Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 6267–6271, September 1987
Medical Sciences

17β-Estradiol acts directly on the clonal osteoblastic cell line UMR106

(alkaline phosphatase/1,25-dihydroxyvitamin D_3/estrogens/bone)

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Communicated by Carl W. Gottschalk, May 8, 1987 (received for review September 30, 1986)

ABSTRACT **We studied the effect of 17β-estradiol (E) on the proliferation and alkaline phosphatase activity of cultured UMR106 cells, a clonal osteoblastic cell line. Growth rates were reduced and alkaline phosphatase activity was increased in cells incubated for 2 days in medium containing E (10^{-8} M). In contrast, E had no effect on the growth rates or alkaline phosphatase of a human fibroblastic cell line, S90E. The effect of E was not observed with low cell density or at confluence. 1,25-Dihydroxyvitamin D_3 antagonized the response to E. Preincubation of the cells with dexamethasone, a potent inducer of differentiation, reversed the effect of E or 1,25-dihydroxyvitamin D_3. These results indicate that cellular and/or extracellular factors such as cell density, the phase of the cell cycle, the state of differentiation, and the presence or absence of other steroids influenced the response of UMR106 cells to E. Serum was removed from the culture medium to minimize the effect of the steroids, growth factors, and nutrients present in serum. A striking stimulation of alkaline phosphatase by E occurred with serum-free conditions. This stimulation was biphasic over an E concentration from 10^{-12} to 10^{-8} M, with the peak response at 10^{-10} M. The action of E on UMR106 cells was metabolite-specific, since the isomer 17α-estradiol produced no effect on proliferation rates or alkaline phosphatase activity. The cyclic AMP response to parathyroid hormone (residues 1–34) was not altered by E treatment of these cells. In contrast, dexamethasone exposure did increase the cyclic AMP response to parathyroid hormone. These results demonstrate a direct effect of E on an osteoblastic cell line. They also raise the possibility that similar or identical actions of E occur in cultured normal osteoblasts.**

Estrogens influence the skeleton, as evidenced by the loss of bone density in postmenopausal women (1, 2) and the preventative action of exogenous estrogens on the loss of bone density in ovariectomized subjects (3). Estrogen administration also produces changes related to bone and mineral metabolism, such as increased intestinal absorption of calcium (4) and alterations in the circulating calciotropic hormones (4, 5). The mechanism of estrogens' action on the skeleton is still unknown. Most investigators have postulated an indirect pathway because of the apparent absence of estrogen receptors in bone (6–9). We report the results of experiments showing an effect of 17β-estradiol (E) on the UMR106 cell line, a well-characterized osteoblastic cell model (10–12).

MATERIALS AND METHODS

Materials. The culture media and additives were obtained from the Tissue Culture Facility of the Lineberger Cancer Research Center, University of North Carolina. The additives included fetal bovine serum, penicillin, streptomycin, insulin, transferrin, selenium, and trypsin, the latter for release of adherent cells. E and 17α-estradiol (17α-E) and dexamethasone were purchased from Sigma. Parathyroid hormone, as the synthetic fragment consisting of amino acid residues 1–34 of the rat hormone [PTH-(1–34)], was obtained from Peninsula Laboratories (San Carlos, CA). Chemicals for the alkaline phosphatase (ALP) assay and the scintillation fluid, Scintiverse E, were purchased from Fisher. Tritium-labeled adenine was purchased from ICN (catalog no. 27001). Tissue culture plates were obtained from Becton Dickinson.

Cell Culture. The UMR106 cells, a clonal line derived from a rat osteosarcoma, were donated by T. J. Martin (University of Melbourne, Melbourne, Australia). These cells were maintained in continuous culture by weekly passage in Dulbecco's modified Eagle's medium (DMEM) and Ham's F12 medium in 1:1 proportions (vol/vol). Fetal bovine serum (5%, vol/vol), penicillin (0.1 unit/ml) and streptomycin (100 μg/ml) were added to the medium. Under these conditions the cells approximately double in number every 24 hr and adhere to the plastic surface.

