Estrogen Suppresses Activation but Enhances Formation Phase of Osteogenic Response to Mechanical Stimulation in Rat Bone

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Abstract

We used a model whereby mechanical stimulation induces bone formation in rat caudal vertebrae, to test the effect of estrogen on this osteogenic response. Unexpectedly, estrogen administered daily throughout the experiments (8–11 d) suppressed, and ovariectomy enhanced, mechanically induced osteogenesis. Osteogenesis was unaffected by the resorption–inhibitor pamidronate, suggesting that the suppression of bone formation caused by estrogen was not due to suppression of resorption. We found that estrogen did not significantly reduce the proportion of osteocytes that were induced by mechanical stimulation to express c-*fos* and IGF-I mRNA; and estrogen suppressed mechanically induced osteogenesis whether administration was started 24 h before or 24 h after loading. This suggests that estrogen acts primarily not on the strain-sensing mechanism itself, but on the osteogenic response to signals generated by strain-sensitive cells. We also found that when estrogen administration was started 3 d after mechanical stimulation, by which time osteogenesis is established, estrogen augmented the osteogenic response. This data is consistent with in vitro evidence for estrogen responsiveness in two phenotypically distinct bone cell types: stromal cells, whose functional activities are suppressed, and osteoblasts, which are stimulated, by estrogen. (*J. Clin. Invest.* 1996. 98:2351–2357.) Key words: estrogen • osteogenesis • bone formation • bone resorption • mechanical stimulation

Introduction

A primary physiological function of the skeleton is mechanical support. Although much of the information needed for this function is provided by the genetic program, bones modify their structure in response to mechanical stimuli, so that it is optimal for the prevailing mechanical environment. It is generally held that mechanical adaptation is initiated when the extensively communicating network of osteocytes embedded in bone, and perhaps bone surface cells, sense changes in strain distribution or intensity, and transmit signals to appropriate bone surfaces, where bone formation or resorption takes place (1–3).

Superimposed on mechanical adaptation are the influences of systemic factors. Thus, estrogen deficiency leads to accelerated bone loss (4–8), which predisposes patients to the fractures commonly seen in postmenopausal osteoporosis. It has been suggested that estrogen maintains bone mass by changing the set-point for the adaptation of bone mass to mechanical loads (9, 10). At the cellular level this could be explained by the hypothesis that the coupling of bone formation to bone resorption is controlled by local mechanical strain, and that estrogen both suppresses the resorption caused by the absence of mechanical load, and facilitates the anabolic response to strain (11, 12).

It has been well established that estrogen maintains bone mass through suppression of bone resorption. Whether estrogen facilitates the anabolic response to strain is unknown. Estrogen appears to be necessary for the beneficial effects of physical activity on bone mineral density in women (13, 14), but the mechanisms underlying such effects and their relationship to mechanical strain remain obscure. It is important to identify the mechanisms by which estrogen and mechanical stimuli interact since they may provide opportunities for therapeutic intervention to imitate or enhance the osteoregulatory response to load bearing.

We have recently developed an experimental model in which pins, inserted into the seventh and ninth caudal vertebrae of 13-wk-old rats, are used to load the eighth caudal vertebra in compression (15). A single, brief application of external loads to the eighth vertebra, sufficient to cause dynamic strains within the physiological range, produces an increase in lamellar bone formation on trabecular surfaces. This model provides an opportunity to assess the effect of estrogen on the anabolic response of rat bone to mechanical stimulation. We found that estrogen suppressed the ability of mechanical stimulation to activate new bone forming surfaces but augmented bone formation once this was under way.

Methods

13-wk-old female Wistar rats (Harlan Olac, Bicester, Oxon, UK, unless otherwise stated) were weight-matched into groups of 6–7 animals per group. Stainless steel pins were inserted into the seventh and ninth caudal vertebrae to enable mechanical stimulation, as previously described (15). In the first experiment rats were mechanically stimulated for 10 d with 30 cycles/day (0.5 Hz) using a load of 50 N, imposed by a cam-operated device, thereby loading the eighth caudal vertebra in compression. For the remaining experiments, rats underwent a single episode of 300 loading cycles (0.5 Hz) at 150 N on the first day of the experiment. This latter load has been found to produce a peak strain of 700 microstrain ($\mu\epsilon$) on the surface of the loaded vertebra. It has been found that peak strains measured in bones during normal locomotion are in the range 1000–3000 $\mu\epsilon$ (16). Thus, our mechanical loading regime is comparable with the mechanical usage experienced by bones during 30–300 cycles of physiological

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activity; the increased bone formation induced by physiological strains is explicable as a response to a new strain direction rather than to a supraphysiological strain magnitude, rate, or cycle number (17). Control groups (for the effects of mechanical stimulation) consisted of nonpinned animals and pinned, nonloaded animals. Pins were immobilized in metal clamps except during mechanical stimulation. Pinning and loading were performed under general anesthesia (2% halothane [Sigma Chemical Co., Poole, UK], 1 liter/min N_2O, and 2 liter/minute O_2). These procedures were carried out between 0800 and 1300 h.

