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Direct and sex-specific stimulation by sex steroids of creatine kinase activity and DNA synthesis in rat bone

(gonadal steroids/diaphysis/epiphysis/ROS 17/2.8 osteosarcoma/osteoporosis)

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ABSTRACT **A direct *in vitro* effect of 17β-estradiol (E_2) was demonstrated on bone and cartilage cell energy metabolism. Sex-specific stimulation by E_2 and testosterone was shown in diaphyseal bone of weanling rats. E_2 (30 nM) caused, within 24 hr, a 70–200% increase in creatine kinase (CK; ATP:creatine *N*-phosphotransferase, EC 2.7.3.2) specific activity in ROS 17/2.8 rat osteogenic sarcoma cells, MC_3T_3-E1 mouse calvaria-derived cells, and rat fetal calvaria cells, and a 40% increase in rat epiphyseal cartilage cells. Stimulation of CK activity by E_2 was dose and time dependent: in ROS 17/2.8 cells, a highly significant increase was found at 3 nM E_2 and a >100% increase in CK activity was found 1 hr after E_2 administration. In female 20-day-old Wistar-derived rats, E_2 (5 μg per rat) increased CK activity in diaphyseal bone by 82% within 1 hr of i.p. injection, with a maximal increase of 200% after 24 hr; neither the weakly estrogenic agonist 17α-estradiol, testosterone, nor progesterone showed this effect. Conversely, in male rat diaphyseal bone, testosterone or dihydrotestosterone increased CK activity after 24 hr by ≈100%, while E_2 was ineffective. In epiphyseal cartilage, both E_2 and testosterone increased CK activity. Stimulation of CK activity by sex hormones was paralleled by significant increases in [^{3}H]thymidine incorporation into DNA. Therefore, it is possible that direct sex-specific actions of gonadal steroids may contribute to stimulating bone growth and maintaining balanced bone turnover.**

Although gonadal steroids have a profound effect on skeletal tissues (1–3), and estrogen deficiency has been established as a major etiologic factor in postmenopausal osteoporosis (1, 2), their influence has been considered to be indirect. This prevailing opinion was due to a lack of evidence for either specific estradiol receptors in bone (4) or direct biologic effects of sex steroids on bone cells (5, 6). The situation has dramatically reversed since recent studies have demonstrated significant, albeit low, concentrations of 17β-estradiol (E_2) receptors (refs. 7–9; ¶) as well as androgen receptors (¶) in bone cells.

The brain type (BB) isoenzyme of creatine kinase (CK; ATP:creatine *N*-phosphotransferase, EC 2.7.3.2; ref. 10) involved in the "energy buffer" system, which regulates cellular concentrations of ATP and ADP, is the major component (11) of the E_2-induced protein of the rat uterus (12). E_2-induced protein synthesis, and more recently, modulation of CK activity, has been a useful marker for studies on the mechanism of action of E_2 in uterus and in other tissues that contain E_2 receptors (13), because of its rapid response to E_2 *in vivo* and *in vitro* (14). Moreover, it is a convenient marker for estrogen-modulated gene expression, since E_2 treatment increases the steady-state level of mRNA for CK BB in the rat uterus (15). The speed and sensitivity of the assay for CK activity makes CK stimulation an efficient response marker to detect the action of E_2 and other hormones (16) in skeletal tissues. In this report, we present evidence that E_2 acts directly on cultured osteoblasts and epiphyseal cartilage cells, leading to increased CK activity as well as increased [^{3}H]thymidine incorporation into DNA. Moreover, we report a rapid and sex-specific action of E_2 and testosterone (T) on these markers in bones of prepubertal rats.

MATERIALS AND METHODS

Cell Cultures. ROS 17/2.8 cells (a subclone of a rat osteogenic sarcoma line; ref. 17) and MC_3T_3-E1 cells (a clone of osteoblastic cells derived from mouse calvaria; ref. 18) were cultured in 35-mm-diameter culture dishes in 2 ml of BGJ_b medium (19) containing 10% (vol/vol) fetal calf serum. Bone cells were prepared from calvaria of 19- to 20-day-old Wistar-derived rat embryos (19). Calvaria were cut into small pieces and cells were released by digestion with 0.25% trypsin/EDTA. Cells were plated (3×10^5 cells per 35-mm-diameter culture dish) and incubated with 2 ml of BGJ_b medium containing 10% (vol/vol) fetal calf serum. Two concentrations of Ca^{2+} were used: 2 mM and 0.2 mM. Previous studies demonstrated that bone cultures grown in a medium containing low [Ca^{2+}] are enriched in parathyroid hormone-responsive osteoblast-like cells (20).

