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Invited Review

Steroid hormones and bone

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Summary. Steroid hormones have an important impact on bone. The mechanism of steroid action on bone cells is through an interaction with specific receptor proteins in the target cells. Steroid receptors are a class of molecules that function as both signal transducers and transcription factors. The receptors each have similar functional domains that are responsible for discrete functions. The mechanism of receptor action is mediated by both genomic and nongenomic pathways. It is believed that in genomic pathway the steroid passes through the cell membrane by passive diffusion and binds to the receptor within the nucleus. Steroids have direct action on osteoblasts via receptor, and the effects of steroids on osteoclastic bone resorption are mediated through osteoblasts which release soluble factors by the action of hormone-receptor binding. However, in addition to indirect effects on osteoclasts, the question whether steroid hormones could exert direct action on osteoclasts as well has not to be answered and findings to date are controversial. It is not doubt that understanding the precise molecular mechanism of steroid action on bone resorption will develop new approaches for the diagnosis and treatment of disease associated with osteoclastic bone resorption.

Key words: Estrogen, Progestins, Androgens, Glucocorticoids, $1\alpha25(OH)_2$ Vitamin D_3, Osteoclast, Osteoblast

Introduction

Steroid hormones are crucial substances for the proper function of the body. The classical steroid hormones have been recognised for many years as being estrogens, progestins, androgens, glucocorticoids and mineralocorticoids. On the other hand, 1α 25 dihydroxy vitamin D_3 [1α 25 $(OH)_2D_3$] vitamin D metabolite, has been accepted as being steroid-like in its mode of action.

The bones are the largest organ in the body. The function of the bones is including the resistance of the mechanical forces due to muscle contraction and gravity, the support of haematopoiesis and participation in mineral homeostasis. It has become apparent that the reproductive steroid hormones, such as estrogens and androgens, have an important impact on bone physiology. They are essential in maintaining bone balance in adults and have a role in maintenance of mineral homeostasis during reproduction. These gonadal steroids appear to be involved in preventing bone loss in women. Other hormones also play major roles in bone metabolism (Raisz et al., 1972a; Lukert and Raisz, 1994; Norman, 1998).

Over the last 15 years, there has been a tremendous increase in the understanding of the mechanisms of action of steroid hormones. The concepts of steroid receptor-mediated action established in studies of uterus, oviduct, breast, and other target tissues have provided the intellectual framework for much of the investigation of hormone action on bone. In this review we will focus on: 1) biology of steroid hormones and their receptors; 2) effect of steroid hormone in bone, particularly in ontogeny, function and cell death of osteoclast and osteoblast.

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General biological action of steroid hormones

Hormones are chemical messengers produced by endocrine glands and secreted into blood, in which they are carried to target organs to exert a specific effect on them. The steroid is a fat soluble organic compound and characterised by its "steroid nucleus". Historically, inclusion of a hormone into the steroid category was based primarily on the chemical structure of the molecule rather than its biological effect or its molecular mechanism of action. The first steroid hormone, an estrogen was isolated in 1929 at a time when the structure of the steroid nucleus was not yet established. Since then, the structure of the steroid nucleus has been elucidated and steroid chemistry has flourished. The steroid hormones have vast biological effects in many different tissues including the regulation of cell proliferation, development and differentiation. In general, the biological actions can be derived into genomic and nongenomic pathways.

Genomic steroid action

Steroids enter cells by passive diffusion and activate receptors which are located predominantly either in the nucleus or in the cytoplasm (Picard and Yamamoto, 1987; Mangelsdorf et al., 1995). The activated receptors are thought to bind to specific target genes and stimulate transcription and subsequently protein synthesis. The genomic action of steroids mainly involves in four steps: 1) ligand-binding induced activation of receptor, 2) steroid receptor-DNA interactions, 3) stable transcription complex formation and 4) recruitment of transcription factors and RNA polymerase.

