for chromatographic separation of amino acids.

Separation of hydroxyproline from proline. The separation of the two amino acids in the hydrolysate was carried out by descending chromatography on Whatman No. 3 paper with a butanol–acetic acid–water (4:1:2 by vol.) system which adequately separated hydroxyproline (OH-proline) from proline after a 20 h run. The chromatograms were dried and cut at the appropriate portion as indicated by ninhydrin and placed in liquid scintillation counting vials containing the scintillation fluid. The radioactivity migrating with the appropriate R_F value for proline was identified by an appropriate standard and taken as a measure of incorporation of proline into both collagen and non-collagen proteins [14]. The radioactive OH-proline, identified similarly, was taken as a measure of new collagen synthesis [15].

Counting radioactivity and quench correction. Scintillation fluid consisted of 3 g of PPO and 100 mg of dimethyl POPOP dissolved in a liter of toluene, to which 300 ml of ethanol per liter was added. Radioactivity was measured with a Packard Tri-Carb Liquid Scintillation Spectrometer and the counts were corrected for quenching or for self-absorption by the use of the channels ratio method of Bruno *et al.*[16].

Statistical analysis. The data in each experiment were subjected to analysis of variances. Standard errors were calculated from the residual term of the analysis of variances. The significance of difference between mean values in each experiment was evaluated by a two-tailed *t*-test (for two groups) or by Hartley's modification of Tukey's [17] test (for multiple comparisons when there were more than two groups).

Drugs and chemicals. UL-[^{14}C]-proline, S.A. 198 mCi/mmol was purchased from ICN (International Chemical and Nuclear Corp.). Parathyroid Hormone (PTH), partially purified through the stage of trichloroacetic acid precipitation, was from Wilson Laboratories. A stock solution of PTH, 0.25 mg/ml with 970 units/mg potency was prepared by dissolving the hormone in 0.001 N HCl. The stock solution was stored at −70°C. Bovine serum albumin, crystalline, was purchased from ICN, puromycin dihydrochloride, testosterone and vitamin A from Sigma Chemical Co., cortexolone from Steraloids, progesterone and 17β-estradiol, chromatographic grade, from Calbiochem, and vitamin D_3 from Aldrich Chemical Co. The spirolactone SC-26304 and dexamethasone were generous gifts from G. D. Searle and Merck, Sharp and Dohme respectively. Solutions of the steroids and vitamin A were prepared in 95% ethanol.

RESULTS

[^{14}C]-Proline incorporation by isolated bone cells

The uptake and incorporation of [^{14}C]-proline by isolated bone cells was monitored over a 2 h period to determine whether protein synthesis could be demonstrated *in vitro* (Fig. 1). A rapid uptake of [^{14}C]-proline from the incubation medium into the free amino acid pool was noted within the first 30 min. and there followed a less rapid but almost linear uptake for the following 90 min. No significant

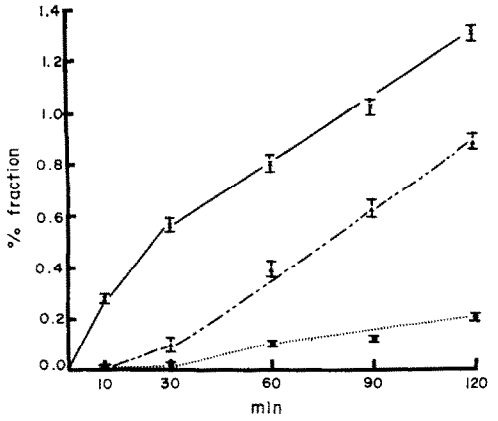

Fig. 1. [^{14}C]-Proline uptake and incorporation by bone cells. Cells were incubated in a medium containing Earle's salts (Grand Island Biological Company), 4.68 g/l TES (N-Tris [hydroxymethyl] methyl-2-amino sulfonic acid) and 10% calf serum. Figures on the ordinate represent the % fraction of the original radioactivity added to the medium at the start of the incubation. All values are mean ± standard error. Each group consisted of at least five observations. ×——×, free amino acid pool; ▲---▲ incorporated proline; ●····● incorporated hydroxyproline.