Rat epiphyseal cells were obtained from vitamin D-deficient 16- to 18-day-old rats, which have a wider epiphyseal cartilage zone than normally fed rats. Epiphyseal cartilage plates were isolated under a binocular dissecting microscope. Cells released by digestion with 0.25% collagenase (Worthington) in phosphate-buffered saline for 60 min at 37°C were cultured as described above for calvaria cells (in 2 mM Ca^{2+}). The cells were identified as chondrocytes by their morphology, by the presence of alkaline phosphatase activity, and by their reaction with antibody against type II collagen (21).

In all experiments, confluent cultures were used. Time from seeding to confluence was 4 days for ROS 17/2 cells, 7 days for calvaria cultures in 2.0 mM Ca^{2+}, and 10 days for calvaria cultures in 0.2 mM Ca^{2+} and for epiphyseal cartilage.

Animals. Batches of weanling (20 days old) female Wistar-derived rats (Hormone Research Departmental Colony, mean weight, 35 g; mean uterine weight, 22 mg) were divided randomly into experimental groups and injected i.p. with one of the following hormones: E_2 (0.5 or 5 μg per rat), 17α-estradiol (5 μg per rat), T (5 or 50 μg per rat), progesterone (1 mg per rat), or with vehicle only (2.5% ethanol in 0.9%

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Abbreviations: E_2, 17β-estradiol; T, testosterone; CK, creatine kinase.

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¶Spelsberg, T. C., Colvard, D. S., Eriksen, E. F., Keeting, P. E. & Riggs, L. B., Third International Conference on the Chemistry and Biology of Mineralized Tissues, October 1988, Chatham, MA, p. 3 (abstr.).

NaCl). Twenty-day-old male rats (mean weight, 35 g) were injected with either T (50 μg per rat), dihydrotestosterone (50 μg per rat), E_2 (5 μg per rat), or vehicle. Rats were fed with pelleted chow ad libitum and were kept on a 14-hr light/10-hr dark schedule in air-conditioned rooms maintained at 23°C. They were killed by cervical dislocation, 24 hr after injection, unless otherwise specified, for CK determinations, or after 22 hr (before removal of organs for 2-hr *in vitro* incubation with [^{3}H]thymidine).

CK Assay. Cells were removed from culture dishes by scraping with a rubber policeman, and epiphyses and diaphyses of the tibia and femur, kidney, uterus (which increased in weight by 70–80% after E_2 treatment), and prostate were washed thoroughly with cold 0.9% NaCl. Cells were sonicated, and tissues were homogenized in ice-cold buffer containing 50 mM Tris·HCl (pH 6.8), 5 mM magnesium acetate, 2.5 mM dithiothreitol, 0.4 mM EDTA, and 250 mM sucrose, using an Ultra Turrax homogenizer (Janke and Kunkel, Staufen i Br., F.R.G.). Supernatant extracts were obtained by centrifugation at 12,000 × *g* for 5 min at 4°C. CK activity was measured at 30°C (11) in a Gilford 250 automatic recording spectrophotometer at 340 nm, using a coupled assay for ATP, in 0.5 ml of incubation mixture containing 50 mM imidazole acetate buffer (pH 6.7), 25 mM creatine phosphate, 20 mM *N*-acetylcysteine, 20 mM D-glucose, 10 mM magnesium acetate, 5 mM EDTA, 2 mM ADP, 2 mM NAD, 2 mM dithiothreitol, 50 μM diadenosine pentaphosphate (adenylate kinase inhibitor), 5 μg of bovine serum albumin, 1.2 units of glucose-6-phosphate dehydrogenase, and 0.8 unit of hexokinase. Protein was determined by Coomassie blue dye binding (22) with bovine serum albumin as the standard. The data presented in the graphs and table are pooled results from two to four independent experiments.

[^{3}H]Thymidine Incorporation into DNA. After 22 hr of treatment of ROS 17/2.8 and epiphyseal cartilage cells with 30 nM E_2, the cell cultures were incubated for an additional 2 hr in the presence of [^{3}H]thymidine (5 μCi/ml; 5 Ci/mmol; 1 Ci = 37 GBq; Amersham). Animals were treated as for CK analysis. After 22 hr of hormonal treatment, the rats were killed and the tissues were removed rapidly, washed, and cut into thin slices or small pieces. The fragments were then incubated for 2 hr with [^{3}H]thymidine (5 μCi/ml) at 37°C in Dulbecco's modified Eagle's medium under an atmosphere of 5% CO_2/95% O_2. [^{3}H]Thymidine incorporation into acid-insoluble material was measured as described (23). DNA was determined by the Burton method (24).