The binding of the steroid to the receptor and the subsequent receptor activation is usually regulated by phosphorylation/dephosphorylaton processes (Barnett et al., 1980; Landers and Speleberg, 1992). Receptors for these steroids usually exist in an inactive form as an 8-10S oligomeric complex in association with several heat shock protein (hsp), such as hsp90, hsp70 and p56 (Denis et al., 1988; Sabbah et al., 1996; Whitesell and Cook, 1996; Pratt and Toft, 1997). Ligand binding (steroid binding) causes dissociation of heat-shock proteins from the receptor complex and induces a structural and functional transformation of receptor from the inactive 8-10S form to an active 4S form (Kost et al., 1989; Sanchez et al., 1990). Transcriptional activation is initiated when the steroid receptor binds to DNA-binding sites. It is clear that DNA-binding domain of steroid receptors provides the specificity for the binding of different receptors to their respective steroid responsive elements (SRE) or hormone responsive elements (HRE). The *cis*-acting regulatory sequences of SRE are usually located within 5'-flanking regions of target genes for steroid receptors (Payvar et al., 1983; Renkawitz et al., 1984; Yamamoto, 1985). The SRE for various receptors have similarities in sequence and contain approximately fifteen base pairs. The consequences of binding are a result of the specificity of action of receptor ligands (Schwabe et al., 1993; Rhodes et al., 1996). As SRE are composed of two half-sites, each half-site binds one monomer of the receptor. In this manner, a more stable complex with high affinity is formed at the target DNA sites, and this will facilitate the initiation of transcription at nearby genes. Only the dimeric form of receptor binds with an affinity sufficient to influence transcription. It is thought that such stable complexes have sufficient residence time at the gene to significantly influence transcription (Tsai et al., 1989; Parker, 1993; Mangelsdorf et al., 1995). On the other hand, it is becoming increasingly apparent that the formation of a stable receptor-DNA complex at an enhancer site can result in recruitment of other transcription factors or RNA polymerase to initiate transcription of target genes (Van Dyke et al., 1989).

Nongenomic steroid action

In addition to the well-recognised genomic action of steroids on cellular development structure and function by activation of gene transcriptions, nongenomic steroid action has not been widely recognised until the last decade. Evidence of nongenomic steroid effects are now being reported all fields of steroid research (Norman, 1998). It is becoming evident that steroids can induce a variety of nongenomic changes in cells, such as opening of ion channels, membrane receptor aggregation and changes in protein phosphorylation status (Mendoza et al., 1995). Other examples of nongenomic steroid action include effect of aldosterone on vascular smooth muscle cells (Wehling, 1995), effect of vitamin D_3 on chondroblasts (Nemere et al., 1998), progesterone action on human sperm (Sabeur et al., 1996) effect of neurosteroid on neuronal function and vascular effects of estrogens (Farhat et al., 1996). The mechanisms by which nongenomic steroid effects are induced are being studied with regard to membrane receptors, secondary messengers, signal perception and signal transduction (Brann et al., 1995; Wehling 1995, 1997). For example, a membrane receptor for $1,25(OH)_2D_3$ has been identified on chondrocytes (Nemere et al., 1998) and chicken intestinal epithelium (Nemere et al., 1994). The receptor has a molecular weight of 66 Kd and seems differ from its nuclear receptor (Norman, 1998). It has been shown that the receptor mediates $1,25(OH)_2D_3$-dependent protein kinase C activation.

Structures of steroid receptors

Almost all of the steroid receptors known are transcriptional regulatory proteins which specifically recognise enhancer response elements of target genes, resulting in control of expression of specific gene networks. These transcription factors are made up of domains that correspond to discrete functions. Many investigations into the molecular cloning of cDNAs for various members of the steroid receptor superfamily demonstrated that the overall structure of these receptors is very similar (Fuller, 1991; King, 1992; Truss and Beato, 1993). However, the receptors range in size from 427 amino acids for VDR to 1,000 amino acids for the mineralocorticoid receptor (MR) and all the receptor species share sequences in the primary sequence and the domain functions. Three major domains have been described in most of steroid receptors: 1) a variable transcriptional-regulating domain (I) involved in trans-activation; 2) a DNA-binding domain (II, III); and 3) a ligand binding (or steroid-binding) domain (IV). A generalised domain structure of receptors belonging to the steroid hormone receptor superfamily is showed in Figure 1.

Transcriptional-regulating domain (I)

The transcriptional-regulating domain, which is at the N-terminal end, has been identified as at least two separate regions on either side of the DNA binding domain (Tasset et al., 1990). The functions of domain, however are poorly understood. It is thought that this region is probably involved in cell specific regulation of

gene transcription, receptor dimer formation and other receptor-protein heterodimer formation (Landers and Spelsberg, 1992).