Where indicated, ovariectomy (ox), or sham ovariectomy (sham-ox), was performed under halothane anesthesia using a dorsal approach, 24 h before mechanical stimulation. Calcein (first label) (30 mg/kg, Sigma) and tetracycline hydrochloride (second label) (25 mg/kg; Lederle Laboratory, Gosport, Hants, UK) were administered intraperitoneally as indicated in the figure legends. 17β-estradiol (E_2) (Sigma Chemical Co.) was dissolved in a vehicle of 5% benzyl alcohol (Sigma Chemical Co.) and 95% corn oil (Sigma Chemical Co.) and administered subcutaneously as indicated. In one experiment some groups of animals were given a single subcutaneous dose of pamidronate (0.3 mg/kg; Yamanouchi Pharmaceuticals, Japan) dissolved in saline vehicle (0.9% NaCl; Sigma Chemical Co.). This dose has previously been found to suppress bone loss and bone turnover in rats after ovariectomy (18, 19) Control animals were given appropriate vehicle subcutaneously. During the experiments, animals were housed in hanging grid cages in groups of 3 or 4 at 21°C with 12:12 h light–dark cycle. Food (rat–mouse diet 1; Special Diet Services, Witham, UK) and water were given ad libitum to all animals.

At the end of the experiment animals were killed using CO_2 gas. Ovariectomy was confirmed by measuring uterine weight. The eighth caudal vertebrae and right tibiae were removed, freed of soft tissue, fixed in 70% alcohol for 48 h, dehydrated through graded alcohols, and embedded undecalcified in London resin (London Resin, Basingstoke, UK). 15-μm-thick mid-coronal sections of the vertebrae were prepared using a Jung-K microtome and mounted unstained for fluorescence microscopy. 7-μm-thick sections were stained with toluidine blue for assessment of static parameters.

The volume of cancellous bone was measured in a standard area (1.8 mm^2) situated at least 0.75 mm from the growth plate to exclude the primary spongiosa and trabeculae connected to the cortical bone. The longitudinal growth rate in the vertebrae of control animals was < 6 μm per day, as a result of which the primary spongiosa does not enter, and growth does not have a significant effect on, the position of the reading frame in these short-term experiments. Dynamic parameters were measured in the same area as that used for assessing bone volume, at an objective magnification of 20. Measurements were made by tracing features of interest viewed in the microscope onto a digitizer pad, through a camera lucida. The digitizer pad was linked to a computer with dedicated bone software (Osteomeasure, Osteometrics, Atlanta, GA). For dynamic parameters, three nonconsecutive unstained sections of bone containing at least 4 cm of total bone surface were measured per vertebra. These measurements included double-labeled surface (dLS/BS) and inter-label distance. The mineral apposition rat (MAR) (μm/day) was obtained by dividing the distance between fluorochrome labels on trabeculae by the label interval (days). The ratio of bone formation rate to bone surface (BFR/BS)[1] was obtained from the product of the MAR and dLS with total surface taken as referent. Similarly the static parameters of osteoblast surface, eroded surface and osteoclast surface were measured on three nonconsecutive sections stained with toluidine blue in an interactive fashion at an objective magnification of 20. These were expressed as a percentage of total bone surface. Histomorphometric data are reported in three-dimensional terms with the use of standard abbreviations (20).

In situ hybridization. To assess the effect of E_2 on the expression of messenger RNA for insulin-like growth factor 1 (IGF-1) or c-*fos* in osteocytes, rats were administered E_2 (40 μg/kg) or vehicle subcutaneously 24 h before mechanical stimulation (300 cycles, 150 N). Animals were anesthetized using 60 mg/kg pentobarbitol sodium (Rhone Merieux, Harlow, Essex, UK) 1 or 6 h after mechanical loading, and killed after intracardiac perfusion–fixation with 4% paraformaldehyde for 15 min. The sixth (nonloaded) and eighth (loaded) caudal vertebrae were removed and postfixed in 4% paraformaldehyde at 4°C for a further 24 h. The bones were then decalcified in 10% EDTA (Sigma Chemical Co.) before embedding in paraffin wax.