Reagents. All reagents used were analytical grade; biochemicals were obtained from Sigma.

Statistical Significance. The significance of differences between experimental and control values was evaluated by Student's *t* test.

RESULTS

Incubation with E_2 for 24 hr of confluent cultures of rat osteosarcoma (ROS 17/2.8) cells, cells from rat calvaria, MC_3T_3-E1 mouse calvaria-derived cells, or rat epiphyseal cartilage cells caused significant increases in CK specific activity in all cell types (Fig. 1), ranging from 1.4- to 2.0-fold. The constitutive CK specific activity in ROS 17/2.8 cells (>1 μmol·min^{-1} per mg of protein) was more than an order of magnitude greater than in the other skeletal-derived cells (Fig. 1). This activity seems correlated with the rate of cell division, which is greatest in the ROS 17/2.8 permanent cell line, higher in calvaria cells grown in 2 mM Ca^{2+} than in 0.2 mM Ca^{2+}, and lowest in epiphyseal cell cultures.

The stimulation of CK activity in E_2 in cultured ROS 17/2.8 cells and in female rat diaphyseal bone was both time (Fig. 2) and dose dependent (Fig. 3 and Table 1). Significant increases

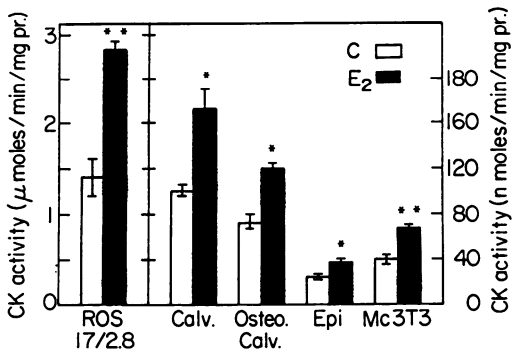

FIG. 1. Stimulation by E_2 of CK specific activity in skeletal-derived cells: rat osteosarcoma cells (ROS 17/2.8), rat calvaria bone cells (Calv.), cultures enriched in osteoblast-like rat calvaria bone cells (Osteo. Calv.), rat epiphyseal cartilage cells (Epi), and mouse calvaria-derived osteoblastic line (Mc3T3). Experimental cultures were incubated for 24 hr with 30 nM E_2. CK was extracted and assayed as described. Results are means ± SEM for n = 4–7 cultures. Statistical analysis was by Student's *t* test of values for vehicle-treated cells (open bars) vs. E_2-treated cells (solid bars). *, $P \leq 0.05$; **, $P \leq 0.01$. pr., Protein.

in CK specific activity occurred as early as 1 hr after stimulation by 30 nM E_2, and a maximal (≈3-fold) increase was sustained between 2 and 24 hr in culture and between 4 and 24 hr *in vivo* after injection of 5 μg of E_2 (Fig. 2). A highly significant response *in vitro* was reached at 3 nM E_2, with a maximal response at 30 nM (Fig. 3). The increase in CK specific activity at 3 nM E_2 represents an increase over a baseline value due, in part, to the estrogenic stimulation contributed by both 10% fetal calf serum and phenol red (20 μg/ml) in the BGJ_b medium. *In vivo*, after injection of 5 μg of E_2 per rat, a 2.7-fold increase in CK activity in diaphyseal bone occurred at 24 hr (Table 1); a lower dose (0.5 μg of E_2 per rat) also caused a significant (1.7-fold) increase.

The hormonal specificity of the CK response was demonstrated in ROS 17/2.8 cells and in the diaphyses of female rats (Fig. 4). In contrast to the highly significant response to E_2, neither 30 nM 17α-estradiol, a weak agonist, nor T (300 nM), had any effect on CK specific activity, while progesterone

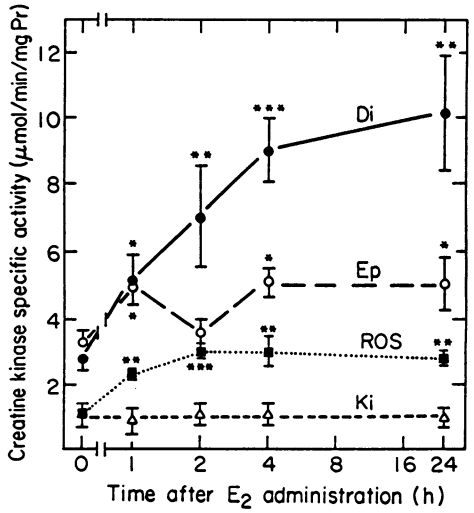

FIG. 2. Time course of CK response to E_2 in skeletal-derived cells and tissues. Cells were treated with 30 nM E_2, and 20-day-old female rats were injected with 5 μg of E_2 per rat. CK was extracted and analyzed as described. Results are means ± SEM (n = 4–10) for ROS 17/2.8 cells (ROS), diaphyseal bone (Di), epiphyseal cartilage (Ep), and kidney (Ki). Statistical analysis was by Student's *t* test of values for vehicle-treated (0 time) vs. E_2-treated cells or tissues. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$. Pr, protein.