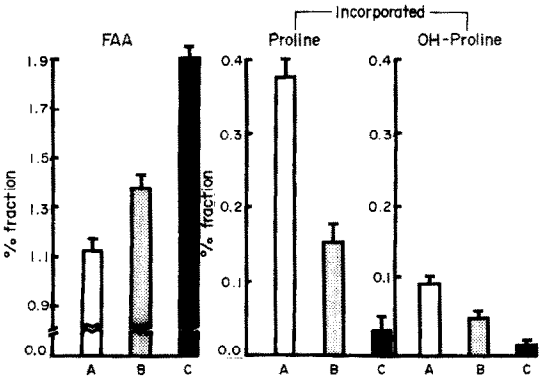

Fig. 2. Effect of puromycin on proline and hydroxyproline incorporation in bone cells. Cells were incubated for 2 h after the simultaneous addition of [^{14}C]-proline and puromycin. Figures on the ordinate represent the % fraction of the radioactivity added to the medium at the start of the incubation. Values are mean ± standard error. Each group consisted of at least three observations. A—control; B—0.01 mM puromycin; C—0.2 mM puromycin. FAA—radioactivity in free amino acid pool; incorporated Proline—cold TCA-inextractable proline pool; Incorporated OH-Proline—cold TCA-inextractable hydroxyproline pool. FAA: A vs B, n.s.; A vs C, $P < 0.01$; B vs C, $P < 0.01$; OH-Proline: A vs B, $P < 0.05$; A vs C, $P < 0.01$; B vs C, $P < 0.05$.

amount of [^{14}C]-proline was incorporated into protein before 30 min., and there was a further delay before [^{14}C]-OH-proline appeared in the TCA insoluble fraction.

The effect of puromycin, an inhibitor of protein synthesis, was tested to validate that the observed incorporation of amino acid was the result of protein synthesis. Puromycin produced a dose-related inhibition in the appearance of [^{14}C]-proline and [^{14}C]-OH-proline in protein which was associated with increased [^{14}C]-proline in the free amino acid pool (Fig. 2).

Effects of dexamethasone on protein synthesis

The minimum effective concentration of dexamethasone required to inhibit [^{14}C]-proline incorporation into protein in a 3 h incubation was 1.5×10^{-7} M (Table 1). After 5 h of incubation, significant inhibition was obtained with 1.5×10^{-8} M dexamethasone. A maximum effect was obtained with 1.5×10^{-7} M. No significant effects of dexamethasone were detected on the free amino acid pool. A time course study of the effects of 1.5×10^{-7} M dexamethasone is shown in Fig. 3. There is a lag period of more than 1 h, following which dexamethasone significantly inhibits [^{14}C]-proline incorporation. The reduction in protein synthesis was not associated with significant differences in labelling of the free amino acid pool at any time point (data not shown) suggesting that the inhibition was not related to amino acid transport. The reduction in protein synthesis at 5 h was significant in both the proline and OH-proline fractions, with the [^{14}C]-proline fraction reduced by 20.3% and the [^{14}C]-OH-proline fraction reduced by 33.3% (Fig. 4).

Table 1. Effect of dexamethasone concentration on uptake and incorporation of [^{14}C]-proline by freshly isolated bone cells

Group	Dexamethasone	FAA	*P*	Protein	*P*
Experiment I					
A	Control	45.2 ± 1.3		53.7 ± 1.9	
B	1.5×10^{-9} M	42.3 ± 1.1	all	49.2 ± 1.7	AB: NS
C	1.5×10^{-8} M	48.5 ± 1.1	NS	48.3 ± 1.7	AC: NS
D	1.5×10^{-7} M	45.1 ± 1.1	vs A	44.2 ± 1.7	AD: <0.05
Experiment II					
A	Control	47.8 ± 2.3		119.1 ± 4.2	
B	1.5×10^{-8} M	54.5 ± 2.0	all[A]	105.3 ± 3.6	AB: <0.05
C	1.5×10^{-7} M	48.0 ± 2.0	NS	93.3 ± 3.6	AC: <0.01
D	1.5×10^{-6} M	46.9 ± 2.0	vs A	98.5 ± 3.6	AD: <0.01
E	1.5×10^{-5} M	49.7 ± 2.0		97.2 ± 3.6	AE: <0.01

Each value represents mean ± S.E. with at least 3 observations in each group. Values are expressed as d.p.m. × 10^{-2}/mg protein. Cells were preincubated with dexamethasone for 1 h in both experiments, and were incubated with isotope for 2 h in Experiment I and 4 h in Experiment II. FAA = free amino acid pool. Protein = TCA-inextractable pool.