Cells were released from the culture plates by treatment with 0.25% trypsin followed by suspension of the released cells in serum-containing medium. After centrifugation (1000 × *g* for 5 min) the cells were washed twice with Hanks' balanced salt solution and resuspended in DMEM/F12 medium with or without fetal bovine serum as noted. Cells were pipetted into the wells of the culture plates (Falcon multiwell plate, catalog no. 93030, Becton Dickinson) and incubated at 37°C in 5% CO_2 and humidified air. When serum-free medium was used, insulin (6.25 μg/ml), transferrin (6.25 μg/ml), and sodium selenate (6.25 ng/ml) were added in addition to the antibiotics. Surface adherence and trypan blue exclusion were not altered by incubation in serum-free medium for up to 3 days.

A second osteoblastic cell line, ROS 17/2.8, was available in the laboratory of T. C. Peng (Department of Pharmacology, University of North Carolina School of Medicine). These cells were included in the experiments involving PTH-(1–34) and cAMP because this cell line manifested an enhanced cAMP response to parathyroid hormone when incubated with dexamethasone (13). The S90E cell line, a human fibroblast line, was generously donated by D. Clemmons (Department of Medicine, University of North Carolina School of Medicine). The ROS 17/2.8 cells and S90E cells were maintained in DMEM with 5% fetal bovine serum, penicillin, and streptomycin.

Cell Counting. The cell number was determined by a method previously reported from this laboratory (14). Brief-

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Abbreviations: E, 17β-estradiol; 17α-E, 17α-estradiol; ALP, alkaline phosphatase; PTH-(1–34), amino acid residues 1–34 of rat parathyroid hormone; 1,25-D_3, 1,25-dihydroxyvitamin D_3.

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ly, it involves aspiration of the medium and addition of a detergent solution to lyse plasma membranes and release nuclei of the adherent cells. The nuclei are counted by a Coulter Counter. These determinations were done in duplicate for each well.

ALP. An established chemical assay (15) was modified so that the enzymatic activity could be measured directly in the wells of the tissue culture plates. The modifications included reduction in the volume of the reagents and use of the outer row of wells for the standard curve. The outer row was cell-free and contained only medium. After the medium was aspirated from each well, diethanolamine (50 mM, pH 10.5, 100 μl) was added. *p*-Nitrophenyl phosphate (2.5 mM, 50 μl) was added to the wells containing cells. Wells that were free of cells received *p*-nitrophenol (1 mM) in stepwise quantities from 0 to 50 μl. Deionized water was added to the wells containing *p*-nitrophenol so that the final volume was 150 μl. The tissue culture plate was incubated for 30 min at 37°C. Then NaOH (0.1 M, 50 μl) was added to each well.

Absorbance of *p*-nitrophenol in the cell-free wells or formed by the conversion of *p*-nitrophenyl phosphate was determined at 405 nm in an enzyme immunoassay plate reader (Bio-Tek, Burlington, VT). The standard curve was constructed from the absorbance values plotted versus the nmol of *p*-nitrophenol per well. The correlation coefficient for absorbance versus amount of *p*-nitrophenol (0–50 nmol) in every experiment was greater than 0.99. The enzymatic activity, expressed as nmol of *p*-nitrophenol formed per 30 min, was determined by observing the absorbance value for a given sample and deriving the amount of *p*-nitrophenol from the standard curve. Activities are expressed as nmol of *p*-nitrophenol produced per 10^4 cells during the 30-min incubation.

Experiments depicted in Tables 4 and 5 included a further modification of the ALP assay so that the enzymatic activity could be measured in a well in which the cell number was determined. In the previous experiments duplicate sets of wells were set up for cell number and ALP assay. The further modification involved aspiration of the medium, addition of 500 μl of Hematoll/Zaptoglobin solution (Curtin–Matheson, Houston) to lyse cell membranes, mixing the solution in each well, and removal of two aliquots (50 μl each), one each for cell counting and ALP assay. The procedures as described above were performed for each aliquot.

cAMP Assay. cAMP production was assayed by a modification of a published method (16). Adherent cells were washed with DMEM and Hepes (25 nM, pH 7.4) after removal of medium. [^{3}H]Adenine (1 μCi in 0.5 ml of DMEM and Hepes; 1 Ci = 37 GBq) was added and the cells were incubated for 2 hr. The radioactive medium was removed and the cells were washed again with DMEM/Hepes. Isobutylmethylxanthine (0.2 mM) in DMEM/Hepes (0.45 ml) was added. Fifteen minutes later PTH-(1–34) dissolved in DMEM/Hepes containing 2% serum from parathyroidectomized rats was added. Addition of trichloroacetic acid (1 ml, 10%) stopped the reaction.