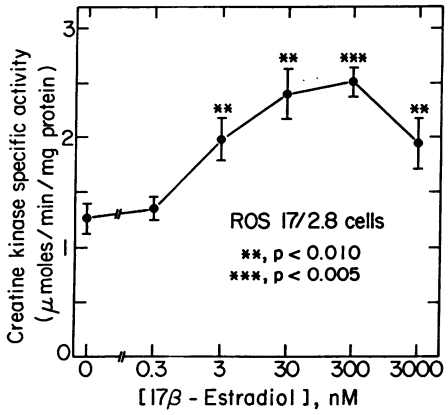

FIG. 3. Dose dependence of the stimulation of CK specific activity in cultured ROS 17/2.8 cells treated with E_2 for 24 hr. CK was extracted and analyzed as described. Results are means ± SEM (n = 4 or 5). Statistical analysis was by Student's t test of values for vehicle-treated vs. E_2-treated cells.

(6000 nM) caused a 1.4-fold increase in CK specific activity in ROS 17/2.8 cells but not in diaphyseal bone. The CK response to E_2 in diaphyseal bone was comparable to that observed in the uterus, the classical E_2 responsive organ (Fig. 5, Table 1). The kidney showed no CK response to E_2.

Compared to diaphyseal bone, the epiphyseal cartilage of female rats showed a smaller, yet significant, response to 5 μg of E_2 per rat (Figs. 2 and 4 and Table 1) but a wider hormonal responsiveness; both T and progesterone, in addition to E_2, caused a significant increase in CK specific activity (Fig. 4). The greater response to E_2 in diaphyseal bone than in epiphyseal cartilage (Figs. 2 and 4 and Table 1) was unequivocal, since these two skeletal tissues showed a similar CK specific activity in control (vehicle injected) rats.

In male diaphyseal bone, T caused a highly significant (2-fold) increase in the specific activity of CK after 24 hr (Fig. 6); in this sex as well, the stimulation appeared greater in diaphyseal bone than in epiphyseal cartilage. The immature prostate also responded significantly to T, while kidney CK specific activity remained unchanged (Fig. 6). The increase in CK activity in diaphysis was also highly significant (60% ± 6%) after treatment with 10 times less T (5 μg per rat). Similarly, 5 μg of T per rat caused the same significant increase as a dose of 50 μg of T per rat, in epiphysis (57 ± 28%) and prostate (79% ± 17%). To rule out the action of T via conversion to E_2, 50 μg of the nonaromatizable androgen dihydrotestosterone was tested in 20-day-old male rats. The highly significant increase in CK activity seen in the diaphysis (98% ± 19%) was the same as that seen with the same concentration of T (Fig. 6). The significant increases caused by dihydrotestosterone in epiphysis (83% ± 32%) and prostate (77% ± 13%), as well as the lack of response in kidney, were also the same as with the identical dose of T. E_2, in

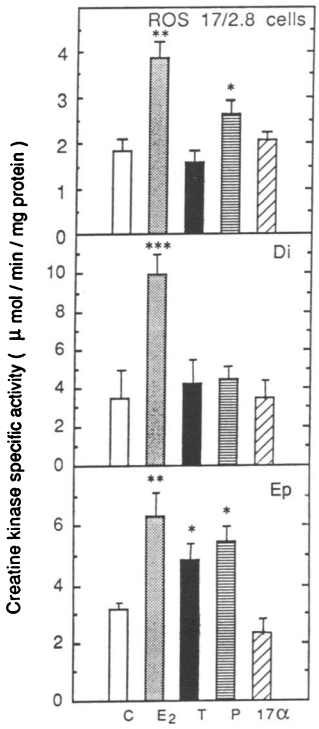

FIG. 4. Hormonal specificity of the induction of CK specific activity by steroid hormones in ROS 17/2.8 cells, diaphyseal bone (Di), and epiphyseal cartilage (Ep) of 20-day-old female rats. ROS 17/2.8 cells were treated for 24 hr with either vehicle (C), 30 nM E_2, 300 nM T, 6 μM progesterone (P), or 30 nM 17α-estradiol (17α). Rats were injected with vehicle (C; 2.5% ethanol in 0.9% NaCl), E_2 (5 μg per rat), T (50 μg per rat), P (1 mg per rat), or 17α (5 μg per rat). After 24 hr, CK was extracted and analyzed as described. Results are means ± SEM (n = 4–10). Statistical analysis was by Student's t test of values for vehicle-treated vs. hormone-treated cells or tissues. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$.