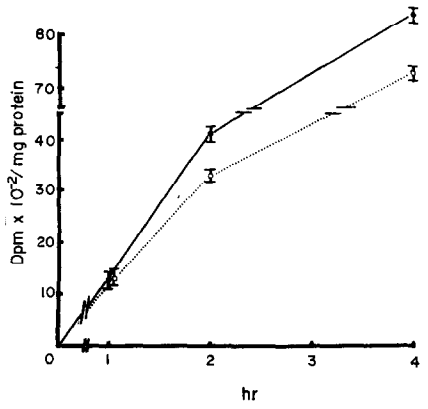

Fig. 3. Time course of the effect of dexamethasone on [^{14}C]-proline incorporation in bone cells. Treated cells were preincubated for 1 h with dexamethasone (1.5×10^{-7} M) before the addition of [^{14}C]-proline. Each value represents the mean ± standard error of 3 observations. Incorporation was significantly inhibited ($P < 0.01$) by dexamethasone at 2 and 4 h. ●——●, control; O···O, dexamethasone.

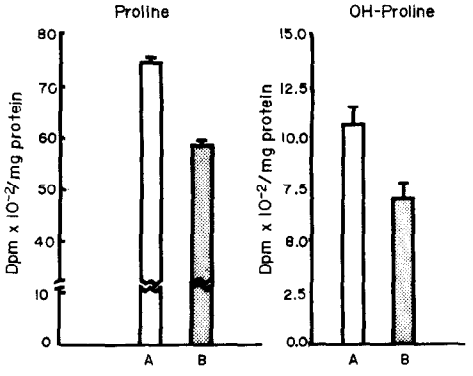

Fig. 4. Inhibition by dexamethasone of the incorporation of [^{14}C]-proline into TCA-inextractable proline and hydroxyproline pools of bone cells. Cells were preincubated with dexamethasone (1.5×10^{-7} M) for 1 h before the addition of [^{14}C]-proline; tissues were then incubated for an additional 4 h. Values are given as mean ± standard error. Control groups contained 3 observations; 4 samples were used for the dexamethasone treatment. A—control; B—dexamethasone. Proline—A vs B, $P < 0.001$; OH-Proline—A vs B, $P < 0.025$.

Effect of parathyroid hormone

PTH, at 0.25 or 0.5 μg/ml, enhanced incorporation of [^{14}C]-proline into the TCA inextractable pool after a 5 h incubation (Table 2). The addition of 1.5×10^{-7} M dexamethasone reversed the effect of PTH on proline incorporation. The combination of PTH and dexamethasone increased the [^{14}C]-proline in the free amino acid pool. PTH alone did not significantly elevate the [^{14}C]-proline in this fraction.

Reversal of the dexamethasone effect by competitors of receptor binding

We previously demonstrated the presence of cytoplasmic receptor proteins for glucocorticoids in these bone cells [2]. If the inhibition of protein synthesis by dexamethasone is a receptor mediated event, competitors for receptor binding sites should alter the dexamethasone effect. To test this hypothesis, compounds which had previously been shown to either compete or not compete with dexamethasone for binding sites in bone cells were added to the cell suspensions separately or in combination with dexamethasone, and effects on [^{14}C]-proline incorporation into protein assayed. The concentration of competitor used was 100-fold the dexamethasone concentration, to simulate the treatment conditions used in the binding experiments. The results are shown in Table 3. Dexamethasone alone at a concentration of 1.5×10^{-7} M significantly inhibited protein synthesis in all experiments as measured by [^{14}C]-proline incorporation (A vs C, Table 3). Uptake into the free amino acid pool was unchanged in all studies. Cortexolone, when added alone at 1.5×10^{-5} M, did not significantly effect protein synthesis in the isolated bone cells; that is, it possessed no agonist activity (A vs B, Table 3). However, when dexamethasone and cortexolone were added simultaneously, protein synthesis was not significantly different from that observed in the controls (A vs D, Table 3). In other

