Journal of Cellular Biochemistry 57:271–279 (1995)

Estrogen Modulation of Osteoclast Lysosomal Enzyme Secretion

M. Kremer, J. Judd, B. Rifkin, J. Auszmann, and M.J. Oursler

Department of Biochemistry and Molecular Biology, Mayo Clinic and Foundation, Rochester, Minnesota 55905 (M.K., J.J., M.J.O.); Division of Basic Sciences, New York University College of Dentistry, New York, New York 10010 (B.R., J.A.)

Abstract Osteoclast-mediated bone resorption is accomplished by secretion of lysosomal proteases into an acidic extracellular compartment. We have previously demonstrated that avian osteoclasts and human osteoclast-like giant cell tumor cells respond in vitro to treatment with 17β-estradiol (17β-E_2) by decreased bone resorption activity. To better understand the mechanism by which this is accomplished, we have investigated the effects of 17β-E_2 treatment on lysosomal enzyme production and secretion by isolated avian osteoclasts and multinucleated cells from human giant cell tumors in vitro. Isolated cells were cultured with bone particles in the presence of either vehicle or steroid. The conditioned media and cells were harvested, and the levels of cathepsin B, cathepsin L, β-glucuronidase, lysozyme, and tartrate-resistant acid phosphatase (TRAP) activities were determined. There was a steroid dose-dependent decrease in secreted levels of these enzymes. Cell-associated levels of cathepsin L, β-glucuronidase, and lysozyme decreased, whereas cell-associated levels of cathepsin B and TRAP increased. These changes were measurable at 10^{-10} M and maximal at 10^{-8} M 17β-E_2. The changes were detectable at 4–18 h of treatment and increased through 24 h of treatment. The response was steroid specific, since the inactive estrogen isomer, 17α-E_2, failed to alter the activity levels. Moreover, the effects of 17β-E_2 were blocked when the cells were treated simultaneously with the estrogen antagonist ICI182–780 in conjunction with 17β-E_2. Human osteoclast-like cells obtained from giant cell tumors of bone responded similarly to estrogen with respect to cathepsin B, cathepsin L, and TRAP activities. However, secretion of β-glucuronidase and lysozyme were not altered by treatment with 10^{-8} M 17β-E_2. These data indicate that estrogen effects on osteoclast resorption activity may be mediated by decreasing the secretion of cathepsin B, cathepsin L, and TRAP. © 1995 Wiley-Liss, Inc.

Key words: osteoclast, estrogen, resorption

Maintenance of physiologic levels of estrogens has long been recognized as an important component of maintaining bone mass in women. The loss of estrogen at menopause results in the rapid loss of bone volume; this loss can be prevented by estrogen therapy at menopause. The mechanism by which estrogen prevents bone loss is unclear. The identification of estrogen receptors in bone forming osteoblasts generated much research on the effects of estrogen on osteoblasts. The consensus today is that there is little effect of estrogen on the bone forming activity of isolated osteoblast-like cells, but 17β-estradiol (17β-E_2) has been shown to stimulate transforming growth factor type β (TGF-β) secretion by these cells in vitro [Oursler et al., 1991a]. Jilka et al. [1992] have presented convincing evidence that the removal of estrogen from mice causes increased bone resorption by an interleukin-6 (IL-6)-mediated process, thus supporting a role for estrogen repression of osteoclast differentiation via an IL 6-mediated pathway. We have demonstrated that isolated avian osteoclasts and human osteoclast-like cells contain estrogen receptors and respond to estrogen in vitro by decreased resorption activity [Oursler et al., 1991b, 1994]. These data suggest that estrogens may also directly influence the activities of the mature osteoclast, but the mechanism by which estrogen modulated resorption activity remains unresolved.

For many years it has been recognized that osteoclasts produce and secrete lysosomal proteases [Delaisse and Vaes, 1992]. Osteoclasts at-

Received May 18, 1994; revised June 26, 1994; accepted June 29, 1994.

Address reprint requests to Dr. M.J. Oursler, Department of Biochemistry and Molecular Biology, Guggenheim 16, Mayo Clinic, Rochester, MN 55905.

© 1995 Wiley-Liss, Inc.

tach to the bone surface and form a sealed extracellular compartment which is acidified by the action of a hydrogen pump, thus forming an acidic milieu into which lysosomal enzymes are secreted. Which of the lysosomal enzymes is involved directly in the resorption process is unclear. Cathepsins B, C, and L as well as acid phosphatases of the tartrate sensitive and resistant forms, β-glycerophosphatase, aryl sulfatase, and β-glucuronidase have all been identified in the extracellular compartment [Eilon and Raisz, 1978; Baron et al., 1985; Rifkin et al., 1991; Karhukorpi et al., 1992; Goto et al., 1993; Sasaki and Ueno-Matsuda, 1993].

Recently, we have documented that estrogen treatment results in a decrease in the steady state levels of the mRNAs of a variety of lysosomal enzymes in both avian osteoclasts and human osteoclast-like multinucleated cells from giant cell tumors of the bone [Oursler et al., 1994, 1993]. The avian cDNA probes used to study avian osteoclasts identified both lysozyme and a lysosomal membrane protein decreases in mRNA levels. The human cDNA probes used to analyze the human giant cell tumor cultures identified cathepsins B and D and TRAP decreases in mRNA levels. Since mRNA changes do not necessarily result in altered protein production, it was important to investigate the effects of estrogen on osteoclast lysosomal enzyme production and secretion. Therefore, to more fully understand the mechanism by which estrogen modulates osteoclast resorption activity, we have assessed the influence of estrogen treatment on the production and secretion of lysosomal enzymes from highly purified avian osteoclasts and human osteoclast-like cells in vitro.