The IGF-I cDNA (21) was radiolabeled with [^{35}S]dATP using a random prime DNA labeling method (Megaprime DNA Labelling Systems, Amersham, Bucks, UK) to a specific activity of no less than 1×10^8 counts per min (cpm)/μg. A rat c-*fos* cDNA (22) was subcloned into pSP72 (Promega, Southampton, UK) using standard procedures (23). The vector was linearized with BamHI and transcribed with T7 polymerase to generate a 2-kb antisense strand. The sense probe was generated by linearizing with EcoRV and transcribing with Sp6. 1μg of linearized vector was Labeled with [^{35}S]UTP using a Sp6-T7 transcription kit (Boehringer Mannheim, Mannheim, Germany).

Mid-coronal sections of the vertebrae, 6 μm thick, were cut and mounted onto glass slides coated with 3-aminopropyltriethoxysilane (Sigma Chemical Co.). The method for IGF-I cDNA in situ hybridization has been described in detail previously (24). For the c-*fos* mRNA in situ hybridization a similar prehybridization procedure was followed. Sections were hybridized against radiolabeled probe (specific activity of 5×10^4 cpm/μl) for 16 h at the calculated melting temperature of 50°C in a humidified environment. Sections were then rinsed twice with 2× SSC (1× SSC = 0.15 M NaCl, 0.015 M Na citrate, pH 7) at room temperature, once with 1× SSC/50% formamide at 50°C, once with 2× SSC at room temperature, followed by incubation for 30 min in RNase A (50 μg/ml) (Sigma Chemical Co.) in 2× SSC at 37°C. Sections were submitted to two more 1× SSC washes at room temperature before a final wash in 0.5× SSC. The sections were dehydrated in graded alcohols containing 0.3 M ammonium acetate and air-dried. For IGF-I cDNA, controls consisted of sections treated with RNase A (100 μg/ml in 2× SSC for 1 h) before hybridization. Hybridization with sense probe served as the control for c-*fos*.

Autoradiography was used to visualize the probe by coating sections with nuclear emulsion (K5) (Ilford, Ilford, UK) and stored for 14 d at 4°C. The slides were then developed, counterstained with hematoxylin and eosin, and mounted. Each hybridization run for each probe incorporated two sections from each of the animals at each time point, to avoid inter-run variability.

Hybridization in osteocytes was assessed in two sections of bone per vertebra. Osteocytes within the area bounded by the 1.5-mm length of cortical bone in the mid-diaphysis were assessed for hybridization for mRNA, and expressed as the number of osteocytes exhibiting hybridization, as a percentage of the total number of osteocytes. A minimum of 600 trabecular and 1,000 cortical osteocytes were counted for each vertebra.

Statistical analysis. All measurements were performed blind. Results for each group of animals are expressed as the mean±SEM. Statistical analysis was performed by comparing paired groups using Fisher's least significant difference method for multiple comparisons in a one-way analysis of variance with StatView 1.02 (Abacus Concepts, Inc., Berkeley, CA). Differences were considered statistically significant at $P < 0.05$.

Results

We expected that E_2 would augment the mechanical response of rat bone. Therefore, in a pilot experiment a loading regime that we have previously found (15) to produce a minimal osteogenic response was used. The dynamic indices of bone formation showed an increase similar to that previously seen with

1. *Abbreviations used in this paper:* BFR, bone formation rate; BS, bone surface.