contrast to its effect on female diaphyseal bone, had no effect on CK activity in male diaphyseal bone (experimental/control ratio, 1.04 ± 0.18). Both E_2 and T stimulated CK activity in epiphyseal cartilage in both female (Fig. 4) and in male rats (E_2 by 1.5-fold and T by 1.7-fold).

To provide a direct measure of sex steroid stimulation of DNA synthesis, the total uptake and incorporation of [^{3}H]thymidine into DNA was measured. In all cases, the slight changes in uptake of [^{3}H]thymidine were insufficient to account for the significant to very highly significant increases found in [^{3}H]thymidine incorporation into DNA (Fig. 5). The stimulation of CK specific activity by E_2 in ROS 17/2.8 cells and in rat epiphyseal chondrocytes was paralleled by a significant increase (40%) in the rate of DNA synthesis in epiphyseal cartilage cells (from 432 ± 72 cpm to 606 ± 91 cpm of [^{3}H]thymidine per μg of DNA; n = 4) and an equally significant increase of 46% in ROS 17/2.8 cells (from 1490 ± 130 to 2180 ± 101 cpm of [^{3}H]thymidine per μg of DNA; n = 5). In rat skeletal tissues as well, the pattern of stimulation of CK activity by sex steroid hormones described above was paralleled by a significant increase in [^{3}H]thymidine incorporation into DNA in both female (Fig. 5) and male 20-day-old rats. The extent of the stimulation by E_2 in female diaphyseal bone (2.2-fold) and epiphyseal cartilage (1.6-fold) was greater than the increase caused by T in these organs of

Table 1. Induction of CK specific activity by E_2 in 20-day-old female rats: dose dependence

E_2 dose, μg per rat	CK specific activity, μmol·min^{-1}·(mg of protein)$^{-1}$			
	Diaphysis	Epiphysis	Kidney	Uterus
0	3.53 ± 0.50	3.14 ± 0.13	1.24 ± 0.79	5.34 ± 0.81
	(1.00 ± 0.13)	(1.00 ± 0.04)	(1.00 ± 0.06)	(1.00 ± 0.15)
0.5	5.88 ± 0.34*	5.05 ± 0.28†	1.33 ± 0.18	6.99 ± 0.11*
	(1.66 ± 0.09)	(1.60 ± 0.05)	(1.07 ± 0.10)	(1.31 ± 0.15)
5.0	9.93 ± 0.76†	6.32 ± 0.70†	1.21 ± 0.11	9.45 ± 0.11†
	(2.71 ± 0.10)	(2.01 ± 0.08)	(0.98 ± 0.08)	(1.77 ± 0.13)

Rats were injected i.p. and organs were analyzed 24 hr later. Results are means ± SEM (n = 5–10). Values in parentheses are the ratios of the experimental to control (vehicle injected) mean values. Statistical analysis was by Student's t test of values for vehicle-treated vs. E_2-treated tissues.

*$P \leq 0.05$.

†$P \leq 0.01$.

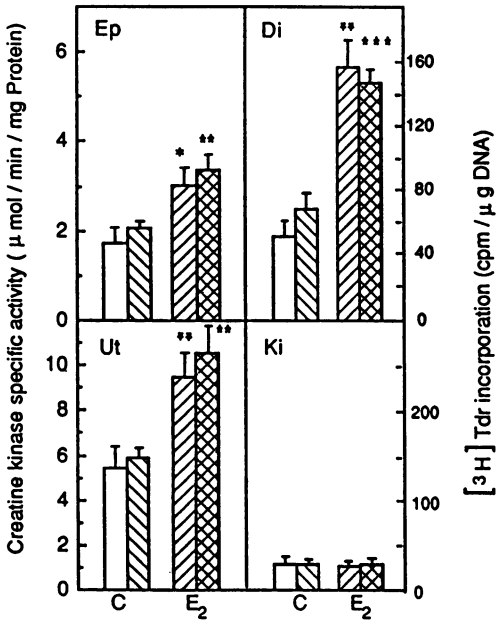

FIG. 5. Organ specificity of the stimulation of CK specific activity and DNA synthesis by E_2 in 20-day-old female rats. Rats were injected with vehicle (C) or with E_2 (5 μg per rat) and CK specific activity and [^{3}H]thymidine ([^{3}H]Tdr) incorporation into DNA were measured as described. The left bar of each pair shows CK specific activity and the right bar shows thymidine incorporation. Results are means ± SEM (n = 10–15) for uterus (Ut), epiphysis (Ep), diaphysis (Di), and kidney (Ki). Statistical analysis was by Student's t test of values for vehicle-treated vs. E_2-treated tissues. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$.