METHODS

Cell Isolation and Culture

All steroids were dissolved in 95% ethanol (vehicle) at a ×1,000 concentration and stored in the dark at −20°C. Osteoclasts and multinucleated cells from a giant cell tumor were isolated as described previously using an osteoclast-directed monoclonal antibody (a gift from P. Osdoby, Washington University) [Collin-Osdoby et al., 1991; Oursler et al., 1991b, 1994]. Isolated cells were cultured in phenol red-free alpha minimal essential media (αMEM) supplemented with 10% (v/v) charcoal stripped fetal calf serum and bone particles (1 mg/10^6 cells) with either vehicle (control) or the indicated concentration of the indicated steroid for the specified time period in a 5% (v/v) CO_2/95% (v/v) air chamber at 37°C. Cells were harvested by scraping the plates while on ice in culture media, followed by centrifugation at 900*g* for 5 min at 4°C. The cell pellets and the conditioned media were stored separately at −70°C until analyzed.

Enzyme Activity Assays

Cell extracts were obtained by resuspension of the pellets in distilled water containing 0.1% (v/v) Brij 35 detergent and sonication. The conditioned media was supplemented with Brij 35 detergent to the same concentration. The extracts and conditioned media were assayed as follows: TRAP activity was measured using an assay based on the work of Brandenberger and Hanson [1953] and Hofstee [1954]. Briefly, the initial rate of hydrolysis of o-carboxyphenylphosphate was determined by following the increase in absorbency at 300 nm resulting from the liberation of sialyic acid. One unit hydrolyses one micro mole of o-carboxyphenylphosphate per minute at 24°C, pH 5.0. The assay is performed in the presence of 1 mM tartrate. Cathepsin activity was measured by incubation with the appropriate substrate, as outlined by Barrett and Kirschke [1981]. Cathepsin B activity was measured by hydrolyzing Na-CBZ-lysine p-nitrophenyl ester (Sigma Chemical Co., St. Louis, MO) as measured by 520-nm absorbance. Cathepsin L was measured by hydrolyzing Na-CBZ-phenylalanine arginine N methionine (Sigma Chemical Co.) using excitation at 370 nm and resultant emission at 460 nm. β-Glucuronidase was assayed by measuring the hydrolysis of phenolphthalein glucuronic acid. One mole of β-glucuronidase will hydrolyze 1 μmole of phenol phenophthalein β-glucuronidate per minute at 37°C and pH 4.5. The absorbance at 540 nm was measured, and a standard curve to determine the micromoles of phenolphthalein liberated was derived. Lysozyme activity was measured by the rate of lysis of *Micrococcus lysodeikticus* as outlined by Shugar [1952]. One unit is equal to a decrease in turbidity of 0.001 per min at 450 nm at pH 7.0, 25°C. To standardize for cell number, aliquots of the cell pellet extracts were analyzed for protein content by the methods of Bradford (Bio-Rad protein detection system). The results were then calculated per mg protein for each sample.

Statistical Analysis

The results are presented as the ratio of treated to control and represent the mean ±SEM of three separate experiments. The effects of the treatment were compared with the control values by one-way analysis of variance (ANOVA); significant treatment effects were further evaluated by the Fisher's least significant difference method for multiple comparisons in a one-way analysis of variance. Tests were carried out using Apple software (Statview II, Abacus Concepts, Inc., Cupertino, CA).

RESULTS

Cathepsin B

Isolated osteoclasts were treated with a broad range of estrogen concentrations for 24 h. A dose-dependent increase in intracellular cathepsin B activity was detectable at 10^{-10} M 17β-E_2 whereas a dose-dependent decrease in activity in the conditioned media was detectable at 10^{-11} M 17β-E_2 (Fig. 1A). The elevation in intracellular accumulation of cathepsin B was detectable by 4 h of culture and a significant decrease in secreted cathepsin B activity was evident after 18 h of culture (Fig. 1B). The specificity of the response was examined by treatment of isolated osteoclasts for 24 h with the inactive estrogen stereoisomer 17α-E_2 and by co-treatment with 17β-E_2 and a specific estrogen antagonist, ICI 182–780 (a gift from Zeneca [formerly I.C.I.]) [Wakeling et al., 1991] (Fig. 1C). These data demonstrate that 17α-E_2 did not effect osteoclast cathepsin B levels in a manner similar to 17β-E_2 and that the specific estrogen antagonist was effective at blocking the influence of 17β-E_2 on osteoclast Cathepsin B accumulation and secretion.

Cathepsin L

Isolated osteoclasts treated for 24 h with 17β-E_2 secreted less cathepsin L into the media