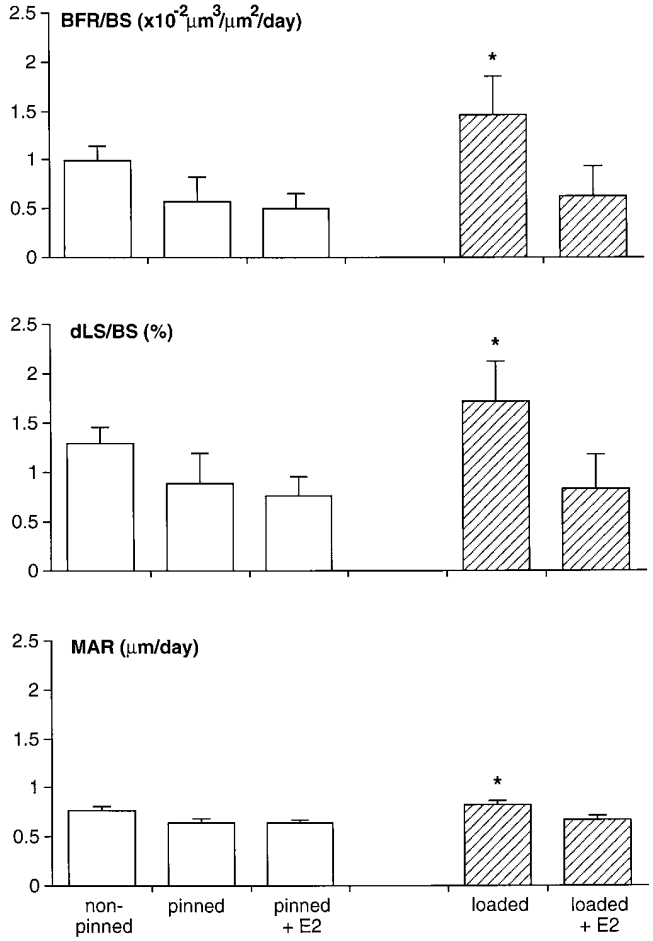

Figure 1. Dynamic histomorphometric parameters of effect of E_2 on response to minimally effective mechanical stimulation of eighth caudal vertebra in the rat. Rats were administered E_2 (40 μg/kg) or vehicle daily for 11 d. 24 h after the first administration, pins were inserted through the seventh and ninth vertebrae, and the eighth vertebra thus loaded in compression as previously described (15) by 30 cycles (0.5 Hz) per day of 50 N by a mechanically operated cam device. Calcein was injected 24 h and tetracycline 8 d after the first episode of mechanical stimulation. Rats were killed 2 d after the tetracycline injection. Initial and final body weights did not differ significantly between groups (range 241–244 grams [initial] and 244–260 grams [final]). Longitudinal growth rates were less than 7 μm/day in all groups. *$P > 0.05$ vs. all other pinned and loaded groups.

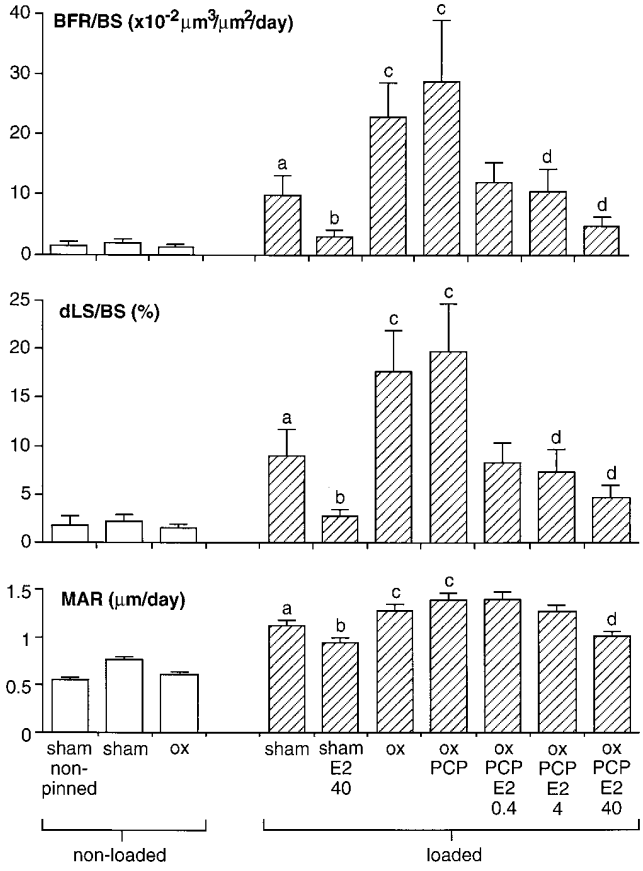

Figure 2. Effect of ovariectomy (*ox*) and E_2 on response of caudal vertebrae to mechanical stimulation. Rats were subjected to ox or sham-ox on day 0. Pamidronate (*PCP*) was administered on day 0 to the groups shown. All animals received daily subcutaneous injections of either E_2 (doses shown in μg/kg per day) or vehicle, starting at day 0. Eighth caudal vertebrae were subjected to mechanical stimulation by 300 cycles (0.5 Hz) of 150 N loading in compression on day 1. Fluorochrome labels were injected on days 2 and 7, and animals were killed on day 8 for assessment of static and dynamic parameters of cancellous bone formation. *a*, $P < 0.05$ vs. nonloaded groups; *b*, $P < 0.05$ vs. loaded controls; *c*, $P < 0.05$ vs. loaded controls; *d*, $P < 0.05$ vs. ox/PCP group.

the same loading regime (15), but, unexpectedly, this response was suppressed by E_2 (Fig. 1).