Table 2. Effects of PTH alone and combined with dexamethasone on protein synthesis in freshly isolated bone cells

Group	Treatment	FAA	*P*	Protein	*P*
Experiment I					
A	Control	1.49 ± 0.05		4.57 ± 0.23	
B	PTH	1.63 ± 0.05	AB:NS	5.61 ± 0.23	AB: <0.05
C	Dex. + PTH	1.75 ± 0.06	AC: <0.05	4.37 ± 0.26	AC:NS
			BC:NS		BC: <0.05
Experiment II					
A	Control	26.7 ± 1.3		69.1 ± 1.1	
B	PTH	27.9 ± 1.3	AB:NS	74.7 ± 1.1	AB: <0.05
C	Dex. + PTH	32.8 ± 1.3	AC: <0.05	63.8 ± 1.1	AC: <0.01
			BC:NS		BC: <0.01

Values are given as mean ± S.E., with at least 3 observations in each group. Cells were preincubated without [^{14}C]-proline for 1 h. After addition of [^{14}C]-proline cells were incubated for an additional 4 h. 0.25 μg/ml PTH was added in Experiment I and 0.50 μg/ml in Experiment II. The concentration of dexamethasone was 1.5×10^{-7} M. Data are expressed in % fraction of original radioactivity added to the incubation medium in Experiment I and d.p.m. $\times 10^{-2}$/mg protein for Experiment II.

Table 3. Effects of combined treatment with dexamethasone and other steroids and vitamin A on incorporation of [^{14}C]-proline into protein by bone cells

Group	Treatment				
	Cortexolone ($N = 6$)	Progesterone ($N = 7$)	SC-26304 ($N = 3$)	Estradiol ($N = 8$)	Vitamin A ($N = 3$)
A—Control	100 ± 4.5	100 ± 2.4	100 ± 2.4	100 ± 4.7	100 ± 4.3
B—Treatment	102.2 ± 4.5	89.3 ± 2.4	88.4 ± 2.4	93.0 ± 4.7	45.3 ± 4.3
C—Dexamethasone	78.7 ± 4.5	81.4 ± 2.4	79.2 ± 2.4	71.6 ± 4.7	76.5 ± 4.3
D—Treatment and Dexamethasone	93.6 ± 4.5	88.5 ± 2.4	91.2 ± 2.4	78.3 ± 4.7	44.9 ± 4.3

Group comparison	Statistical analysis (P values)				
	Cortexolone	Progesterone	SC-26304	Estradiol	Vitamin A
A vs B	NS	<0.05	<0.05	NS	<0.01
A vs C	<0.01	<0.01	<0.01	<0.01	<0.01
A vs D	NS	<0.05	NS	<0.01	<0.01
B vs D	NS	NS	NS	<0.05	NS
C vs D	<0.05	NS	<0.05	NS	<0.01

Dexamethasone was added at 1.5×10^{-7} M and the competing compounds at 1.5×10^{-5} M. Cells were preincubated with steroids for 30 min. to 1 h before addition of [^{14}C]-proline. Incubation with [^{14}C]-proline was for an additional 4 or 5 h. Values calculated as d.p.m./mg protein were normalized for tabulation (and for facilitating comparison) by adjusting the control values to 100 S.E. d.p.m./mg protein. Therefore the values in this table are "relative d.p.m./mg protein".

words, the inhibitory effect of dexamethasone was reversed by adding cortexolone (C vs D, Table 3).

Progesterone at 1.5×10^{-5} M significantly decreased [^{14}C]-proline incorporation when given by itself although the effect was not as great as that of dexamethasone. In combination with dexamethasone, progesterone treatment resulted in a reversal of the dexamethasone inhibition to the level of protein synthesis seen with progesterone alone. These results suggest that progesterone is a weak agonist as well as an antagonist in this system, a so called sub-optimal inducer [18, 19].

The spirolactone compound SC-26304 produced results similar to those seen with progesterone. When administered alone, this drug caused an inhibition of protein synthesis. In combination with dexamethasone there was a reversion of the inhibitory effect from the level seen with dexamethasone alone to that of SC-26304 alone. These results, as those of progesterone, indicate that SC-26304 is a sub-optimal inducer in this system.