DNA binding domain (II and III)

The DNA binding domain II of 66 to 68 amino acids is a highly conserved region in the middle of the molecule, and also is the region of the receptor that provides the specificity for binding of different receptors to their respective HREs (Berg, 1989; Umesono and Evans, 1989). The salient feature of this domain is its zinc finger, which is a stretch of about 25 amino acids with four cysteine residues that co-ordinate zinc and form a loop or finger. Steroid receptors have two such zinc fingers within the DNA binding domain. The first finger is for the recognition of DNA sequences whereas the second finger stabilises the binding of the receptor to the HRE. Each finger consists of 12 to 13 amino acids, and the two fingers are separated by a linker region of 15 to 17 amino acid (Umesono and Evans, 1989) and encoded by separate exons (Green and Chambon, 1988; Ponglikitmongkol et al., 1988; Fairall et al., 1993).

The DNA blinding domain III, also termed the "hinge" region, contains a short flanking sequence on the C-terminal side of the DNA-binding domain. This region is speculated to interact with other proteins such as transcription factors to form heterodimers which enhance affinity for the DNA binding site (Landers and Spelsberg, 1992; Mader et al., 1993).

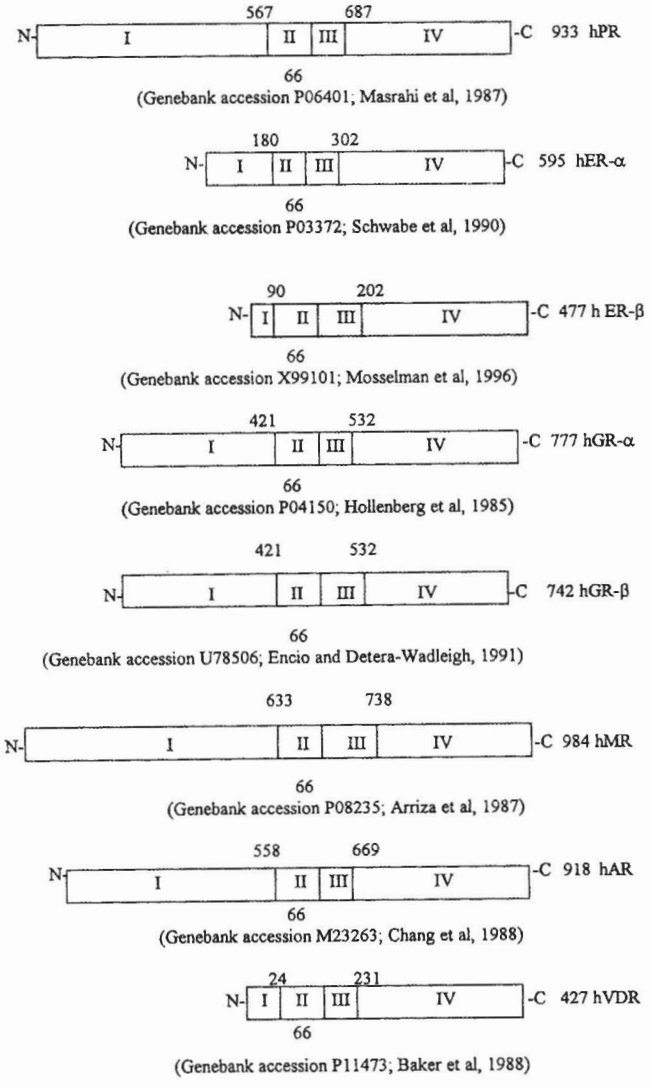

Fig. 1. Structure of the steroid hormone receptor superfamily. A model comaring receptor size and illustrating the conserved sequences of receptors for steroid receptor superfamily including the receptor for progesterone (PR), estrogen (ER), glucocorticoid (GR), mineralcorticoid (MR), androgen (AR) and vitamin D (VDR). I: regulatory domain; II: DNA-binding domain, III: "Hinge domain"; IV: steroid-binding domain. The receptors range in size from 66-427 amino acids for VDR to 984 amino acids for MR. All of receptors have 66 amino acids in domain II. The numbers above the schematic refer to amino acid positions (Modified from Landers and Spelsberg 1992).

Ligand binding domain (IV)

The steroid-binding domain, containing around 250 amino acids, is also highly conserved and located at the C-terminal end of the protein (Yamamoto, 1985; Evans, 1988). Several single amino acid changes within the domain can result in loss of binding (Carson-Jurica et al., 1990). Binding of steroid ligand appears to be a pre-requisite for specific DNA binding and transcriptional activation (Carson-Jurica et al., 1990; Danielian et al., 1992). The presence of ligand may also protect or stabilise steroid binding sites under thermal inactivation conditions (Moudgil and Hurd, 1987) and/or influence affinity of receptor for DNA (Skafar and Notides, 1985). In addition, the ligand binding domain may also mediate interactivity with the heat shock protein hsp 90, which forms a complex with the receptor in the cytoplasm (Pratt and Toft, 1997).