To determine the extent to which E_2 suppressed mechanical responsiveness the experiment was repeated, using a more intense loading regime. Similar to our experience above, a single episode of mechanical stimulation by 300 cycles (0.5 Hz) of 150 N loading induced a sixfold increase in BFR/BS which was suppressed by E_2 (40 μg/kg per day) (Fig. 2). Consistent with a suppressive action by estrogen, ovariectomized rats showed a significantly increased response to mechanical stimulation. Because ovariectomy induces bone resorption, which itself stimulates bone formation, perhaps through resorption-induced sensitization of the skeleton to mechanical stimulation (11, 25), we suppressed resorption in some groups with pamidronate. BFR/BS was not significantly changed in ovariectomized animals administered with pamidronate, suggesting the increased bone formation in ovariectomized rats is not attributable to an increase in bone resorption caused by ovariectomy. E_2 showed dose-dependent suppression of mechanical responsiveness in such animals (Fig. 2).

None of the groups showed significant changes in body weight or bone volume during these short experiments. As expected, uterine weights were reduced by ovariectomy and normalized by E_2 at 4 μg/kg (data not shown). Static indices of bone formation (ObS/BS, NOb/BS) showed a similar pattern to that observed in the dynamic histomorphometric measurements, consistent with stimulation of bone formation by mechanical stimulation, and suppression of this response by E_2 (data not shown). Osteoclast indices did not show significant changes ($P > 0.05$).

It is known that bone formation and bone resorption are closely coupled. If mechanically induced bone formation were preceded by resorption, resorption surfaces should be ob-

served to be substantially increased soon after mechanical stimulation, preceding the increase in mineralizing surface. We therefore assessed the eroded surface in animals before, and at 6, 24, and 48 h after loading (300 cycles, 0.5 Hz, 150 N): previous experiments (15, 24) suggest that the osteogenic response is maximal within 48–72 h of mechanical stimulation. We found no significant ($P > 0.05$) change in eroded surface before this time (ES/BS (%) time 0, 0.58±0.08; 6 h, 0.81±0.24; 24 h, 0.43±0.15; 48 h, 0.41±0.15).

We have recently found that cancellous bone formation induced by mechanical stimulation is preceded by expression of mRNA for c-*fos* and IGF-1 in osteocytes (3, 24). The relationship between this response and bone formation is not known, but the mRNA expression represents a quantifiable measure of osteocytic responsiveness to mechanical stimulation. To determine whether the E_2-mediated suppression of mechanically induced bone formation was associated with a change in osteocytic responsiveness, we subjected the eighth caudal vertebra of animals pre-treated with E_2 (40 μg/kg) or vehicle to a single episode of loading (150 N for 300 cycles at 0.5 Hz). Animals were killed 1 or 6 h later for assessment of osteocytic expression of mRNA for c-*fos* and IGF-1 respectively by in situ hybridization. We found that in E_2-treated animals, 32±5 (mean±SEM, eight animals) percent of cortical osteocytes exhibited hybridization for IGF-1 mRNA, compared to 47±10% vehicle-treated animals. For c-*fos* mRNA, corresponding values were 42±5.5 and 39±9 respectively (eight animals per group). Neither difference was significant. Neither c-*fos* nor IGF-1 mRNA was detected in any osteocytes from nonloaded vertebrae.

These data suggest that E_2 does not suppress the osteogenic response through an action on osteocytes. To clarify the effects of E_2 further, we performed two experiments. To test whether E_2 has an action at the time of strain imposition, we administered a single dose (40 μg/kg) 3 h before mechanical stimulation. This regime induces peak E_2 levels in serum by 2 h, with a serum half-life of approximately 8 h (data not shown). We found (Table I) no significant effect on the osteogenic response. In the second experiment, we repeated an assessment of the effects of E_2 on mechanical responsiveness, in animals in which daily E_2 administration started 24 h after, or 24 h before, mechanical loading. It has previously been shown that osteocytic IGF-1 mRNA expression is decreasing by 24 h after loading (24). Dynamic parameters of bone formation showed suppression of the osteogenic response by E_2 administration, whether commenced before or after mechanical stimulation (BFR [mean±SEM], nonpinned 1.6±0.8; pinned 1.3±0.4; pinned + E_2 (40 μg/kg per day) 0.7±0.3; loaded (300 cycles, 0.5 Hz, 150N) 10.0±3.0; loaded + E_2 commencing 24 h before load 0.6±0.2; loaded + E_2 commencing 24 h after load 0.3±0.2). Dynamic parameters of bone formation in the tibiae of these animals confirmed our previous finding (15), that BFR/BS is increased by E_2 in the tibial metaphysis in short-term experiments (data not shown). We have previously found that this increase is transient, and is followed by a decrease in bone formation in longer experiments (19). This early stimulation and later inhibition of bone formation by E_2 is consistent with a model in which E_2 increases the activity of already-active osteoblasts, while inhibiting induction of new sites of bone formation.