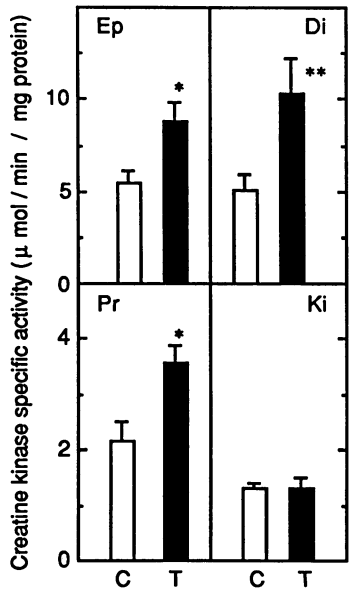

FIG. 6. Organ specificity of stimulation of CK specific activity by T in 20-day-old male rats. Rats were injected with vehicle (C) or with T (50 μg per rat), and CK activity was extracted and analyzed as described. Results are means ± SEM (n = 10) for epiphysis (Ep), diaphysis (Di), kidney (Ki), and prostate (Pr). Statistical analysis was by Student's t test of values for vehicle-treated or T-treated tissues. *, $P \leq 0.05$; **, $P \leq 0.01$.

male rats (1.4-fold in both diaphyseal bone and epiphyseal cartilage; data not shown).

DISCUSSION

Utilizing the stimulation of CK BB specific activity as a sensitive marker for estrogen action, and parallel stimulation of [^{3}H]thymidine incorporation into DNA, we have found that E_2 has a direct effect on three different rodent bone cell cultures, while neither human foreskin fibroblast cultures (M. Symons and A.M.K., unpublished observations) nor NIH 3T3 cells (M. Tal and A.M.K., unpublished observations) showed stimulation of CK specific activity by E_2. In addition, we have revealed a sex-specific response to E_2 and T in rat diaphyseal bone.

Specificity in the CK response to E_2 was demonstrated by the observation that neither 17α-estradiol (an isomer with 1–2 orders of magnitude less activity than E_2) nor T had an effect on CK BB activity in osteoblast-like ROS 17/2.8 cells or in diaphyseal bone of female rats (Fig. 4). The finding that progesterone (at a much higher concentration than E_2), increased CK specific activity in cultured bone-derived cells requires further study, since progesterone has been reported to have a beneficial effect on bone loss in postmenopausal osteoporosis (25). In male diaphyseal bone, in contrast to female bone, T but not E_2 stimulated CK specific activity. The CK responses in diaphyseal bone, to E_2 in females and to T in males, were comparable to those in the uterus and prostate, the classical responsive organs for estrogens and androgens, respectively.

The low concentration of estrogen receptors found in bone cells (refs. 7–9; ¶) may explain more than just the previous failures to find direct effects of estrogens on bone cells. It may also be part of the reason that the dose–response relationship for stimulation of CK by estrogen in cultured bone cells (Fig. 3) apparently covers the higher end of the physiological range of estrogen concentrations. Similarly, there was a greater stimulation of diaphyseal CK *in vivo* by 5 μg of E_2 than by 0.5 μg of E_2 per rat (Table 1), which is closer to the generally accepted physiological dose. T was also more active in diaphyseal bone at a high dose (50 μg per rat) than at a lower dose (5 μg per rat). Moreover, the significant increase in CK specific activity in ROS 17/2.8 cells at 3 nM E_2 was determined over and above a baseline value due, in part, to estrogenic stimulation contributed by both 10% fetal calf serum and 20 μg of phenol red per ml in the BGJ_b medium.

In contrast to the sex-specific effects of E_2 and T on diaphyseal bone, both hormones increased CK activity (and [^{3}H]thymidine incorporation into DNA) in epiphyseal cartilage of both male and female rats, albeit with a higher response to E_2 in females and to T in males. These results parallel the recent report of Corvol *et al.* (26) of a direct stimulatory effect of E_2 and T on $^{35}SO_4^{2-}$ incorporation into newly synthesized proteoglycans in fetal rabbit chondrocytes.