Effects of steroid hormones in bone

In general, steroids have a role in maintenance of mineral homeostasis and are essential in maintaining bone balance in adults. Other factors such as local growth factors and cytokines also participate the regulation of bone metabolism (see review by Zheng et al., 1992). Several excellent reviews have summarised the effect of steroids on bone metabolism and calcium homeostasis (Prior, 1990; Kasperk and Ziegler, 1992; Hewison, 1995). Hence, we mainly focus on the effect of steroids on bone-forming cells, the osteoblasts and bone resorbing cells and the osteoclasts.

Effects of steroids on osteoblasts

Estrogens

The major effect of estrogen on bone is to increase bone formation and decrease bone resorption. Estrogen action on bone cells, however is complicated. Earlier

opinion that the effect of sex steroids on bone was indirect due to a lack of evidence for specific estrogen receptors in bone cells (Chen and Feldman, 1978; Van Paassen et al., 1978). However, it was dramatically changed when several studies after 1988 demonstrated the detection of high affinity of receptors for estrogen (ER) in osteoblastic cells (Eriksen et al., 1988; Komm et al., 1988). Since then, others have reported that the presence of ER in human osteosarcoma cell lines (Etienne et al., 1990), mouse MC3T3-E1 (Masuyama et al., 1992) and rat UMR-106-01 osteoblastic cells (Davis et al., 1994).

The effects of estrogens on proliferation and differentiation of cells from osteoblastic lineage has been widely explored by numerous investigators. Unfortunately, the results often appear to be contradictory. For example, estrogens have been reported to enhance proliferation in primary and immortalised foetal rat calvarial cells (Ernst et al., 1989), mouse MC3T3-E1 cells (Masuyama et al., 1992; Majeska et al., 1994) and human SAOS-2 cells (Saggese et al., 1993); to inhibit proliferation in primary human osteoblastic cells (Bodo et al., 1991; Watts and King, 1994), or to have no effect on proliferation in primary osteoblastic cells (Keeting et al., 1991; Richard et al., 1993), human TE85 osteoblastic cells (Ikegami et al., 1994) and mouse MNT stromal osteogenic cells (Mathieu and Merregaert, 1994). Contradictory effects of estrogens on bone matrix protein expression have been reported including increased expression in MC3T3-E1 cells (Majeska et al., 1994), primary human osteoblast cells (Benz et al., 1991a; Scheven et al., 1992), TE85 cells (Komm et al., 1988), and primary and immortalised foetal rat calvarial culture (Ernst et al., 1989), decreased expression in primary human foetal osteoblastic cells (Zang et al., 1994) or no effect in human primary osteoblastic cells (Keeting et al., 1991), and MG-63 human osteosarcoma cells (Lajeunesse, 1994). All these conflicting results can probably be attributed to the wide variety of osteoblastic cell culture models studied and the range of species from which the cells were derived.

On the other hand, estrogen regulates the production of cytokines by osteoblast which lead to the inhibition of bone resorption. The prevailing hypothesis is that the binding of estrogens to receptors in osteoblasts results in inhibition of the release of osteoclast stimulatory factors including GM-CSF, MCSF, tumour necrosis factor α (TNF-α), IL-1 and IL-6 (Lorenzo et al., 1987; Thomson et al., 1987; Pfeilschifter et al., 1989; Ishimi et al., 1990; Corboz et al., 1992) and stimulation of the production of osteoclast inhibitory factors such as transforming growth factor ß (TGF-ß), IL-4 and the insulin-like growth factors (IGF) (Dieudonne et al., 1991; Oursler et al., 1991a; Riancho et al., 1993; Kassem et al., 1996).

Progesterone

Compared to estrogens, the effects of progesterone on bone physiology have not been studied extensively. Progesterone receptors (PR) have been detected in primary human osteoblast cells (Colvard et al., 1989; Benz et al., 1991b; Orwoll et al., 1991; Wei et al., 1993). Progesterone appears to act directly on bone by binding to the receptor or indirectly through competition for glucocorticoid receptor and, as a result, effectively blocking glucocorticoid responses (Chen et al., 1977). Furthermore, it has been reported that estrogens stimulate increased progesterone binding to the receptor on osteoblasts (Eriksen et al., 1988; Harris et al., 1995). Progesterone can enhance proliferation of human primary osteoblastic cells (Scheven et al., 1992) and osteoblast-like cells (Tremollieres et al., 1992), it also inhibits IL-6 secretion in human osteoblast cells and mouse bone marrow stromal cells (Girasole et al., 1992).