The effects of 1.5×10^{-5} M estradiol differed from the preceding compounds. Estradiol showed only minimal binding in our previous studies [2] and in the present experiments behaved as an inactive compound. It failed to show any agonist effects of its own and did not exhibit any antagonism of the dexamethasone effect. On the other hand, vitamin A (1.5×10^{-5} M), which also failed to compete for dexamethasone binding sites [2], was a potent inhibitor of protein synthesis when given alone. It is of interest that dexamethasone plus vitamin A is no more active than vitamin A alone. Total bone cell protein was not significantly altered by any of the treatments.

DISCUSSION

The data presented demonstrate that freshly isolated bone cells possess the capacity to incorporate precursor amino acids into protein *in vitro* (Fig. 1). Identification of OH-proline in the product indicates that collagen is being synthesized in addition to non-collagen protein. The rate and pattern of amino acid uptake and incorporation are similar to those previously reported for cell-free whole embryo extracts [20] or intact isolated fetal rat calvaria [14], with a delay of about an hour before [^{14}C]-OH-proline appears in the TCA insoluble fraction. Puromycin, in a dose dependent fashion, inhibits [^{14}C]-proline incorporation into both proline and OH-proline protein fractions with a simultaneous increase in the free amino acid fraction (Fig. 2). These results support the contention that the amino acid incorporation reflects protein synthesis.

The effects of dexamethasone on this system become manifest after a latent period of 1–2 h (Fig. 3). Subsequently, over time, there is a dose dependent inhibition of amino acid incorporation into both proline and OH-proline fractions. The glucocorticoid effect is comparable to results previously reported for hydrocortisone in cultured bone cells [4]. The absence of any detectable increase in the free amino acid pool in our studies may be related to the small magnitude of the effect on protein synthesis as well as dexamethasone-induced decreases in the formation of small soluble peptides present in this pool. Recently, Dietrich and Raisz[21] have reported that short term exposure to glucocorticoids stimulates collagen synthesis in isolated rat calvaria. This effect of glucocorticoids was dose-dependent and not anta-

gonized by cortexolone. The basis for the difference between their findings and ours is not known. The isolated cells could represent a different cell population from those in the intact tissue, if, for example, certain cell types did not survive the isolation procedure. These dual effects could explain why we did not observe clear dose-response relationships with increasing concentrations (Table 1). If the glucocorticoid was producing both stimulatory and inhibitory effects in the isolated cells, and the stimulatory effects had a higher threshold concentration, a flat dose-response curve could result. Our studies do not unequivocally rule out the possibility that the observed effects of glucocorticoids are due to an increase in the precursor pool of unlabelled proline; however, the lack of significant effects on total bone cell protein makes this less likely.

The inhibition of protein synthesis is steroid specific, being elicited by only certain steroids (Table 3). Cortexolone, at 100-fold the dexamethasone concentration, completely abolished the dexamethasone effect. Since we have previously demonstrated that, at this concentration ratio, the majority of dexamethasone binding to receptors is blocked [2], these data support the concept that the inhibitory action of dexamethasone on protein synthesis is a receptor-mediated event. When cortexolone was added to the isolated cells in the absence of dexamethasone, it exerted no effect on the [^{14}C]-proline incorporation indicating that this drug functions as a pure antagonist. Cortexolone has also been shown to block the dexamethasone inhibition of PTH induced bone resorption in organ cultures of bone [7].

Progesterone and SC-26304 both antagonized the inhibitory action of dexamethasone, but in both cases the antagonism was not complete. In contradistinction to cortexolone, these two steroids did exhibit agonist activity when administered in the absence of dexamethasone. And in fact, the final activity of the dexamethasone plus progesterone and the dexamethasone plus SC-26304 combinations approached the level of agonist activity of progesterone and SC-26304 given independently. In other words, the inability to achieve complete inhibition of the dexamethasone effect was a result of the intrinsic agonist activity manifested by these drugs. Progesterone has previously been demonstrated to act as a glucocorticoid antagonist with perhaps minimal agonist activity in the induction of tyrosine aminotransferase in hepatoma tissue culture cells [19, 22]. SC-26304 functions mainly as a mineralocorticoid antagonist [23], but has been shown to possess agonist activity in toad bladder short circuit current experiments [24]. Further study of the effects of the spirolactones as agonists in glucocorticoid systems is presently underway.

