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BONE AND CARTILAGE RESPONSIVENESS TO SEX STEROID HORMONES

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Summary—Gonadal steroids influence the skeletal growth and metabolism both during the pubertal growth spurt and in adulthood with aging It is now generally agreed that sex steroid effect on skeletal tissues is due to indirect and direct actions In this presentation, *in vitro* effects of sex steroids on cartilage cells are reported by comparison with those observed on bone cells

INTRODUCTION

The role of gonadal steroids on skeletal tissues is obvious from the remarkable increase of growth rate which occurs at the time of gonadal maturation in both sexes [1] In adulthood, the relationship between postmenopausal osteoporosis and estrogen deficiency which was first described by Albright *et al.* [2] is now well established The lack of estrogen is associated with increased bone remodeling rate, accelerated bone loss, and a negative calcium balance [3–5] Estrogen replacement therapy is generally considered to maintain bone mass by preventing bone loss more than by restoring bone after it has been lost [6–11] but it has been also suggested that estrogen may have anabolic effects in osteoblasts [12] In a recent study, physiological concentrations of 17βestradiol (E2) were administered to ovariectomized rats in which bone resorption has been almost completely suppressed by biphosphonates The authors observed a significant increase in trabecular bone volume in E2 treated animals [13]

The role of androgens in the prevention of osteoporosis is less clear, although male hypogonadism is associated with osteoporosis [14, 15] Nevertheless, the mechanism by which sex steroids interact with cartilage and bone cells remains poorly understood

INDIRECT AND DIRECT EFFECTS OF SEX STEROIDS ON SKELETAL TISSUES

It is now generally agreed that sex steroid effect on skeletal tissues is due to an indirect action combined with a direct effect For many years attempts to demonstrate sex steroid effects on cartilage or bone cells *in vitro* were unsuccessful, suggesting that the action of these hormones on skeletal tissues is indirect [16, 17] Indeed, the indirect action of sex steroids on skeletal growth during puberty is well established Both experimental and clinical evidence indicates that the increased steroid secretion at puberty is associated with stimulation of GH secretion, which in turn stimulates insulin growth factor-1 (IGF1), at least partly accounting for the increase growth rate and bone maturation [18, 19]

However, recent *in vivo* and *in vitro* observations suggest that sex steroids may have a direct effect on skeletal growth, independent of GH and IGF1 Laron dwarfs, who suffer from a functional defective GH receptor gene and have high circulating values of GH but no endocrine generation of IGF1, show a definite pubertal growth spurt in spite of their lack of circulating IGF1 [20] Other clinical data indicate that estrogens have a dual effect upon growth Doses of ethynil-estradiol as high as 400–800 ng/kg/day do not affect the bone growth rate of girls with Turner Syndrome, while the relatively low dose of 100 ng/kg/day of estrogens produces a maximal growth response, as evaluated by a doubling of the base-line ulnar growth rate, with no increase in the circulating IGF1 [21]

In rats, ovariectomy causes osteopenia and has been used as a model for postmenopausal bone loss [11] The local infusion of E2 delivered directly into ovariectomized rat femur trabecular bone *in vivo*, restored the trabecular bone volume dose dependently [22] showing that estrogen local treatment reverted the skeletal

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changes produced by ovariectomy as was already demonstrated previously by systemically E2 administration [10, 11]

PRESENCE OF ESTROGEN RECEPTORS IN CARTILAGE AND BONE

Recently, we have reported the presence of high affinity nuclear binding sites for E2 in rabbit growth plate cartilage tissue and cells [23] A low concentration of high affinity estrogen receptors has been also found in primary cultures of human [24] and rat [25] osteoblast-like cells, and in orteosarcoma-derived cell lines These findings together with the failure to demonstrate E2 receptors in bone cells from osteoporotic women [26] strongly argue for a direct interaction of estrogens with skeletal tissues The low concentration of the estrogen receptors found in all skeletal tissues studied may explain the previous failures to find direct effects of this hormone on bone or cartilage cells

The evidence for androgen receptors in bone or cartilage is still less convincing despite recent reports of specific androgen binding sites in postnatal rabbit cartilage cells [27] and in human fetal cartilage [28] Whether or not androgens could act via the estrogen receptors remains questionable