The present demonstration of direct effects of gonadal steroids on bone-derived cells in culture (Figs. 1–4) is consistent with the finding of E_2 receptors in bone cells (refs. 7–9; ¶) and the recent report (27) that E_2 has a direct effect on alkaline phosphatase activity in the UMR106 line of osteosarcoma cells. However, the mechanism(s) by which gonadal steroids exert their sex-specific effects on rat diaphyseal bone *in vivo* may be direct, indirect, or both. A direct sex-specific response of bone cells to gonadal steroids could be mediated by differences in the concentrations of their receptors or by different postreceptor response patterns in bone cells of males and females. The extremely rapid (within 1 hr) response of CK activity in female diaphyseal bone to i.p. injection of E_2 (Fig. 2) accords with the interpretation of a direct effect of E_2, in light of the findings that mRNA for CK can be stimulated within 1 hr in immature rat uteri (15) and within 2 hr (the earliest time tested) in rat bone *in vivo*.[‖]

[‖]Sömjen, D., Weisman, Y., Harell, A., Binderman, I. & Kaye, A. M., Israel Endocrine Society Annual Meeting, January 1988, p. 22 (abstr.).

However, it is possible that part or all of the sex-specific responses to gonadal steroids in rat diaphyseal bone are indirectly mediated by other hormones. In the rat, there are marked sex-specific patterns of growth hormone (28) as well as gonadotropin (29, 30) secretion coupled with sex-specific differences in the response to gonadal steroids of several enzymes in rodent liver and kidney (28). Some sex-specific effects are blunted by hypophysectomy (28, 31, 32), suggesting that they may be mediated indirectly. To what extent a similar indirect mechanism contributes to the sex-specific responses to E_2 and T in diaphyseal bone remains to be tested.

It is also an open question whether estradiol can have a significant physiological effect directly at the level of the cartilage (26) or if its effect *in vivo* is mediated solely by modulation of growth hormone secretion (28). Our observation that E_2 increases CK activity and [^{3}H]thymidine incorporation into DNA in cultured rat epiphyseal cartilage cells suggests that E_2 has a direct effect on chondrocytes or chondroblasts. This suggestion is supported by the report mentioned above (26) of a direct stimulatory effect of E_2 on proteoglycan synthesis in fetal rabbit chondrocytes.

Although, in considering postmenopausal osteoporosis, it has been thought that the primary effect of E_2 on bones is inhibition of bone resorption (33, 34), it has also been proposed that estrogen may increase bone formation by stimulation of osteoprogenitor cell proliferation (35), and, indeed, stimulation by E_2 of bone formation has been observed in a model of matrix-induced osteoinduction (36).

The role of androgens in the prevention of osteoporosis is much less clear, although male hypogonadism is associated with osteoporosis (3, 37), and short-term replacement therapy with T in hypogonadal males resulted in increased rates of bone formation and mineralization (3, 38). The view that E_2 and T stimulate bone formation is supported by our present observation that these hormones increase [^{3}H]thymidine incorporation into DNA in ROS 17/2.8 cells and in bone tissue *in vivo*, in parallel to their stimulation of CK specific activity.

In conclusion, this study provides evidence that E_2 and T have a direct metabolic effect on rat bone and cartilage cells and a sex-specific trophic effect on diaphyseal bone growth. These findings therefore raise the question of sex-specific effects of gonadal steroids on bone metabolism and growth in other species, including the human, during normal growth and in maintenance of bone density (39) in later life.