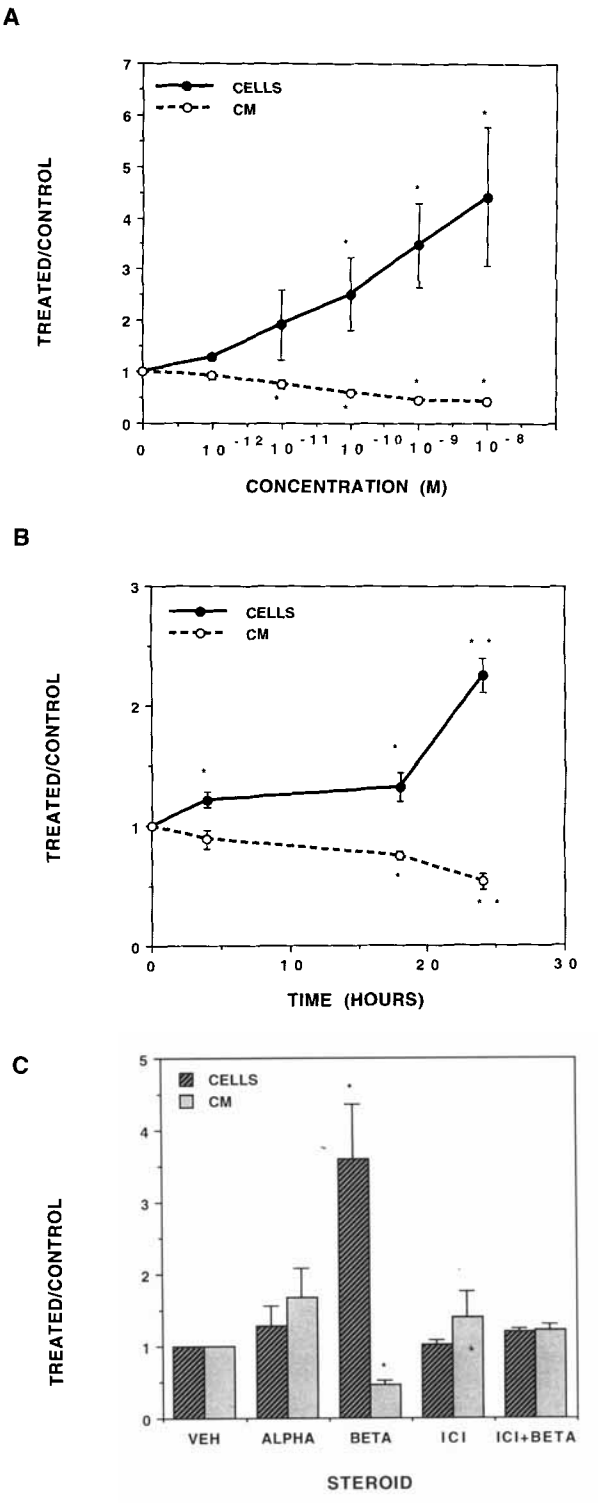
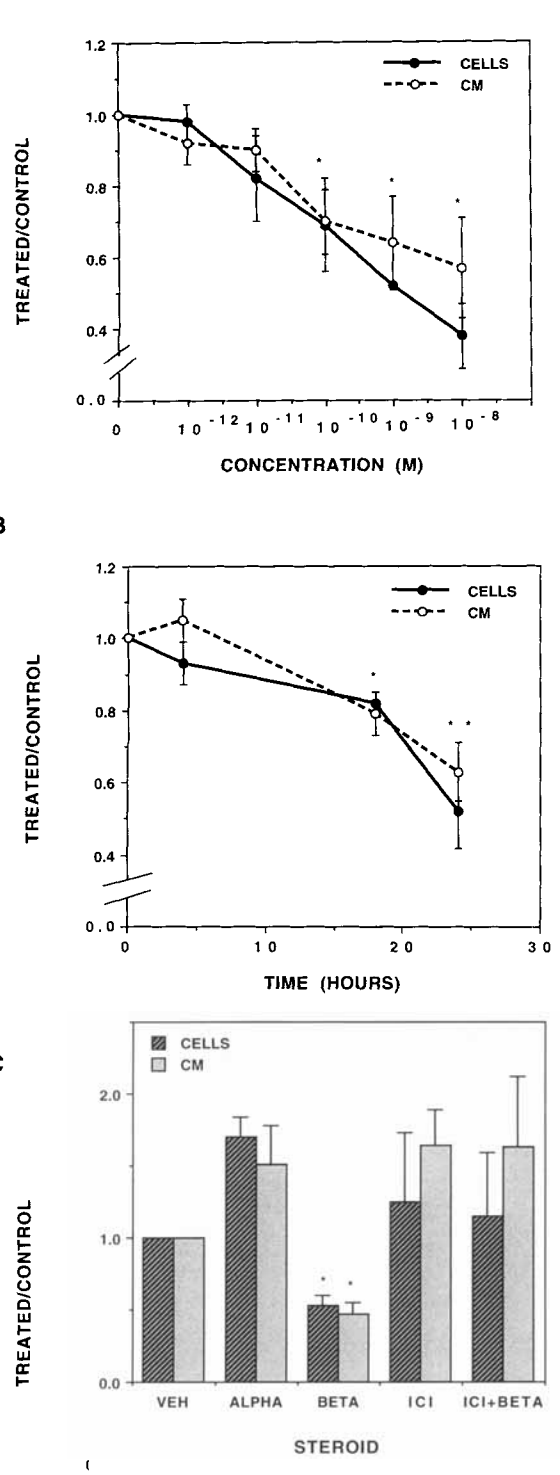


Fig. 1. Cathepsin B response. Isolated osteoclasts were treated with the indicated concentration of 17β-E_2 (**A**) or 10^{-8} M 17β-E_2 for the indicated time (**B**). **C:** Isolated osteoclasts were treated with either vehicle (VEH), 10^{-9} M 17a-E_2 (ALPHA), 10^{-9} M 17β-E_2 (BETA), 10^{-7} M ICI 182–780 (ICI), or 10^{-9} M 17β-E_2 and 10^{-7} M ICI together (ICI + BETA) for 24 h. Conditioned media and cell extracts were analyzed for cathepsin B activity, as outlined under Methods. Results were standardized for cell number by calculating activity per mg cell protein and are presented as the ratio of treated to control (vehicle treated). $^*P < 0.01$; $^{**}P < 0.005$.



and also contained less intracellular enzyme activity (Fig. 2A). These effects were measurable at 10^{-10} M 17β-E_2 and maximal at 10^{-8} M 17β-E_2. The responses were detectable after 18 h of treatment (Fig. 2B). The response was specific for 17β-E_2, since either treatment with 17α-E_2 or cotreatment with 17β-E_2 and the estrogen antagonist did not result in a similar decrease in either secreted or cell-associated cathepsin L activity (Fig. 2C).