Androgens

Like other steroid receptors, androgen receptors have been identified in osteoblastic cells (Colvard et al., 1989; Masuyama et al., 1992). Androgens are known to act directly on osteoblasts, by binding to androgen receptors stimulation of proliferation and differentiation of osteoblastic cells *in vitro* (Kasperk et al., 1990; Benz et al., 1991b; Masuyama et al., 1992; Weisman et al., 1993). In a similar fashion to estrogens, androgens also act on osteoblastic cells to produce autocrine/paracrine factors, such as TGF-ß and IGF-1 or to decrease production of cytokines such as IL-1 or IL-6 (Kasperk et al., 1990; Pilbeam and Raisz, 1990; Bellido et al., 1995). Murine marrow-derived stromal cells has also been reported to express the androgen receptor. In addition, the production of IL-6 by murine marrow-derived stromal cells has been shown to be inhibited by testosterone (Bellido et al., 1995).

Glucocorticoids

Excessive glucocorticoid *in vivo* decreases bone formation and causes osteonecrosis. The reduction of bone formation is associated with a decrease in serum levels of osteocalcin, a marker of osteoblastic function (Peretz et al., 1989) and a reduction of cancellous bone and bone density. As osteoblasts contain glucocorticoid receptor (GR), it is possible that the glucocorticoids may have direct effects on bone osteoblastic lineage (Chen et al., 1977; Manolagas and Anderson, 1978; Haussler et al., 1980). On the other hand, glucocorticoids inhibit gonadotropin and sex steroid production and the resulting hypogonadism could cause a decrease in bone mass. In addition, inhibitory effects of glucocorticoids on growth hormone production may also lead to decreased bone formation (Luo and Murphy, 1989) providing further evidence that indirect action of glucocorticoids on bone formation also exists.

In vitro, glucocorticoids can either stimulate or inhibit bone formation. The effects of glucocorticoids are often dependent on the type of experimental model, the concentration of hormone used, the timing of

hormone addition and the stage of differentiation of the cells. Glucocorticoids can induce the differentiation of cells of the osteoblastic lineage into mature cells expressing the osteoblastic phenotype, such as osteocalcin and alkaline phosphatase production (Bellows et al., 1987; Leboy et al., 1991; Cheng et al., 1994). Conversely, it has also been reported that glucocorticoids decrease functions of mature osteoblasts and that dexamethesone inhibits osteoblast differentiation (Lian et al., 1997). Glucocorticoids decrease replication of cells that are capable of production of bone collagen. They also inhibit type I collagen and osteocalcin gene expression, but increase interstitial collagenase expression (Delany et al., 1995). Furthermore, it has been shown that glucocorticoids contributed to the regulation of insulin-like growth factor I and II (IGF-I and II) in bone (Gabbitas et al., 1996). It inhibits the production of IGF-binding protein. In addition, it has recently been shown that glucocorticoid induces osteocyte apoptosis (Weinstein et al., 1998). The disruption of the trabecular network by osteocyte apoptosis leads to microdamage accumulation and increases bone fragility. This data further highlights the critical role of inhibitory actions of glucocorticoids on bone matrix and bone mass and explains why excessive glucocorticoids cause osteoporosis.

1α, 25 $(OH)_2D_3$

Vitamin D_3 from skin or diet is metabolised sequentially to 25-hydroxyvitamin D_3, and then to 1α,25-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$] which stimulates intestinal calcium absorption and bone calcium mobilisation. The active metabolite of vitamin D_3, $1\alpha,25(OH)_2D_3$ is necessary for normal mineralization of the skeleton and is a major regulator of both bone formation and bone resorption. The actions of 1,25 $(OH)_2VitD_3$ on bone are complex and both direct and indirect effects have been identified (see review by Norman, 1998).