Table 1. Effect of E on proliferation and ALP activity of UMR106 and S90E cells

Cells	Addition	Cell no. × 10^{-4}	ALP activity, nmol/10^4 cells
UMR106	Vehicle	5.30 ± 0.23	27.9 ± 1.3
	E, 10^{-10} M	5.04 ± 0.24	25.6 ± 2.0
	E, 10^{-8} M	4.54 ± 0.20*	43.1 ± 1.5*
	E, 10^{-6} M	4.11 ± 0.21*	36.7 ± 1.9*
S90E	Vehicle	6.66 ± 0.10	11.3 ± 0.90
	E, 10^{-10} M	6.72 ± 0.17	10.4 ± 0.62
	E, 10^{-8} M	6.07 ± 0.20	9.2 ± 1.02
	E, 10^{-6} M	6.76 ± 0.15	8.7 ± 0.84

The values are expressed as means ± SEM of triplicate determinations after a 2-day incubation. Vehicle was 95% ethanol (10 μl). *$P < 0.05$ versus control (vehicle) value.

The media were aspirated and centrifuged (1000 × *g* for 10 min) to sediment particles. ATP and cAMP were separated by Dowex chromatography followed by alumina chromatography (16). After scintillation counting of the samples, the percent of the [^{3}H]ATP pool converted to [^{3}H]cAMP was calculated as {cpm [^{3}H]cAMP/(cpm [^{3}H]cAMP + cpm [^{3}H]-ATP)} × 100.

Data Analysis. The results were analyzed by an analysis of variance, Fisher's least differences, and Scheffe's estimate of probability, using a TRS-80-II minicomputer and a statistical program.

RESULTS

Table 1 documents the effect of E on the proliferation and ALP activity of UMR106 and S90E cells incubated for 2 days in medium containing fetal bovine serum (5%, vol/vol) and E or vehicle. Compared to the effects produced by the vehicle alone, a statistically significant reduction in the proliferation of UMR106 cells and a concomitant increase in ALP of these cells occurred with E concentrations of 10^{-8} and 10^{-6} M. In contrast to this effect of E on UMR106 cells, no significant change in either proliferation or ALP occurred in the S90E cells.

Table 2 shows the effect of E (10^{-8} M), 1,25-dihydroxyvitamin D_3 (1,25-D_3) (10^{-9} M), or a combination on the proliferation and ALP of the UMR106 cells after incubation for 1, 2, and 4 days in medium containing fetal bovine serum (5%) and the test substances or the vehicle. From day 1 to day 2 of the incubations, the cell number in the untreated group doubled, indicating a phase of rapid proliferation. Thereafter the cell number increased 50% from day 2 to day 4, and confluence of the monolayer occurred on day 4. The ALP activity of the untreated cells increased slightly during the phase of rapid proliferation, from a mean of 33.7 nmol after 1 day of incubation to a mean of 37.2 nmol after 2 days (Table 2). After 4 days the mean ALP value was 58.8 nmol, a 58% increment over the activity after 2 days.

Treatment with E (10^{-8} M) produced a statistically significant reduction in cell number and increase in ALP after 2

Table 2. Effect of E, 1,25-D_3, or a combination of the two on proliferation and ALP activity of UMR106 cells