β-Glucuronidase

In an analogous manner to the above cathepsin L results, treatment of isolated osteoclasts with a broad range of estrogen concentrations resulted in a steroid dose-dependent decrease in cell-associated with secreted β-glucuronidase levels that was detectable at 10^{-10} M 17β-E_2 and maximal at 10^{-8} M 17β-E_2 (Fig. 3A). This response was measurable after 18 h of treatment and was specific for 17β-E_2 (Fig. 3B,C).

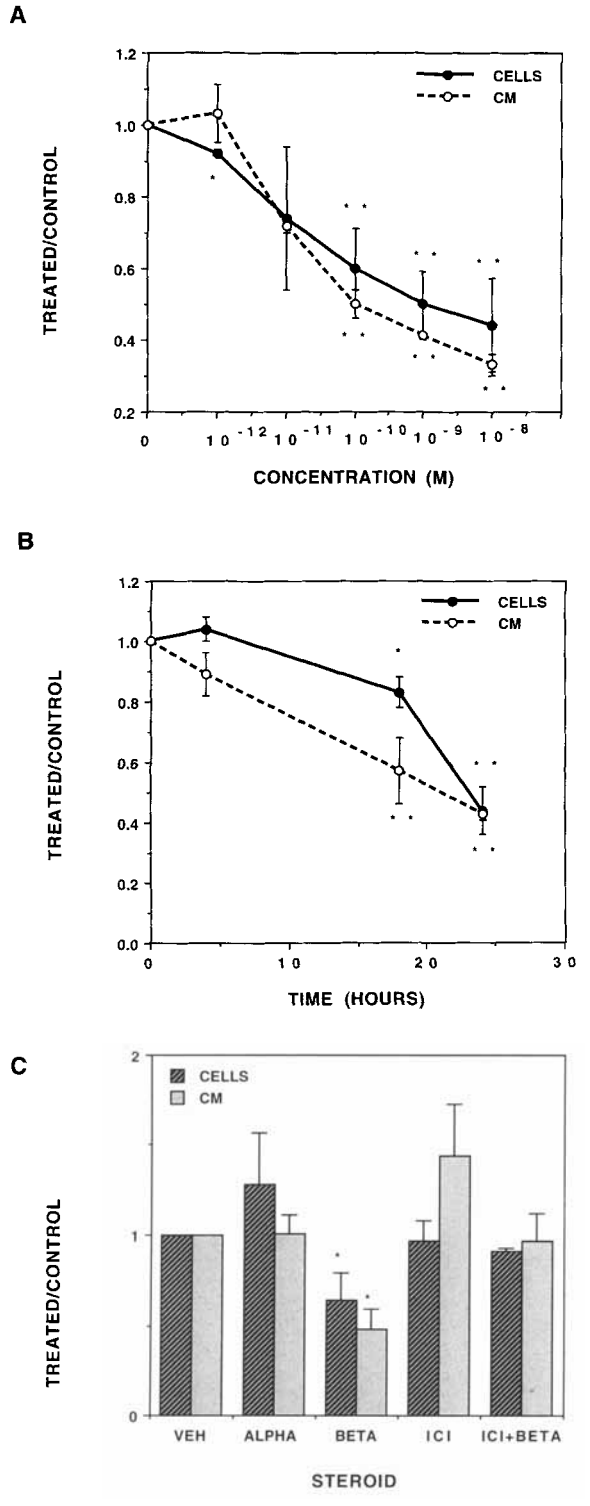
Lysozyme

Cell-associated and secreted levels of lysozyme activity were decreased by treatment with 17β-E_2 and this steroid dose-dependent effect was detectable at 10^{-11} M steroid and maximal at 10^{-8} M 17β-E_2 (Fig. 4A). Moreover, the response was detectable after 18 h of culture and was steroid specific (Fig. 4B,C).

TRAP

TRAP cell-associated enzyme activity levels increased, while the level of secreted activity decreased (Fig. 5A). In response to 17β-E_2 treatment, this effect was steroid dose dependent and measurable at 10^{-11} M 17β-E_2 and maximal at 10^{-8} M 17β-E_2. The influences of 17β-E_2 were detected following 18 h of culture and appeared to be steroid specific (Fig. 5B,C).

Fig. 2. Cathepsin L response. Isolated osteoclasts were treated with the indicated concentration of 17β-E_2 (**A**) or 10^{-8} M 17β-E_2 for the indicated time (**B**). **C:** Isolated osteoclasts were treated with either vehicle (VEH), 10^{-9} M 17α-E_2 (ALPHA), 10^{-9} M 17β-E_2 (BETA), 10^{-7} M ICI 182–780 (ICI), or 10^{-9} M 17β-E_2 and 10^{-7} M ICI together (ICI + BETA) for 24 h. Conditioned media and cell extracts were analyzed for cathepsin L activity, as outlined under Methods. Results were standardized for cell number by calculating activity per mg cell protein and are presented as the ratio of treated to control (vehicle treated). $^*P < 0.01$; $^{**}P < 0.005$.