It is well accepted that vitamin D deficiency is associated with rickets in children and osteomalacia in adults (Holick, 1996; Weisman and Hochberg, 1997). However, it is still not clear exactly what $1\alpha,25(OH)_2D_3$ has on the bone mineralization process. The main action of $1\alpha,25(OH)_2D_3$ on bone appears to be an indirect one through its effects on the intestine where it enhances calcium and phosphate absorption and thus provides mineral components to bone forming cells. On the other hand, $1\alpha,25(OH)_2D_3$ has also been shown to promote osteoblast proliferation and to stimulate osteoblast differentiation (Chen et al., 1986; Owen et al., 1991). $1\alpha,25(OH)_2D_3$ has been demonstrated to regulate the production of numerous parameters of osteoblast function related to bone matrix formation and mineralization, such as collagen, alkaline phosphatase, osteopontin, osteocalcin, and matrix Gla protein (Haussler et al., 1970; Raisz et al., 1978; Price and Baukol, 1980; Fraser et al., 1988; Noda et al., 1990). With the exception of type I collagen synthesis which is down regulated by $1\alpha,25(OH)_2D_3$, other gene expressions associated with bone formation by osteoblasts are up regulated by $1\alpha,25(OH)_2D_3$. It has been shown that a vitamin D_3 responsive element is the promoter region of various osteoblastic including osteocalcin, osteopontin and alkaline phosphatase genes (Yoon et al., 1988; Zheng et al., 1992; Norman et al., 1998). The receptor for vitamin D_3 (VDR) in osteoblastic cells has been widely reported (Kream et al., 1977; Chen et al., 1986). Hence, it seems that osteoblast phenotypic expression is mediated by the direct actions of $1\alpha,25(OH)_2D_3$.

Effects of steroids on osteoclasts

Estrogens

Although there is general agreement that cells of the osteoblast lineage respond to estrogen directly, estrogen regulation of osteoclastic bone resorptive activity is controversial. Oursler and colleagues (1991b, 1993a,b, 1994) showed evidence of estrogen receptor in enriched osteoclasts isolated from human giant cell tumour of bone and chicken. By using a quantitative pit resorption assay, an inhibiting effect of 17-ß estadrial on bone resorption was demonstrated (Oursler et al., 1993a, 1994). Other investigations also used highly purified rabbit osteoclast preparations to demonstrate expression of ER together with estrogen-induced inhibition of bone resorption and promotion of apoptosis (Kameda et al., 1997). On the other hand, although our previous data showed that estrogen loss in ovariectomized rats enhanced gene expression of osteoclastic bone resorptive enzymes including tartrate resistant acid phosphatase (TRAP) and Carbonic anhydrase II (CA II), whereas administration of 17-ß estradiol to these animals reduced gene expression of these enzymes (Zheng et al., 1995b), we have failed to demonstrate the presence of estrogen receptor in osteoclasts (Zheng et al., 1995a; Collier et al., 1998). In fact, the majority of data has shown that using in-situ techniques estrogen receptor was not detected in osteoclast, suggesting indirect action of estrogen in bone resorption (Malawer et al., 1984; Zheng et al., 1995a, Arnett et al., 1996; Kusec et al., 1998). Using a method of osteoclast microisolation, we have previously shown that estrogen receptor α mRNA was not detectable in multinucleate osteoclasts isolated from human giant cell tumour of bone (Collier et al., 1998). However, the receptor mRNA was readily detectable in microisolated mononuclear cells, which consist of osteoclast precursor cells and stromal-like tumour cells. In-situ hybridisation of estrogen receptor-α has confirmed the result of microisolation assay that estrogen receptor-α does not exist in osteoclasts at the detectable level (Collier et al., 1998). Furthermore, by using bone resorption pit assay 17-ß estrodial had no effect on bone resorption by microisolated osteoclasts. Overall, the data relating to the estrogen action on

osteoclasts is inconsistent. In particular, our results differs from the previous work of Oursler and colleagues, who demonstrated direct action of estrogen in osteoclasts. In view of the high sensitivity of the RT-PCR technique used by Oursler and colleagues and the fact that their preparation contained 5-10% mononuclear cells from giant cell tumour of bone (Oursler et al., 1994), it is possible that ER mRNA may have derived from the contaminating mononuclear cells. Furthermore, the inconsistency of the estrogen action on osteoclastic bone resorption may be due to substantial differences in the cell preparation, such as, the differences in purity. In short, we believed that anti-resorptive effect of estrogen in vivo is predominantly mediated indirectly via osteoblast/stromal cells in the bone microenvironment. Osteoclasts are not the direct estrogen traget cells. In support of this, Hughes and colleagues (1996) recently reported that estrogen promoted indirectly apoptosis of murine osteoclast, an effect apparently mediated by TGF-ß.