Addition	Cell no. × 10^{-4}			ALP activity, nmol/10^4 cells		
	Day 1	Day 2	Day 4	Day 1	Day 2	Day 4
Vehicle	6.56 ± 1.11	11.9 ± 0.58	18.9 ± 0.70	33.7 ± 1.4	37.2 ± 2.2	58.8 ± 1.8
E, 10^{-8} M	6.48 ± 0.34	9.88 ± 0.12*	18.9 ± 0.98	38.6 ± 2.5	50 ± 3.1*	60.8 ± 4.1
1,25-D_3, 10^{-9} M	6.54 ± 0.11	12.7 ± 0.61	17.2 ± 0.82	36.9 ± 1.8	36.6 ± 3.2	52.7 ± 2.1
1,25-D_3 + E	6.44 ± 0.25	11.9 ± 0.33	16.5 ± 0.37	37.0 ± 2.0	34.7 ± 1.7	53.1 ± 0.8

Cells were cultured 1, 2, or 4 days in medium containing 5% fetal bovine serum, antibiotics, and the test substances or vehicle. The values represent the mean ± SEM of triplicate determinations.
*$P < 0.05$ versus control value on the same day.

Table 3. Effects of E, 1,25-D_3, or a combination on proliferation and ALP activity of UMR106 cells preincubated in the presence of dexamethasone

Dexamethasone	Addition	Cell no. $\times 10^{-4}$	ALP activity, nmol/10^4 cells
−	Vehicle	4.95 ± 0.19	32.8 ± 2.2
−	E, 10^{-8} M	3.94 ± 0.40*	40.1 ± 2.1*
−	1,25-D_3, 10^{-8} M	4.19 ± 0.21*	34.4 ± 2.3
−	E + 1,25-D_3	4.86 ± 0.23	30.6 ± 1.6
+	Vehicle	3.72 ± 0.54†	57.0 ± 1.4†
+	E, 10^{-8} M	4.26 ± 0.26*	49.0 ± 1.3*
+	1,25-D_3, 10^{-8} M	5.08 ± 0.22*	37.4 ± 1.5*
+	E + 1,25-D_3	5.55 ± 0.30*	33.5 ± 0.9*

Cells were incubated for 24 hr in medium containing vehicle or 10^{-7} M dexamethasone. The medium was removed after this incubation period and the cells were washed twice with serum-free medium. Fresh medium containing fetal bovine serum (5%, vol/vol) was then added and the 2-day incubation was begun. The values are expressed as means ± SEM of triplicate determinations after the 2-day incubation.
*$P < 0.05$ versus control values.
†$P < 0.05$ versus control values for cells not treated with dexamethasone.

days of incubation. These effects were not observed after 1 and 4 days of incubation. The presence of 1,25-D_3 (10^{-9} M) in the medium did not alter the cellular growth or ALP, a finding consistent with our prior experience using this concentration of 1,25-D_3 and these conditions.† The addition of 1,25-D_3 in combination with E blocked the fall in cell number and the rise in ALP associated with exposure to E alone. This inhibition of the E effect after 2 days of incubation suggested an antagonism between these two steroids on the UMR106 cells.

Dexamethasone is a potent inducer of differentiation in osteoblast-enriched bone cell preparations (17) and osteoblastic cell lines (18, 19). We next tested the hypothesis that UMR106 cells induced to differentiate by exposure to dexamethasone might be more or less responsive to E. The results of this experiment are depicted in Table 3. When the cells were not exposed to dexamethasone for 24 hr prior to the treatment with E, 1,25-D_3, or a combination, the results shown in Table 3 were similar to those shown in Table 2 even though a 10^{-8} M concentration of 1,25-D_3 was chosen in this experiment. As anticipated, E reduced the cell number and increased ALP after a 2-day incubation. 1,25-D_3 also reduced cell number but did not significantly increase ALP of the cells. The combination of the two steroids produced results indistinguishable from those of controls without steroids, confirming the previous experiments (Table 2) and demonstrating again the antagonistic interaction of E and 1,25-D_3.

The preincubation with dexamethasone followed by a 2-day incubation with vehicle alone produced a significant fall in cell number and a striking increase in ALP (Table 3). The treatment with E during the 2-day incubation following differentiation by dexamethasone produced a reversal of the previously observed E actions. Cells pretreated with dexamethasone and then exposed to E increased in number and contained less ALP after 2 days of incubation. A similar response was produced by the sequential exposure to dexamethasone and 1,25-D_3 (Table 3). However, the combination of E and 1,25-D_3, both at 10^{-8} M, after dexamethasone priming increased proliferation further and reduced the ALP to the level associated with the untreated cells incubated with vehicle alone during the entire incubation. These unexpected

†Gray, T. K., Shapiro, R., D'Amico, C. N. & Dodd, R. C., Abstracts of the American Society for Bone and Mineral Research, June 15–18, 1985, Washington, DC, A58.