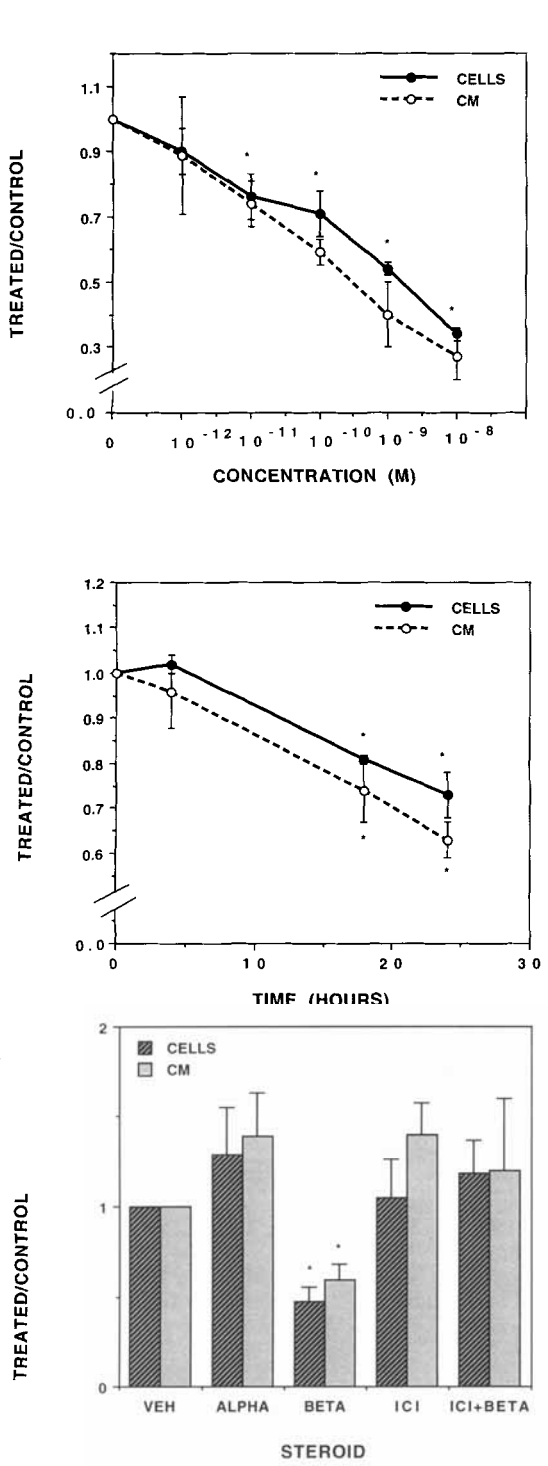
Giant Cell Tumor Cell Responses

Isolated multinucleated cells from the giant cell tumors increased cell-associated levels of cathepsin B, β-glucuronidase, and TRAP following treatment with 10^{-8} M 17β-E_2 (Fig. 6). The levels of secretion of cathepsin B, cathepsin L, and TRAP were decreased, whereas the levels of secretion of β-glucuronidase and cell-associated cathepsin L were unchanged. Moreover, there appeared to be no measurable effects of 17β-E_2 treatment on lysozyme and total acid phosphatase levels.

DISCUSSION

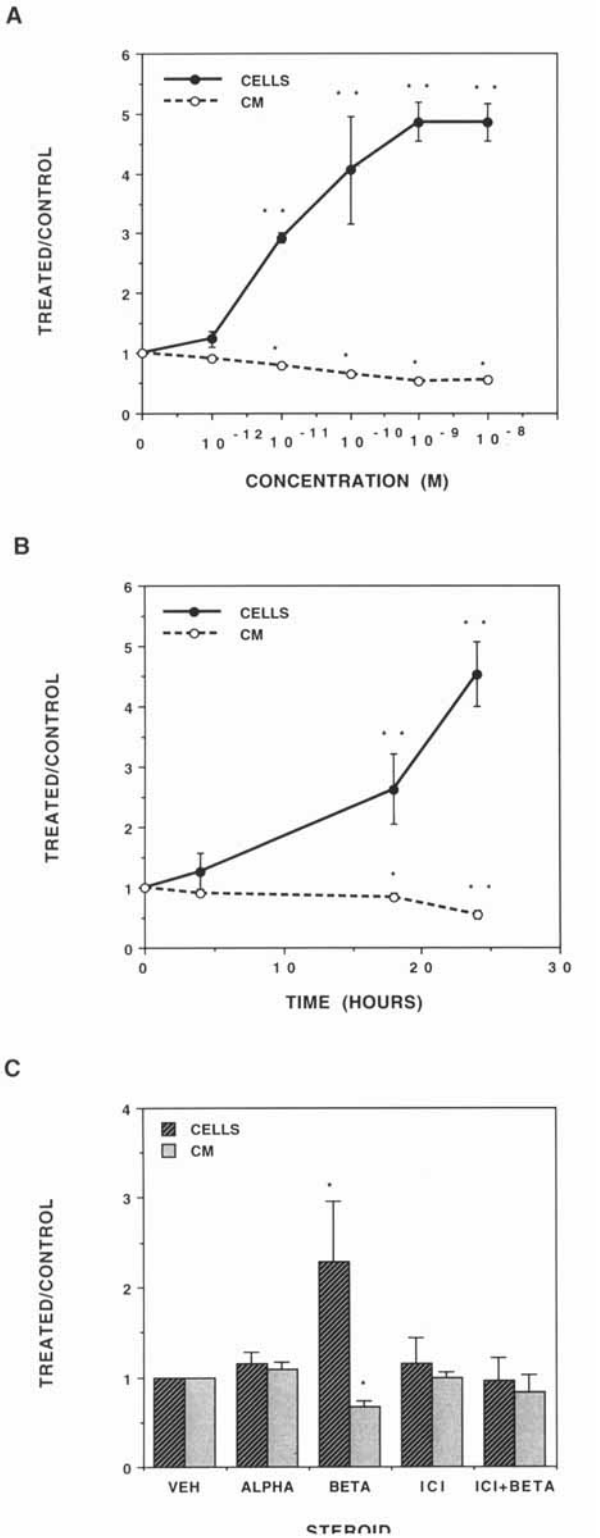
The data presented here demonstrate that estrogen decrease secretion of cathepsin B, cathepsin L, β-glucuronidase, lysosome, and TRAP in culture of avian osteoclasts. Intracellular levels of cathepsin L, β-glucuronidase and lysozyme similarly decrease. By contrast, there is a steroid dose-dependent elevation in the concentration of cathepsin B and TRAP within these cells. The response pattern was somewhat different in isolated human osteoclast-like cells. When a comparison is made between the estrogen responses presented here between authentic avian osteoclasts and human osteoclast-like cells, an interesting pattern appears. In both cell populations, the cellular-associated cathepsin B and TRAP increase, while the secreted levels of these enzymes decreased. The patterns observed with the other lysosomal enzymes are not in accord with the observed response in osteoclasts. These variations may reflect phenotypic differences in estrogen responsiveness. Alternatively, the differences may be the result in differences in the paracrine/autocrine culture environment between the two phenotypes. This is not unlikely given the recent observations of multiple stimulatory factors being produced by the tumor cells in culture [Hasegawa et al., 1993; Oreffo et al., 1993].