Estradiol, which did not compete for dexamethasone receptor binding sites [2], failed to demonstrate either agonist or antagonist potency. Vitamin A, which also does not interact with the dexamethasone receptors, nevertheless, is a potent inhibitor of protein synthesis, apparently via a separate pathway.

Glucocorticoid receptors have recently been characterized in bone cells in primary culture [3]. Those studies, like the current ones, were carried out with a heterogeneous cell population, although three separate cell populations isolated through timed digestion were not different in dexamethasone binding parameters from the total population. The dissociation constant for dexamethasone and the number of binding sites per bone cell were similar to those determined for freshly isolated cells [2]. Inhibition of cell growth was used as the parameter of glucocorticoid action. As in the present study, dexamethasone did not produce complete inhibition and a plateau in the dose-response curve was obtained at 1.3×10^{-7} M dexamethasone. These results were interpreted to suggest a subpopulation of cells which were resistant to the inhibitory action of the steroids, an interpretation which could be applied to the present findings, as well. Comparison of the effects of steroids on cell growth revealed that triamcinolone acetonide was slightly more potent than dexamethasone; cortisol and corticosterone were progressively less potent. Progesterone had no effect on cell number when added alone to the cell cultures. However, when 1.3×10^{-6} M progesterone was added in combination with 1.3×10^{-8} M dexamethasone, the inhibitory effect of the glucocorticoid was partially blocked. The effects of the steroids on cell growth were consistent with their effects on cytosol and nuclear extract binding. Cortexolone was an effective competitor for binding but its effects on growth were not examined.

Turning to the interaction of glucocorticoids with drugs that cause bone resorption, vitamin A stimulates resorption of fetal bones *in vitro* at concentrations in the 0.3 to 3.0×10^{-5} M range [5, 6, 25]. Hydrocortisone, at 10^{-6} M, antagonized these effects of vitamin A. The results of the current studies in which dexamethasone and vitamin A both decreased protein synthesis suggest that the interaction of the two agents on bone resorption is not due to mutually antagonistic effects on overall bone protein synthesis. Glucocorticoids likewise antagonize the *in vitro* bone-resorbing activity of PTH. In the current studies, PTH stimulated incorporation of [^{14}C]-proline into protein and this effect was antagonized by dexamethasone at concentrations of the two agents in the same range as those effective in culture. Although this could indicate inhibition of protein synthesis as the underlying mechanism for the PTH-glucocorticoid interaction, this conclusion must be made with caution in view of the above mentioned results with vitamin A. Also, the previously reported effects of PTH on amino acid uptake and protein synthesis are related to the duration of exposure. Although amino acid transport may be stimulated with short term incubation [26], the latter effect is an inhibition of collagen synthesis [27, 28]. It appears that in order to definitively resolve the nature of the interaction of PTH

and glucocorticoids on resorption, the effects of the agents must be studied on the synthesis of proteins specifically involved in the resorptive events.

Estradiol, progesterone and cortexolone have previously been examined for their effects on bone resorption *in vitro* [6, 7, 29, 30]. Neither progesterone nor cortexolone at 10^{-5} M mimicked the antagonistic effects of hydrocortisone on PTH-induced resorption [7]. Although estradiol [6, 29] and progesterone [29] at high concentrations have been shown to inhibit PTH-induced bone resorption *in vitro*, more physiologic concentrations were ineffective [6, 7, 30]. Only one study [7] has thus far examined the interaction of another steroid (cortexolone) with a glucocorticoid on bone resorption *in vitro*. More of such studies, carried out with progesterone, estradiol and SC-26304 would help in evaluating the significance of the observed steroid interaction on binding and protein synthesis for the process of bone resorption.

It is generally accepted that glucocorticoids can cause osteoporosis. It is tempting to relate the inhibitory effect on bone protein and collagen synthesis to the clinical disorder, although no specific test of this concept has been made. The results presented here support the contention that glucocorticoids have important direct actions on bone cells in addition to possible indirect effects on bones mediated by changes in calcium homeostasis elsewhere in the body.

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