Table 4. Effect of E on proliferation and ALP activity of UMR106 cells incubated for 3 days in serum-free medium

Addition	Cell no. $\times 10^{-4}$	ALP activity, nmol/10^4 cells
Vehicle	9.65 ± 0.75	23.6 ± 0.86
E, 10^{-12} M	8.40 ± 0.30	29.6 ± 0.68
E, 10^{-11} M	9.25 ± 0.30	21.8 ± 0.42
E, 10^{-10} M	5.30 ± 0.40†	49.6 ± 2.0†
E, 10^{-9} M	7.30 ± 0.40*	31.6 ± 0.4*
E, 10^{-8} M	7.80 ± 0.25*	29.8 ± 1.8*

Cell counts and ALP activities were determined in aliquots of cell lysates from the same well. The values are expressed as means ± SEM.
*$P < 0.025$ versus control value.
†$P < 0.01$ versus control value.

responses of the dexamethasone-primed cells to E, 1,25-D_3, or the combination indicated that the state of differentiation and perhaps the inducing agent influenced the response to E of the UMR106 cells.

To minimize the effects of steroids present in the fetal bovine serum, experiments were performed using culture medium supplemented with insulin, transferrin, and selenium but no serum. In these experiments cells were incubated in serum-free medium for 1 day prior to the commencement of the experiment in an attempt to lower the intracellular content of estrogens derived from the prior exposure to serum during the plating procedure and the previous continuous culture in serum-containing medium. After this day the medium was removed and replaced by fresh serum-free medium. The incubation period was also extended to 3 days because of the anticipated reduction in the rate of cell proliferation during culture in serum-free medium.

Incubation of UMR106 cells in serum-free medium containing E over a range of concentrations (10^{-12} to 10^{-8} M) led to changes in the proliferation rates and ALP activities (Table 4). E concentrations from 10^{-10} to 10^{-8} M were associated with statistically significant reductions in cell number relative to the group exposed to vehicle. Likewise, statistically significant increases in ALP were associated with these same concentrations of E. Interestingly, the peak ALP activity was associated with 10^{-10} M rather than the highest E concentration tested, 10^{-8} M.

Table 5 compares the effect of E and its isomer 17α-E on the proliferation and ALP activity of these cells under serum-free conditions. No statistically significant changes in cell number or ALP were observed after the 3-day incubation in the presence of 17α-E. In contrast, E reduced cell number and increased ALP activity at the three concentrations tested, 10^{-11} to 10^{-9} M. The reduction in cell number was nearly uniform for the three doses. Once again the peak ALP

Table 5. Effects of 17α-E and E on proliferation and ALP activity of UMR106 cells incubated for 3 days in serum-free medium

Addition	Cell no. $\times 10^{-4}$	ALP activity, nmol/10^4 cells
Vehicle	7.10 ± 0.35	19.6 ± 3.8
17α-E, 10^{-11} M	8.55 ± 0.55	17.2 ± 2.8
17α-E, 10^{-10} M	7.10 ± 0.20	23.6 ± 4.4
17α-E, 10^{-9} M	6.80 ± 0.25	23.2 ± 9.8
Vehicle	7.20 ± 0.70	19.4 ± 2.0
E, 10^{-11} M	3.95 ± 0.35*	52.2 ± 3.0*
E, 10^{-10} M	3.50 ± 0.20*	56.2 ± 2.4*
E, 10^{-9} M	3.90 ± 0.40*	35.6 ± 3.6*

Cell counts and ALP activities were determined in aliquots of cell lysates from the same well. The values are means ± SEM.
*$P < 0.01$ versus control value.