Fig. 3. β-Glucuronidase response. Isolated osteoclasts were treated with the indicated concentration of 17β-E_2 (**A**) or 10^{-8} M 17β-E_2 for the indicated time (**B**). **C:** Isolated osteoclasts were treated with either vehicle (VEH), 10^{-9} M 17α-E_2 (ALPHA), 10^{-9} M 17β-E_2 (BETA), 10^{-7} M ICI 182–780 (ICI), or 10^{-9} M 17β-E_2 and 10^{-7} M ICI together (ICI + BETA) for 24 h. Conditioned media and cell extracts were analyzed for β-glucuronidase activity, as outlined under Methods. Results were standardized for cell number by calculating activity per mg cell protein and are presented as the ratio of treated to control (vehicle treated). $^{*}P < 0.01$; $^{**}P < 0.005$.



Osteoclasts firmly attach to the bone surface, forming a sealed compartment between the cell and the bone [Jones et al., 1985]. The plasma membrane juxtaposed to this bone surface becomes lined with hydrogen ATPase molecules that pump H^+ ions into the extracellular space [reviewed by Blair and Schesinger, 1992]. The pH of this compartment can reach as low as 4.7, creating an environment in which acidic lysosomal enzymes are functional [Silver et al., 1988]. Many lysosomal enzymes are directionally secreted into this compartment, including β-glycerol phosphatase, aryl sulfatase, β-glucuronidase, and multiple cathepsins [Eilon and Raisz, 1978; Baron et al., 1985; Rifkin et al., 1991; Karhukorpi et al., 1992; Goto et al., 1993; Sasaki and Ueno-Matsuda, 1993]. Although osteoclasts synthesize and secrets a number of lysosomal enzymes, the precise mechanism by which the bone is degraded remains unclear. Several lysosomal enzymes have been selected for these studies on the basis of evidence in the literature that these enzymes are likely to be involved in bone resorption. TRAP is the most abundantly synthesized and secreted enzyme made by osteoclasts [Hammarstrom et al., 1971]. Moonga et al. [1990] have shown that there is a correlation between TRAP inhibition and decreased resorption activity. Moreover, antibodies to TRAP have been used to inhibit resorption activity in vitro [Zaidi et al., 1989]. Everts et al. [1988] showed that inhibition of cysteine proteases decreased resorption levels, however, there is conflicting evidence on which cathepsins are involved in osteoclast-mediated resorption. Goto et al. [1993] immunolocalized both cathepsin B and cathepsin L in the extracellular resorption lacunae, whereas there was a high level of intracellular cathepsin D in the osteoclasts. Rifkin et al. [1991] demonstrated 25-fold higher levels of cathepsin L than cathepsin B associated with

Fig. 4. Lysozyme response. Isolated osteoclasts were treated with the indicated concentration of 17β-E_2 (**A**) or 10^{-8} M 17β-E_2 for the indicated time (**B**). **C:** Isolated osteoclasts were treated with either vehicle (VEH), 10^{-9} M 17α-E_2 (ALPHA), 10^{-9} M 17β-E_2 (BETA), 10^{-7} M ICI 182–780 (ICI), or 10^{-9} M 17β-E_2 and 10^{-7} M ICI together (ICI + BETA) for 24 h. Conditioned media and cell extracts were analyzed for lysozyme activity, as outlined under Methods. Results were standardized for cell number by calculating activity per mg cell protein and are presented as the ratio of treated to control (vehicle treated). *$P < 0.01$.



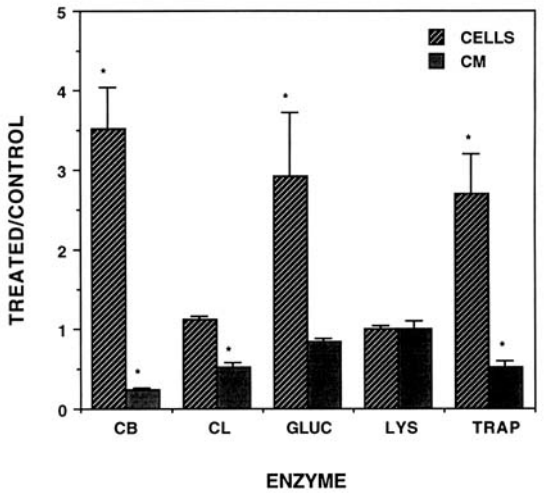


Fig. 6. Giant cell tumor response. Isolated multinucleated cell from three giant cell tumors of the bone were treated with either vehicle or 10^{-9} M 17β-E_2 for 24 h. Conditioned media and cell extracts were analyzed for enzyme activity, as outlined under Methods. Results were standardized for cell number by calculating activity per mg cell protein and are presented as the ratio of treated to control (vehicle treated). *$P < 0.01$.