Table I. Effect of a Single Dose of E_2 3 h before Mechanical Loading, on Mechanical and Dynamic Morphometric Assessment of Responsiveness of Cancellous Bone of Eighth Caudal Vertebrae

	Pinned	Load	Load E_2 (−3 h)
BFR/BS (10^{-2} μm³/μm² per day)	1.9±0.9	19.5*±4.8	13.8*±5.7
dLS/BS (percent)	2.0±1.0	13.2*±2.8	9.1*±2.9
MAR (μm/day)	0.9±0.1	1.4*±0.1	1.4*±0.2
Osteoblast surface (ObS/BS) (percent)	0.1±0.1	1.9*±0.68	2.8*±0.7
No. of osteoblasts (No Ob/BS) (percent)	0.04±0.03	1.1*±0.4	1.5*±0.3
Eroded surface (ES/BS) (percent)	2.6±0.4	2.5±0.3	2.9±0.6
Osteoclast surface (OcS) (percent)	< 0.1	< 0.1	< 0.1

Rats were injected with E_2 (40 μm/kg) or vehicle 3 h before mechanical stimulation (300 cycles, 0.5 Hz, 150 N). Animals were administered fluorochrome labels 3 to 7 d later, and killed after 8 d. *$P < 0.05$ vs. pinned.

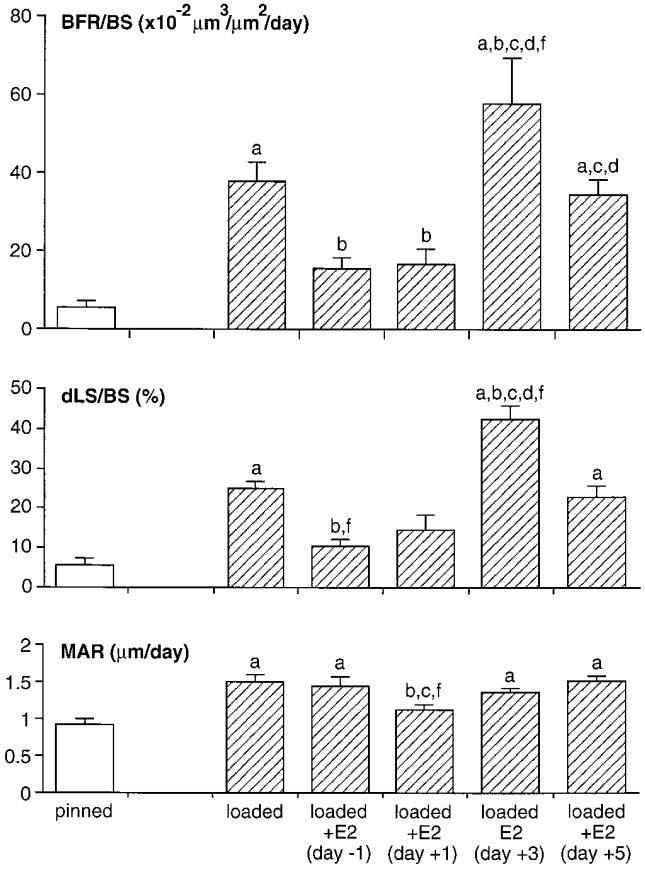

Figure 3. Effect of commencement of E_2 at varying times relative to mechanical stimulation, on osteogenic response of rat (Tuck & Son, Battlesbridge, Essex, UK) vertebrae to mechanical load. Rats were stimulated by 300 cycles of 150N at 0.5 Hz. E_2 (40 μg/kg per day) was commenced 1 day before, or 1, 3, or 5 d after loading. Fluorochrome labels were administered 3 and 7 d after loading; the experiment was terminated 8 d after loading. *a,* $P < 0.05$ vs. pinned; *b,* vs. loaded control; *c,* vs. E_2 d-1; *d,* vs. E_2 d + 1; *f,* vs. E_2 d + 5.