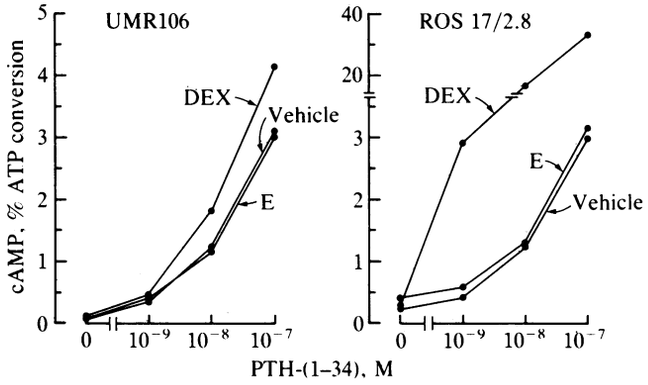

FIG. 1. Basal and PTH-(1–34)-induced cAMP in UMR106 or ROS 17/2.8 cells. DEX, dexamethasone.

response was associated with the 10^{-10} M concentration. In contrast to the results in Table 4, a significant increase in ALP was associated with the 10^{-11} M concentration of E in this experiment.

Basal cAMP concentration and the PTH-1(1–34)-induced rise in cAMP were studied in UMR106 cells and in a second osteoblastic cell line, ROS 17/2.8. Both cell lines were incubated for 2 days with E (10^{-8} M), dexamethasone (10^{-6} M), or the vehicle and then assayed for cAMP before and after acute PTH-(1–34) challenge. Fig. 1 depicts the percent conversion of radiolabeled ATP to radiolabeled cAMP in the basal state and after stimulation by PTH-(1–34) at one of three doses. The basal cAMP levels in the ROS 17/2.8 cells were slightly higher than those in the UMR106 cells. The cAMP response to PTH-(1–34) in the cells incubated with vehicle was nearly identical (Fig. 1). Incubation with E did not change the basal cAMP levels or the response to PTH-(1–34) in either cell line. However, dexamethasone treatment of the ROS 17/2.8 cells markedly increased the cAMP response to PTH-(1–34). In contrast, UMR106 cells exposed to dexamethasone manifested little or no change in the cAMP response to PTH-(1–34) relative to the E or vehicle groups.

DISCUSSION

The mechanism of action of E on bone is uncertain despite studies over the past three decades reporting effects on bone in rodents and quail and on mineral metabolism in humans (1–5, 20–28). The studies in rodents and quail have found effects of exogenous estrogens on osteoblasts, their precursors, or both (20–28). Endosteal proliferation and metaphyseal sclerosis were observed in several studies, implying an anatomic localization of the estrogen effect. A stimulatory action of estradiol and progesterone on bone formation was observed in an established model of matrix-induced osteoinduction (29). Nevertheless these studies could not distinguish the direct or indirect effects of estradiol on the osteoblasts.

Several attempts to find E receptors in bone were not successful (6–9). Despite this lack of success, we reexamined the possibility that E might act directly on osteoblasts by studying its effect on UMR106 cells, a clonal line derived from a rat osteosarcoma (10). This cell line expresses many phenotypic characteristics of normal osteoblasts (10–12), and in these experiments we utilized UMR106 cells as surrogate osteoblasts. Our results show that E acted directly, stimulating the ALP activity (corrected for the cell number) in a dose-dependent manner. The ALP was corrected for the cell number at the end of each incubation period as emphasized by Chen and her associates (17) to avoid misinterpretations of altered enzyme activity due to changes in cell number rather than enzyme content per cell. We have expressed the enzyme activity as nanomoles of substrate hydrolyzed per 30 min per 10^4 cells, and we have designated this corrected measurement as the ALP activity. Generally the ALP activity of UMR106 cells and osteoblasts has been used as a gauge of differentiation, with higher enzyme activity indicating enhanced cell differentiation (17–19). In view of this fact, E apparently induced the differentiation of UMR106 cells specifically. The response is specific for both cell type and metabolite because the fibroblasts did not respond in the manner of the UMR106 cells and the isomer 17α-E was inactive when tested at 10^{-11} to 10^{-9} M under serum-free conditions. E treatment did not alter the responsiveness of the UMR106 cells or the ROS 17/2.8 cells to PTH-(1–34), while dexamethasone augmented this response in both, although to a larger degree in the ROS 17/2.8 cells than in the UMR106 cells.