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1. Riggs, B. L., Jowsey, J., Goldsmith, R. S., Kelly, P. J., Hoffman, D. L. & Arnaud, C. D. (1972) *J. Clin. Invest.* **51**, 1659–1663.
2. Lindsay, R., Hart, D. M., Forrest, C. & Baird, C. (1980) *Lancet* **ii**, 1151–1153.
3. Francis, R. M., Peacock, M., Aaron, J. E., Selby, P. L., Taylor, G. A., Thompson, J., Marshall, D. H. & Horsman, A. (1986) *Bone* **7**, 261–268.
4. Chen, T. L. & Feldman, D. (1978) *Endocrinology* **102**, 236–244.
5. Caputo, C. B., Meadows, D. & Raisz, L. G. (1976) *Endocrinology* **98**, 1065–1068.
6. Van Paassen, H. C., Poortman, J., Borgart-Creutzburg, I. H. C., Thijssen, J. H. H. & Duursman, S. A. (1978) *Calcif. Tissue Res.* **25**, 249–254.
7. Komm, B. S., Terpening, C. M., Benz, D. J., Graeme, K. A., Kox, M., Greene, G. L., Gallegos, A., O'Malley, B. W. & Haussler, M. R. (1988) *Science* **241**, 81–84.
8. Eriksen, E. F., Colvard, D. S., Berg, N. J., Graham, M. L., Mann, K. G., Spelsberg, T. C. & Riggs, B. L. (1988) *Science* **241**, 84–86.
9. Kaplan, F. S., Fallon, M. D., Boden, S. D., Schmidt, R., Senior, M. & Haddad, J. G. (1988) *N. Engl. J. Med.* **319**, 421–425.
10. Watts, D. S. (1973) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), Vol. 8, part 2, p. 383.
11. Reiss, N. A. & Kaye, A. M. (1981) *J. Biol. Chem.* **256**, 5741–5749.
12. Notides, A. & Gorski, J. (1966) *Proc. Natl. Acad. Sci. USA* **56**, 230–235.
13. Kaye, A. M. (1983) *J. Steroid Biochem.* **19**, 33–40.
14. Katzenellenbogen, B. S. & Gorski, J. (1972) *J. Biol. Chem.* **247**, 1299–1305.
15. Walker, M. D. & Kaye, A. M. (1981) *J. Biol. Chem.* **256**, 23–26.
16. Kaye, A. M., Reiss, N. A., Weisman, Y., Binderman, I. & Sömjen, D. (1986) in *Myocardial and Skeletal Muscle Bioenergetics*, ed. Brautbar, N. (Plenum, New York), pp. 83–101.
17. Majeska, R. J., Rodan, S. B. & Rodan, G. A. (1980) *Endocrinology* **107**, 1494–1503.
18. Sudo, H., Kodama, H., Amagai, Y., Yamamoto, S. & Ksai, S. (1983) *J. Cell Biol.* **96**, 191–198.
19. Binderman, I., Duksin, D., Harell, A., Sachs, L. & Katchalsky, E. (1974) *J. Cell Biol.* **61**, 427–439.
20. Binderman, I. & Sömjen, D. (1982) in *Current Advances in Skeletogenesis: Development, Biomineralization, Mediators and Metabolic Bone Diseases*, eds. Silberman, M. & Slavkin, H. C. (Excerpta Medica, Amsterdam), pp. 338–342.
21. Levy, J., Shimshoni, Z., Sömjen, D., Berger, E., Fine, N., Silberman, M. & Binderman, I. (1989) *In Vitro*, in press.
22. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
23. Sömjen, D., Binderman, I. & Weisman, Y. (1983) *Biochem. J.* **214**, 293–298.
24. Burton, K. (1956) *Biochem. J.* **62**, 315–323.
25. Lindsay, R., Hart, D. M., Purdie, D., Ferguson, M. M., Clark, A. S. & Kraszenski, A. (1978) *Clin. Sci. Mol. Med.* **54**, 193–195.
26. Corvol, M. T., Carrascosa, A., Tsagris, L., Blanchard, O. & Rappaport, R. (1987) *Endocrinology* **120**, 1422–1429.
27. Gray, T. K., Flynn, T. C., Gray, K. M. & Nabell, L. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6267–6271.
28. Jansson, J. O., Eden, S. & Isaksson, O. (1985) *Endocrinol. Rev.* **6**, 128–150.
29. MacLusky, N. Y. & Naftolin, F. (1981) *Science* **211**, 1294–1303.
30. Frantz, A. G. & Rabkin, R. T. (1965) *J. Clin. Endocrinol. Metab.* **25**, 1470–1480.
31. Denef, C. (1974) *Endocrinology* **94**, 1577–1582.
32. Gustafsson, J. A. & Sterenberg, A. (1974) *Endocrinology* **95**, 891–896.
33. Budy, A. M., Urist, M. R. & MacLean, F. C. (1952) *Am. J. Pathol.* **28**, 1143–1148.
34. Riggs, B. L., Jowsey, J., Kelly, P. J., Jones, J. D. & Maher, F. T. (1969) *J. Clin. Invest.* **48**, 1065–1072.
35. Ernst, M., Schmid, C., Frankenfeldt, C. & Froesch, E. R. (1988) *Calcif. Tissue Int.* Suppl. **42**, 14–30.
36. Seeman, E., Melton, L. J., III, O'Fallon, W. M. & Riggs, B. L. (1983) *Am. J. Med.* **75**, 977–983.
37. Burnett, C. C. & Reddi, A. H. (1983) *Calcif. Tissue Int.* **35**, 609–614.
38. Baran, D. T., Beryfeld, M. A., Teitelbaum, S. L. & Avioli, L. V. (1978) *Calcif. Tissue Res.* **260**, 103–106.
39. Riggs, B. L. & Melton, L. J., III, eds. (1988) *Osteoporosis: Etiology, Diagnosis, and Management* (Raven, New York), p. 501.