Table II. Effect of Commencement of E_2 Administration to Rats at Varying Times Relative to Mechanical Stimulation, on Static Morphometric Measurements of Cancellous Bone of Eighth Caudal Vertebrae

	Osteoblast surface (ObS/BS) (percent)	Number of osteoblasts (No Ob/BS) (percent)	Eroded surface (ES/BS) (percent)	Osteoclast surface (OcS) (percent)
Pinned	0.6±0.2	0.4±0.1	3.7±1.0	0.1±0.1
Load	3.6*±0.8	1.8*±0.4	2.3±0.3	0.1±0.1
Load E_2 (day −1)	1.1‡§‖±0.2	0.6‡§±0.1	2.7±1.0	0.1±0.1
Load E_2 (day +1)	1.0‡§‖±0.2	0.6‡§±0.1	1.7±0.5	< 0.1
Load E_2 (day +3)	5.3*±1.4	2.5*‖±0.6	2.4±0.7	< 0.1
Load E_2 (day +5)	3.2*±0.7	1.5*±0.3	1.4*±0.2	< 0.1

Rats (Tuck & Son, Battlesbridge, Essex, UK) were stimulated by 300 cycles at 0.5 Hz. E_2 (40 μm/kg per day) was commenced 1 d before, or 1, 3, or 5 d after loading. The experiment was terminated 8 d after loading. *$P < 0.05$ vs. pinned; ‡vs. load; §vs. load E_2 day +3; ‖vs. load E_2 day +5.

We therefore tested the effect of E_2 on the function of already-active osteoblasts, by commencing E_2-administration 3 d after loading, at which time we had previously found that matrix protein gene expression is maximal (24). As before, mechanical stimulation (300 cycles, 0.5 Hz, 150 N) induced a substantial increase in indices of bone formation (BFR (mean±SEM) pinned 1.6±0.4; loaded 21.4±5.6). Unlike the previous results, however, E_2 administration commencing 3 days after loading induced a significant increase in BFR/BS (35.6±8.4) compared to loaded control rats (pinned control + E_2 at day 3: 1.0±0.3).

This was confirmed in a similar experiment in which E_2 administration was started the day before, 1, 3 or 5 days after loading. Again, we found that when E_2 was commenced the day before, or the day after loading, the osteogenic response was significantly inhibited (Fig. 3, Table II). However, when E_2 was commenced 3 d after loading, a significant enhancement of the osteogenic response was observed. This was not seen when E_2 was commenced after 5 d: this might be because E_2 was commenced in this group too late for an osteogenic response to be detected, 2 d after the first fluorochrome; or because the osteogenic response to a single episode of mechanical stimulation is largely complete after 5 d (24).

Discussion

We have previously found that dynamic loading induces bone formation on the trabecular surfaces of the loaded rat tail vertebra (15). It has been found that peak strains measured in bones during physiological activity are in the range 1,000–3,000 microstrain (16). Thus, the strains imposed by our mechanical loading regime (700 microstrain) are comparable to those experienced by bone during physiological usage. Strains of physiological magnitude induce bone formation in other species also, and it is presumed that the bone formation so induced is a mechanically adaptive response to a strain of physiological magnitude, exerted in a direction or distribution to which the bone is unaccustomed. This process is thought to be the basis for mechanical adaptation in bone (2).

In this communication, we have exploited this model to test the hypothesis that estrogen alters the set-point for mechanical adaptation (9, 12) by facilitating the anabolic response of bone to mechanical stimulation. We expected that E_2 would augment the mechanical response of rat bone, and therefore we initially utilized a minimal mechanical stimulus. To our surprise, E_2 suppressed, rather than stimulated, bone formation. We therefore tested the sensitivity of ovariectomized animals, given a range of E_2 doses, to a more intense mechanical stimulus. Because ovariectomy induces bone resorption, which might sensitize the skeleton to mechanical stimulation (11, 25), we suppressed resorption in some groups, with a dose of bisphosphonate previously shown by us to suppress bone loss after ovariectomy (18). As predicted by the first experiment, we found that ovariectomy sensitized the trabecular bone to mechanical stimulation. This sensitization was unaffected by bisphosphonate, but was suppressed in a dose-dependent manner by E_2. It thus seems likely that estrogen itself, rather than some other ovarian hormone, such as progesterone, which can be suppressed by E_2 administration, is responsible for the inhibition of mechanical sensitivity. Moreover, the increased mechano-sensitivity of ovariectomized animals, and its reversal by low doses of E_2, suggest that physiological levels of estrogen can suppress the response of the rat vertebral metaphysis to mechanical stimulation.