osteoclasts; furthermore, a selective inhibitor of cathepsin L successfully inhibited bone resorption in vitro. This observation is additionally supported by the data from Kakegawa [1993] that inhibition of cathepsin B activity did not inhibit resorption, whereas inhibition of cathepsin L did inhibit resorption. Collectively, these data support a role for cathepsin L in osteoclast-mediated bone resorption, but there is also evidence that cathepsin B may play a key role in this process. Using a functional assay based on collagenolytic activity at an acid pH, Blair et al. [1993] purified an acidic collagenase from highly purified osteoclasts; sequence data have confirmed it to be cathepsin B. The theory that there are roles for multiple proteases in osteoclast-mediated resorption is supported by the recent work of Page et al. [1993], in which the

Fig. 5. TRAP response. Isolated osteoclasts were treated with the indicated concentration of 17β-E_2 (**A**) or 10^{-8} M 17β-E_2 for the indicated time (**B**). **C:** Isolated osteoclasts were treated with either vehicle (VEH), 10^{-9} M 17α-E_2 (ALPHA), 10^{-9} M 17β-E_2 (BETA), 10^{-7} M ICI 182–780 (ICI), or 10^{-9} M 17β-E_2 and 10^{-7} M ICI together (ICI + BETA) for 24 h. Conditioned media and cell extracts were analyzed for TRAP activity, as outlined under Methods. Results were standardized for cell number by calculating activity per mg cell protein and are presented as the ratio of treated to control (vehicle treated). *$P < 0.01$; **$P < 0.005$.

experimental degradation of bone matrix by purified cathepsin B and cathepsin L was compared to a mixture of proteases obtained from a giant cell tumor of bone. There was some degradation of osteonectin and α2HS-glycoprotein by the purified enzymes, but much more degradation was observed the tumor-derived mixture. Moreover, each cathepsin produced a different pattern of osteocalcin degradation. It could be concluded from these data that the variety of proteins found in the bone matrix requires a mixture of enzymatic activities to degrade them efficiently. This hypothesis is supported by our finding that a mixture of protease inhibitors is required to neutralize the proteolytic activity of isolated osteoclasts which is involved in the activation of latent TGF-β [Oursler, 1994].

The observation that the secretion of cathepsin B and TRAP was decreased by estrogen treatment while the intracellular concentration of these lysosomal enzymes increased is intriguing. There are several possible explanations for these findings, including increased synthesis of enzyme that has not been secreted, decreased rates of enzyme degradation, and impaired transport of enzymes to the cell surface with subsequent secretion. It is interesting that the accumulation of intracellular cathepsin B and TRAP accelerates at 18–24 h of treatment. These data argue strongly that this accumulation is dependent on cellular events (e.g., synthesis of effector proteins) that must occur for a subsequent increased rate of accumulation of these enzymes within the cells. Baron et al. [1988] demonstrated that osteoclasts target lysosomal enzymes to the resorption lacuna in a vectoral manner when actively resorbing. Moreover, inhibition of the resorption process by calcitonin appeared to disrupt secretion of aryl sulfatase [Baron et al., 1990]. This treatment appeared to result in decreased synthesis of lysosomal enzymes as well as decreased secretion of previously synthesized enzymes. Thus, modulation of lysosomal enzyme secretion by calcitonin may be at the level of both synthesis and export of the enzymes.

We have previously shown that treatment with 17β-E_2 decreased steady-state mRNA levels of lysozyme in avian osteoclasts and TRAP, cathepsin B, and cathepsin D in human giant cell tumor cells [Oursler et al., 1993, 1994]. The decreases in steady state mRNA levels were in the range of 30–60%. Thus, the decreased cell-associated and -secreted cathepsin L and lysozyme may be mainly the result of decreased steady-state mRNA levels. The increase in cell-associated cathepsin B and TRAP, coupled with the decreased secretion of these activities, suggests that estrogen may act at multiple levels to modulate enzyme secretion. In this manner, estrogen may impair osteoclast-mediated bone resorption by directly acting on the mature osteoclast to prevent secretion of lysosomal enzymes.

ACKNOWLEDGMENTS

This work was supported by NIH grants R29AR41114 and R01DE09576.

REFERENCES

Baron R, Neff L, Brown W, Courtoy PJ, Louvard D, Farquhar MG (1988): Polarized secretion of lysosomal enzymes: Co-distribution of cation-independent mannose-6-phosphate receptors and lysosomal enzymes along the osteoclast exocytic pathway. J Cell Biol 106:1863–1872.

Baron R, Neff L, Louvard D, Courtoy PJ (1985): Cell-mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of a 100-kD lysosomal membrane protein at the osteoclast ruffled border. J Cell Biol 101:2210–2222.

Baron R, Neff L, Brown W, Louvard D, Courtoy PJ (1990): Selective internalization of the apical plasma membrane and rapid redistribution of lysosomal enzymes and mannose-6-phosphate receptors during osteoclast inactivation by calcitonin. J Cell Sci 97:439–447.

Barrett A, Kirschke H (1981): Proteases of diverse original and function, cathepsin B, cathepsin B, cathepsin H, and cathepsin L. Methods Enzymol 80:535–561.

Blair HC, Schlesinger PH (1992): The mechanism of osteoclast acidification. In Rifkin BR, Gay CV (eds): "Biology and Physiology of the Osteoclast." Boca Raton, FL, CRC Press, pp 259–287.