On the other hand, the possibility of direct estrogen action on osteoclast precursor cells exists. Previous study by Huang et al. (1998) demonstrated that putative mononuclear osteoclast precursor cells express estrogen receptor mRNA. Estrogen has been shown to modulate the proliferation of early osteoclast progenitor cells, through a stromal cell-independent mechanism that involves apoptosis (Shevde and Pike, 1996). A significant increase in the number of osteoclast progenitor cells was observed following ovariotomy and correlated directly with an increase in the number of osteoclast-like cells generated in marrow cultures. Thus, it appears that estrogen has a direct effect on the differentiation of osteoclast precursor cells. Osteoclast precursor cells may possess functional estrogen receptors which are lost with differentiation or during cell fusion. A hypothetical model of estrogen action in osteoclasts and their precursor cells is presented in Figure 2.

Progesterone and Androgens

Direct *in vitro* actions of progesterone and androgens on isolated osteoclasts have received little attention. Since progesterone receptor was identified in human osteoclasts (Pensler et al., 1990), it is possible that a direct modulation of bone resorption by progesterone may occur. However, there have been few studies of the effect of progestrone on gene expression in bone cells. Similarly, evidence that mouse osteoclast-like cells contain androgen receptors suggests that osteoclasts may be likely targets for direct androgen action (Mizuno et al., 1994). However, it has been demonstrated by Tobias and Chambers (1991) that androgen has no effect on bone resorption of rat osteoclasts. Interestingly, a more recent report by Weinstein et al. (1997) has provided data suggesting that the effects of androgen deficiency on bone remodelling and bone mineral density are mediated by cells of the osteoblastic lineage (Weinstein et al., 1997). Thus, it appears that available evidence strongly supports the hypothesis that an indirect role of androgens inhibiting bone resorption may exist.

$1,25\ (OH)_2VitD_3$

It has been known for many years that $1,25\ (OH)_2\ VitD_3$ stimulates bone resorption *in vitro* and *in vivo* (Tanaka and Deluca, 1971; Raisz et al., 1972a; Reynolds et al., 1976; Holtrop et al., 1981). Early studies showed that $1\alpha,25(OH)_2D_3$ not only promotes differentiation of mouse myeloid leukaemia cells into mature macrophages from which osteoclasts are derived (Abe et al.,

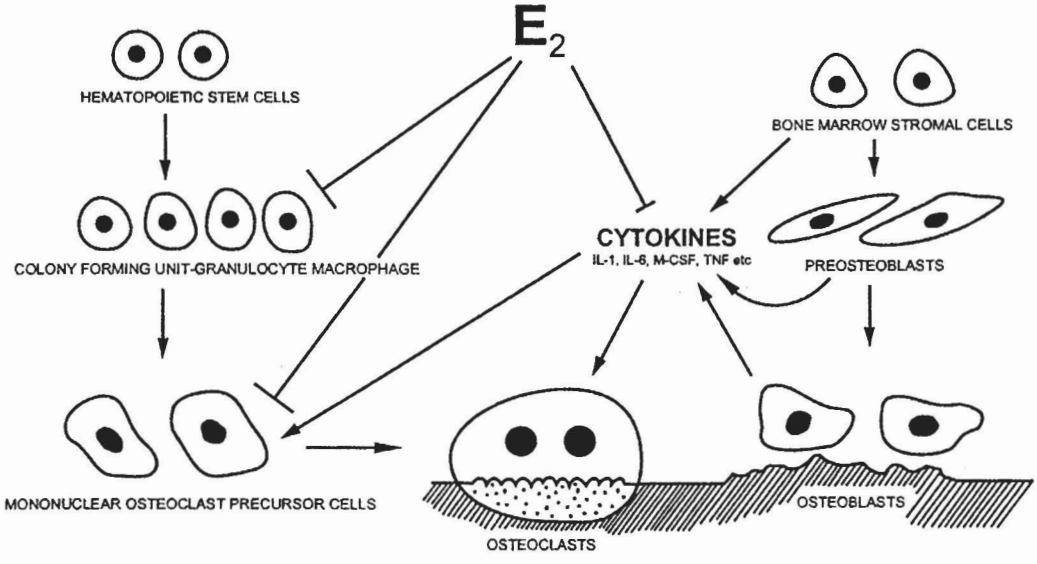

Fig. 2. Hypothetical model of estrogen action in osteoclasts and their precursor cells. The left side of the diagram represents differentiation of hematopoietic stem cells into the colony forming unit-granulocyte macrophage (CFU-GM) and osteoclasts. The right side of the diagram represents the differentiation of bone marrow stromal cells towards osteo-blastic lineage. Estrogen has direct inhibition on the proliferation and the accumulation of bone-resorbing enzymes in early progenitor cells and possibly on fusion of mature osteoclast precursor cells. On the other hand, estrogen has an indirect effect on osteoclastogenesis by the suppression of production of various cytokines in osteoblasts/stromal cells. ----|: inhibition; →:activation.