Suppression of mechanically induced bone formation by estrogen might be explained by the well-documented coupling between bone formation and bone resorption that occurs during bone turnover. In both rats and humans, estrogen deficiency causes increased bone resorption, followed by increased bone formation; and suppression of resorption, whether by estrogen, bisphosphonate, or calcitonin, suppresses bone formation (26–30). Thus, suppression of bone formation by estrogen is generally considered to be due to its ability to suppress bone resorption. However, mechanically induced bone formation appears not to be dependent upon prior bone resorption (31, 32), possibly because coupling itself might occur through resorption-enhanced mechanical stimuli (11, 25). Indeed, mechanical stimulation suppresses bone resorption (15, 33). In the present experiment, we noted no inhibition of the mechanical response by bisphosphonate. Moreover, we observed no increase in eroded surface, which did not exceed 1% of the bone surface, during the first 48 h after loading, while ~ 25% of the trabecular surface shows evidence of bone formation by 72 h (24). These observations suggest that estrogen suppresses the activation of bone formation in a way that differs from other resorption-inhibitors, through an unknown mechanism. Thus, activation of bone surfaces for both resorption and formation might be directly suppressed by estrogen. We speculate that estrogen deficiency might facilitate both processes through a shared mechanism, such as enhanced cytokine expression, as has been suggested for ovariectomy-induced bone resorption (34).

It is generally held that osteocytes are likely to play a key role in the mechanical responsiveness of bone. Consistent with this, we have recently found that c-*fos* and IGF-1 mRNA expression in osteocytes precedes mechanically induced bone formation (3, 24). To determine whether estrogen acted to suppress osteocytic responsiveness, we assessed the proportion of osteocytes showing detectable c-*fos* and IGF-1 expression after mechanical stimulation, in the presence and absence of estrogen. We found no significant difference in the proportions. However, for IGF-1 at least, our results do not exclude a

suppressive action of E_2 on osteocytic responsiveness. We also found that bone formation was not affected when E_2 was administered as a single injection 3 h before mechanical stimulation, and bone formation was suppressed to a similar degree in animals starting estrogen administration 24 h before, and 24 h after, mechanical stimulation. This suggests that estrogen suppresses the induction of osteogenesis not through, or not only through, an action on the strain-sensing mechanism itself, but on an early component of the osteogenic response to signals generated by strain-responsive cells.

Suppression of the osteogenic response by E_2 was especially surprising, but even a failure of E_2 to amplify the response of bone to mechanical stimulation would have been unexpected, because the general view is that E_2 maintains bone mass by altering the set-point for mechanical adaptation. It is conceivable that E_2 maintains bone mass entirely through suppression of resorption. However, there is evidence that E_2 can also stimulate bone formation (18, 35–38). In the rat, the stimulation is transitory, and is followed by suppression of bone formation to the subnormal levels typically observed after prolonged estrogen administration (19). This pattern of response raises the possibility that the anabolic action of estrogen might primarily increase the activity of committed, already active osteoblasts. When estrogen was commenced 3 days after mechanical stimulation, at which time matrix protein gene expression is at its greatest, it augmented the response of bone to mechanical stimulation. A similar synergistic interaction between mechanical stimulation and estrogen on (established) osteogenesis has been observed in vitro (39) This interaction might explain why there is an early stimulation of dynamic indices of bone formation in the (mechanically used) tibia (18), but not in (nonloaded) tail vertebrae, while the action of E_2 to suppress induction of new episodes of osteogenesis might account for the long-term suppression of bone formation by E_2, seen in rats and humans. The data are also consistent with recent fracture healing studies, in which rats with higher estrogen levels developed a less exuberant but ultimately stronger callus (40, 41).

A substantial body of evidence supports the notion that estrogen maintains bone mass by sensitizing the skeleton to mechanical stimuli (see 9, 11, 12). If this were the primary role of estrogen, however, we would not expect the hormone to suppress the induction of bone formation in response to loading, nor should long term administration of the hormone suppress bone formation to subnormal levels in osteopenic rats and women. Suppression of not only resorption, but also, independently, of induction of bone formation, suggests that the primary role of estrogen is to cause the skeleton to resist rapid changes in mass — to stabilize rather than to maintain bone mass. Superimposed on this function, estrogen appears to have the ability to sensitize already-active osteoblasts to mechanical stimuli. Therefore, while estrogen deficiency causes rapid bone loss through increased turnover accompanied by relatively deficient bone formation, estrogen administration should gradually increase bone mass, through a net anabolic effect operating on a background of slow bone turnover.

The cell biological basis for these responses remains unknown, but it may be significant that estrogen responsiveness has been documented in vitro in bone cells of two distinct phenotypes: committed osteoblastic cells, which respond with increased matrix protein gene expression (42–44); and bone marrow stromal cells, which have recently been shown to be of the same lineage as osteoblasts (45), and possess E_2 receptors (46), and which respond to estrogen with suppression of synthesis of cytokines (see 34, 47). These cytokines, or related cytokines or responses, might be involved in the activation after ovariectomy of responsiveness of bone surfaces to not only bone-resorbing but also bone-forming stimuli. The data presented in this communication may provide a physiological context for a cellular and molecular analysis of these responses, and have implications for therapeutic strategies for the reversal of osteopenia.

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