Our results suggest that the direct action of E on the UMR106 cells depends on a complex set of cellular and/or extracellular events. The ALP response to E occurred consistently during the rapid proliferative phase of UMR106 culture but not during the first day, when cell density was low, or on the fourth day, when the cells were confluent. This temporal pattern suggests that cell density and the cell cycle influence responsiveness to E. This type of response is not unique to E, since it has already been described in osteoblasts for 1,25-D_3 (18, 28). Exposure of rapidly proliferating ROS 17/2.8 cells, another osteoblastic cell line, to 1,25-D_3 increased the ALP activity, but this response was not present in confluent cells (18). Likewise 1,25-D_3 increased the ALP activity of proliferating MC3T-E1 cells, a murine osteoblastic cell line, but not when these cells were confluent (28). The responsiveness of the MC3T3 cells correlated with the expression of 1,25-D_3 receptors in these cells (28). Variation in the expression of 1,25-D_3 receptors in osteoblasts during the cell cycle has been reported (30). The nature of the UMR106 response to E is very similar to the responses of osteoblastic cells or cell lines to 1,25-D_3. Given this similarity, it is possible that E receptors, if present in the UMR106 cells, vary their expression during the phases of proliferation and confluence and thereby determine to a large extent the cellular response.

The interactions between E, 1,25-D_3, and dexamethasone observed in these experiments are both interesting and puzzling. The antagonism between E and 1,25-D_3 seems clear, and it suggests that the UMR106 cells exposed to both substances are not capable of manifesting the changes due to E alone, presumably because the cellular responses to each affect or inhibit the response to the other. The more interesting and puzzling result is the fact that UMR106 cells induced to differentiate by exposure to dexamethasone manifested a response to E opposite that seen in cells not treated with dexamethasone. This observation indicates that the state of differentiation and possibly the inducing agent determine the response to E. Given the temporal pattern of the E response and the dexamethasone experiments, we suggest that the UMR106 response to E depends on multiple factors, including cell density, proliferation rate or phase of the cell cycle, the state of differentiation, and the presence or absence of growth factors and other steroids. We hypothesize that synchronization of these factors might produce a maximal response to E and that loss of synchrony might reduce the magnitude of the response or, under certain circumstances, lead to a reversal of the E effect.

The removal of serum from the culture medium allowed us to observe the effect of E on the UMR106 cells in the absence of the growth factors, steroids, and nutrients derived from serum. Under these conditions a stimulation of ALP activity, the marker of differentiation, was clearly seen when E concentrations from 10^{-12} to 10^{-8} M were present in the

medium. The ALP response to E appeared to be biphasic under serum-free conditions, reaching its peak at 10^{-10} M (Tables 4 and 5).

Removal of serum also influenced the E-dependent inhibition of UMR106 proliferation observed in earlier experiments that employed culture medium containing fetal bovine serum. The suppression of cell growth by E under serum-free conditions was not dose dependent. It seems unlikely that this is a nonspecific or toxic action of E, since its isomer, 17α-E, produced no significant change in cell proliferation when tested over the same dose range.

The lack of a dose response in the suppressed cellular growth versus the biphasic pattern of ALP stimulation suggests to us that the molecular mechanisms underlying these two processes are different and distinct. The apparent dose-dependent inhibition of proliferation observed when serum was present in the culture medium is another example of the complex interactions between extracellular factors that modulate the E response.

The demonstration of a direct action of E on the UMR106 cells raises several important questions. Do these cells contain E receptors? We assume that they do and we anticipate variation in receptor expression during the cell cycle analogous to the 1,25-D_3 receptors. Does E act directly on normal osteoblasts? Do normal osteoblasts contain E receptors? The results presented here should stimulate investigators in the area of bone cell biology to address these questions.

The authors thank Ms. Dail White for her assistance in the preparation of this manuscript. S. Garner performed the cAMP assays in the laboratory of Dr. T. C. Peng. These studies were supported in part by National Institutes of Health Grant AM-33320 (T.K.G.) and by National Institutes of Health Training Grant 5 T32 AM07129-11) (T.C.F.).

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