1981) but also induces activation and fusion of some macrophages (Abe et al., 1984; Roodman et al., 1985; Quinn et al., 1997; Scheven et al., 1997). It has been suggested that $1\alpha,25(OH)_2D_3$ induction of bone resorption occurs by stimulation of differentiation and fusion of osteoclast progenitor cells into osteoclasts (Suda et al., 1992). A recent study showed that $1\alpha,25(OH)_2D_3$ induces the production of osteoclast differentiation factor (ODF), a key factor that regulates osteoclastogenesis (Tsukii et al., 1998). Moreover, $1\alpha,25(OH)_2D_3$ stimulates formation of the ruffled membrane indicating that it also activates differentiated osteoclasts (Holtrop and Raisz, 1979; Holtrop et al., 1981). To date, $1\alpha,25(OH)_2D_3$ has been to used to induce the formation of osteoclasts form bone marrow cells in a number of species including cats, baboons, humans, mice and rats (Ibbotson et al., 1984; Fuller and Chambers, 1989; Quinn et al., 1996). The exact mechanism of $1\alpha,25(OH)_2D_3$ action in cellular differentiation, however is not fully understood. Most actions of $1,25(OH)_2VitD_3$ are thought to be mediated via its receptor (Minghetti and Norman, 1988). As previous study has failed to detect Vitamin D receptor in avian osteoclasts (Merke et al., 1986) and the accelerated resorption by osteoclasts stimulated by $1,25\ (OH)_2VitD_3$ occurs only in the presence of osteoblasts, it has been generally accepted that the effect of $1,25\ (OH)_2D_3$ on osteoclast formation and bone resorption are mediated via osteoblasts or stromal cells. On the other hand, Vitamin D receptor mRNA has recently been demonstrated in human osteoclasts using both *in situ*-RT-PCR and in situ hybridisation, suggesting that $1\alpha,25(OH)_2D_3$ could exert direct effects on bone resorbing osteoclasts (Mee et al., 1996; Huang et al., in press).

Glucocorticoids

Histomorphometric studies of glucocorticoid-induced osteoporosis revealed an increase in the number of osteoclasts and bone-resorbing sites (Bressot et al., 1979). On the other hand, *in vitro* investigations performed to assess the role of glucocorticoids in bone resorption have yielded conflicting results. Some earlier studies suggested that glucocorticoids inhibit bone resorption (Raisz et al., 1972b; Tobias and Chamber, 1989), while more recent studies reported that glucocorticoids stimulate bone resorption (Gronowicz et al., 1990; Lowe et al., 1992; Conaway et al., 1997). Osteoclastic bone resorption is believed to be initiated by the activation of existing osteoclasts and the generation of new osteoclasts. Nevertheless, the mechanisms by which glucocorticoids regulate osteoclast formation and activation are unclear and the preferred mode of action of glucocorticoids remains uncertain. It was reported that glucocorticoids inhibited the recruitment and differentiation of bone resorbing cells, but stimulated previous studies demonstrated that the activation of quiescent osteoclastic cells (Defranco et al., 1992). In addition, our data also demonstrated that glucocorticoid promotes osteoclast apoptosis in rat isolated osteoclast cultures (Collier et al., unpublished data). By contrast, dexamethasone has been shown to enhance the formation of osteoclast-like cells in mouse bone marrow cell cultures (Shuto et al., 1994; Kaji et al., 1997). Shuto et al. (1994) suggested that dexamethasone stimulated osteoclast-like cell generation by inhibiting the production of granulocyte macrophage-colony stimulating factor (GM-CSF). On the other hand, more recent studies by Kaji et al. (1997) demonstrated that dexamethasone acted directly on haematopoietic blast cells to enhance osteoclast-like cell formation.

In conclusion, the mechanism of steroid action on bone cells is through an interaction with specific receptor proteins in the target cells. The receptor action is mediated by both genomic and nongenomic pathways. It is believed that steroids have direct action on osteoblasts via receptor, and the effects of steroids on osteoclastic bone resorption are mediated through osteoblasts or stromal cells by the production of soluble/membrane factors. However, in addition to indirect effects on osteoclasts, the question whether steroid hormones could exert direct action on osteoclasts as well has not to be answered and findings to date are controversial.

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