Blair HC, Teitelbaum SL, Grosso LE, Lacey DL, Tan H-L, McCourt DW, Jeffrey JJ (1993): Extracellular matrix degradation at acid pH. Biochem J 290:873–884.

Brandenberger H, Hanson R (1953): Spectrophotometric determination of acid and alkaline phosphatases. Helv Chim Acta 36:900.

Collin-Osdoby P, Oursler MJ, Webber D, Osdoby P (1991): Osteoclast-specific monoclonal antibodies coupled to magnetic beads provide a rapid and efficient method of purifying avian osteoclasts. J Bone Miner Res 6:1353–1365.

Delaisśe J-M, Vaes G (1992): Mechanism of mineral solubilization and matrix degradation in osteoclastic bone resorption. In Rifkin BR, Gay CV (eds): "Biology and Physiology of the Osteoclast." Boca Raton, FL: CRC Press, pp 289–314.

Eilon G, Raisz LG (1978): Comparison of the effects of stimulators and inhibitors of resorption on the release of lysosomal enzymes and radioactive calcium from fetal bone in organ culture. Endocrinology 103:1969–1975.

Everts V, Beertsen W, Schroder, R (1988): Effects of the proteinase inhibitors leupeptin and E-64 on osteoclastic bone resorption. Calcif Tissue Int 43:172–178.

Goto T, Tsukuba T, Kiyoshima T, Nishimura Y, Kato K, Yamamoto K, Tanaka T (1993): Immunohistochemical localization of cathepsins B, D, and L in the rat osteoclast. Histochemistry 99:411–414.

Hammarstrom LE, Hanker JS, Toverud SU (1971): Cellular differences in acid phosphatase isoenyzmes in bone and teeth. Clin Orthop 78:151–167.

Hasegawa T, Hirose T, Seki K, Sano T, Hizawa K (1993): Transforming growth factor alpha and CD68 immunoreactivity in giant cell tumours of bone: A study on the nature of stromal and giant cells, and their interrelations. J Pathol 170:305–310.

Hofstee BHJ (1954): Direct and continuous spectrophotometric assay of phosphomonoesterases. Arch Biochem Biophys 51:139.

Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC (1992): Increased osteoclast development after estrogen loss: mediation by Interleukin-6. Science 257:88–91.

Jones SJ, Boyde A, Ali NN, Maconnachie E (1985): A review of bone cell and substratum interactions. Scanning 7:5–24.

Kakegawa H, Nikawa T, Tagami K, et al. (1993): Participation of cathepsin L on bone resorption. FEBS 321:247–250.

Karhukorpi E-K, Vihko P, Vaananen K (1992): A difference in the enzyme contents of resorption lacunae and secondary lysosomes of osteoclasts. Acta Histochem 92:1–11.

Moonga BS, Moss DW, Patchell A, Zaidi M (1990): Intracellular regulation of enyzme secretion from rat osteoclasts and evidence for a functional role in bone resorption. J Physiol 429:29–45.

Oreffo, ROC, Marshall J, Kirchen M, Garcia C, Gallwitz WE, Chavez J, Mundy GR, Bonewald LF (1993): Characterization of a cell line derived from a human giant cell tumor that stimulates osteoclastic bone resorption. Clin Orthop 296:229–241.

Oursler MJ (1994): Osteoclast synthesis and secretion and activation of latent transforming growth factor β. J Bone Miner Res 9:443–452.

Oursler MJ, Cortese M, Keeting P, Anderson M, Bonde S, Riggs BL, Spelsberg TC (1991a): Modulation of transforming growth factor-beta production in normal human osteoblast-like cells by 17 beta-estradiol and parathyroid hormone. Endocrinology 129:3313–3320.

Oursler MJ, Osdoby P, Pyfferoen J, Riggs BL, Spelsberg TC (1991b): Avian osteoclasts as estrogen target cells. Proc Nat Acad Sci USA 88:6613–6617.

Oursler MJ, Pederson L, Pyfferoen J, Osdoby P, Fitzpatrick L, Spelsberg TC (1993): Estrogen modulation of avian osteoclast lysosomal gene expression. Endocrinology 132: 1373–1380.

Oursler MJ, Pederson L, Fitzpatrick L, Riggs BL, Spelsberg TC (1994): Human giant cell tumors of the bone (osteoclastomas) are estrogen target cells. Proc Nat Acad Sci USA 91:5227–5231.

Page AE, Hayman AR, Andersson LMB, Chambers TJ, Warburton MJ (1993): Degradation of bone matrix proteins by osteoclast cathepsins. Int J Biochem 25:545–550.

Rifkin BR, Vernillo AT, Kleckner AP, Auszmann JM, Rosenberg LR, Zimmerman M (1991): Cathepsin B and L activities in isolated osteoclasts. Biochem Biophys Res Commun 179:63–69.

Sasaki T, Ueno-Matsuda E (1993): Cystein-proteinase localization in osteoclasts: An immunocytochemical study. Cell Tissue Res 271:177–179.

Shugar D (1952): Measurement of lysozyme activity and the ultraviolet inactivation of lysozyme. Biochim Biophys Acta 8:302–308.

Silver IA, Murrills RJ, Etherington DJ (1988): Microelectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. Exp Cell Res 175:266–276.

Wakeling AE, Dukes M, Bowler J (1991): A potent specific pure anitestrogen with clinical potential. Cancer Res 51: 3867–3873.

Zaidi M, Moonga B, Moss DW, MacIntyre I (1989): Inhibition of osteoclastic acid phosphatase abolishes bone resorption. Biochem Biophys Res Commun 159:68–71.