DIRECT BIOLOGICAL EFFECTS OF SEX STEROIDS ON SKELETAL TISSUES

In cartilage, a dose-dependent stimulating effect of dihydrotestosterone (DHT) and E2 (10–11 to 10–9 M) was observed on ^{35}S incorporation into proteoglycans synthesized by rabbit [29] as well as human [30] chondrocytes in primary culture The stimulating effect of both hormones was age-dependent Cartilage cells derived from animals or young children in the early phase of puberty responded best than when cells were extracted from rabbits a few days after birth, or from children during the first year after birth It is unlikely that the age-dependency of the *in vitro* responsiveness of cartilage cells to sex steroids could be due to a variation of the number of sex steroid receptors in cartilage during postnatal growth since, at least in rabbits, the number of E2 binding sites remained stable in cartilage tissue from birth to puberty [23] The previous *in vivo* exposure of cartilage cells to circulating androgens or estrogens may well be responsible for this age-dependency Human cartilage cells taken from children up to 1-year-old did not respond It is now well known that during this age period there is an increase in the circulating concentrations of sex steroid hormones in both sexes This could perhaps saturate the sex steroid receptors, which are present in cartilage at much lower concentrations than in other classical target tissues

Rabbit [31] and human [30] cartilage tissue has been shown to convert testosterone (T) to DHT and to a lesser extent, to E2 These data indicate that cartilage tissue *in vivo* contains both 5α-reductase and aromatase activities The effect of androgens on cartilage may thus be partly mediated through their transformation into estrogens

In bone, E2 was shown to increase alkaline phosphatase activity in the UMR-106 osteoblast cell line [32], to enhance replication and collagen mRNA level in rat calvaria cells *in vitro* [33, 34], to stimulate thymidine incorporation and creatine kinase activity in rat bone-derived cells in culture [35] and to decrease parathyroid hormone (PTH)-stimulated adenylate cyclase activity in the human osteosarcoma cell line Saos-2 [36] These data are probably of physiological relevance, since the effects were observed at nanomolar concentrations of E2 as in cartilage cells [29, 30]

IGF1 AS AN ESTROGEN MEDIATOR IN CARTILAGE AND BONE

E2 appears to regulate IGF1 synthesis in a selected number of target tissues such as the uterus [37], ovary [38] and mammary gland [39] without increasing IGF1 serum levels IGF1 is considered to be one of the important local growth factors which regulate cartilage and bone cell replication and/or differentiation [40] There is a local production of IGF1 by cartilage cells *in vivo* [41, 42] and *in vitro* [43] and relatively large amounts of IGF1 are stored in bone [44, 45] Whereas the relevance of IGF1 as a GH mediator has been strongly suggested in cartilage [46], the involvement of IGF1 as a E2 mediator has been more documented in bone cells IGF1 was shown to be accumulated in conditioned medium of the clonal osteoblastic cell line UMR-106 grown in the presence of E2 [47] E2 was shown to increase IGF1 mRNA levels in osteoblastic cells from calvariae and long bone *in vitro* [34] In general, serum levels of IGF1 decline with age but do not differ in

patients with postmenopausal osteoporosis from those in age-matched controls [48] It is thus possible that E2 regulates IGF1 synthesis in bone without increasing IGF1 serum levels as already shown in other selected target tissues [37–39]

As compared with other growth factors, IGF1 is remarkable in that it is continuously produced and accumulated in high concentrations in extracellular spaces under normal conditions, with the possibility of being biologically active in an autocrine–paracrine manner and/or subsequently degraded [49] IGF1 binding proteins (IGFBP) are also secreted with IGF1 in a tissue- and cell-specific manner including in osteoblasts [50], and are proposed to either inhibit or enhance the local effects of IGF1 [51–53] In bone cells, the synthesis of IGF1BPs appears to be regulated by E2 and other hormones [54] These additional factors, as well as other locally produced growth factors such as transforming growth factor-β [47] may modify further the cellular response to E2

CONCLUSION

There is now a great deal of evidence that sex steroids have a direct metabolic effect on cartilage and bone cells Recent data strongly suggest that the sex steroids are partly anabolic via the local regulation of IGF1 factors The age- and sex-dependency of the steroid effects is still not explained One can suggest that the specific nature of certain subpopulations of cells or their maturation state (quiescent or proliferative) may play an important role in the tissue responsiveness to the steroid hormones

Although most of the recent data were observed *in vitro* on cultured cells, they underly the effect of sex steroids on skeletal tissues seen *in vivo*

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