Vertebral Bone Resorption In Vitro: Effects of Parathyroid Hormone, Calcitonin, 1,25 Dihydroxyvitamin D₃, Epidermal Growth Factor, Prostaglandin E₂, and Estrogen

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Summary. We have developed and characterized a new bone resorption system to test the effect of estrogen on vertebral bone in vitro. Neonatal mouse vertebral bones prelabeled with ⁴⁵Ca were maintained in stationary tissue culture for 60–108 hours at 37°C in 5% CO₂/air. Each vertebral bone measured approximately 1 mm × 1 mm. Hormonal treatments were added directly to the incubation medium. The morphological appearance of these bones, before and after the onset of resorption, was examined by scanning electron microscopy. Bone resorption was measured by determining the % of the bone ⁴⁵Ca released into the incubation medium. Vertebral bone resorption was stimulated in a dose-dependent manner by parathyroid hormone (1–100 nM), 1,25 dihydroxyvitamin D₃ (0.0325–3.25 nM), and prostaglandin E₂ (3–3000 nM). Epidermal growth factor (300 ng/ml) produced a small stimulation of bone resorption which was not inhibited by indomethacin (0.5 μM). Likewise, indomethacin (0.5 μM) did not inhibit PTH-stimulated vertebral bone resorption. Calcitonin (6.6 nM) produced a 79% inhibition of bone resorption induced by PTH (10 nM), whereas estradiol (up to 3 μM) did not inhibit bone resorption. Our results demonstrate that estrogen does not have a direct effect on vertebral bone tissue in vitro. This new bone culture system is a sensitive assay for the direct effects of resorptive agents on vertebral bone.

Key words: Vertebrae — Estrogen — Resorption — Bone — Parathyroid hormone.

Vertebral bone is the site most frequently involved in osteoporosis-induced bone deformation [1]. Vertebrae contain a large amount of trabecular bone, and excessive loss of trabecular bone is observed in osteoporotic patients [1, 2]. After oophorectomy there is accelerated bone loss from the spine as well as from other bone sites [3]. Estrogen replacement therapy has been shown to be effective in prevention of the bone loss after oophorectomy [3] and that occurring postmenopausally [4].

The mechanism by which estrogen produces its effect(s) on bone has not been determined. Attempts to locate estrogen receptors in bone have not demonstrated their presence [5, 6]. In vitro studies which have utilized calvaria (intramembranous bone) and limb bones (endochondral bone) have failed to show an effect of estrogenic compounds on bone resorption or collagen synthesis [7–9]. The direct effect of estrogen on vertebral bone tissue in vitro has not been examined.

The present study was undertaken to develop and characterize a vertebral bone resorption system and to test the effects of estrogen in this system.

Materials and Methods

Materials

Parathyroid hormone (1-34, bovine) (PTH) was obtained from Bachem (Torrance, CA). 1,25 dihydroxyvitamin D₃ (1,25(OH)₂D₃) was the generous gift of Dr. Milan Uskokovic, Hoffman LaRoche. Prostaglandin E₂ (PGE₂) was the generous gift of Dr. Norman Brink, Merck Pharmaceutical, and salmon calcitonin (sCT) was generously supplied by Dr. James Bastian of Armour Pharmaceutical (Kankakee, IL). Epidermal Growth Factor was purchased from Collaborative Research, Inc. (Lexington, MA).
Indomethacin (final solvent concentration in the culture medium equals 0.1% absolute alcohol) was obtained from Sigma Chemical Comp. (St. Louis, MO). Estradiol 17β (E₂) was obtained from Steraloids, Inc. (Wilton, NH).

Bone Tissue Preparation and Culture

Neonatal mice (4–6 days old) were subcutaneously injected with 20 μCi ⁴⁵CaCl₂ 24 hours prior to sacrifice and removal of tail. The caudal vertebrae were excised from the mouse tail and the first 11 calcified vertebrae were discarded. The next four vertebrae (i.e., #12, #13, #14, and #15) were consecutively excised. The calcified region of each vertebra was dissected, and used in stationary tissue culture. The bone was transferred onto a 3 mm x 3 mm Gelman filter (0.45 μm pore size). The bones were precultured for 3–5 hours in culture medium before being transferred to the final culture medium with or without hormonal treatments added. Vertebrae were cultured on Gelman filters (3 mm x 3 mm) on stainless steel screens in glass culture dishes for 60–108 hours at 37°C in 5% CO₂air. Each culture dish contained 500 μl of Dulbecco’s modified Eagle’s medium (DMEM, Whittaker M.A. Bioproducts, Inc., Walkersville, MD) to which hormonal treatments were directly added. The DMEM was supplemented with 15% heat inactivated (56°C for 1 hour) horse serum, 10 U/ml heparin, and 100 U/ml penicillin. After 48 hours of culture and at every subsequent 24 hours of culture, the progress of the bone resorption was determined by the morphological appearance of the bones under a dissection microscope at a 16 x magnification. At the end of the culture period the vertebral bones were extracted overnight in 500 μl of 0.1 N HCl. ⁴⁵Ca extracted from the bones and ⁴⁵Ca released from the bones into the incubation medium during the incubation were measured by liquid scintillation spectrometry with a Beckman (Model 7500) liquid scintillation spectrometer. Results are expressed as the percent of the bone ⁴⁵Ca released.

Preparation of Vertebral Bones for Scanning Electron Microscopy

Vertebral bones, dissected and cultured as described above, were washed twice with 0.15 M cacyolate buffer and fixed in 1% glutaraldehyde (0.15 M cacodylate buffer, pH 7.2) for a 30-minute fixation at room temperature. The bones were washed twice with 0.15 M cacodylate buffer, pH 7.2, followed by a post-