Minireview: Aromatase and the Regulation of Estrogen Biosynthesis—Some New Perspectives

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There is a growing awareness that androgens and estrogens have general metabolic roles that are not directly involved in reproductive processes. These include actions on vascular function, lipid and carbohydrate metabolism, as well as bone mineralization and epiphyseal closure, in both sexes. In postmenopausal women, as in men, estrogen is no longer solely an endocrine factor, but instead is produced in a number of extragonadal sites and acts locally at these sites in a paracrine and intracrine fashion. These sites include breast, bone, vascularity, and brain. Within these sites, aromatase action can generate high levels of E2 locally without significantly affecting circulating levels. Circulating C19 steroid precursors are essential substrates for extragonadal estrogen synthesis. The levels of these androgenic precursors decline markedly with advancing age in women, possibly from the mid to late reproductive years. This may be a fundamental reason why women are at increased risk for bone mineral loss and fracture and possibly decline of cognitive function, compared with men. Aromatase expression in these various sites is under the control of tissue-specific promoters regulated by different cohorts of transcription factors. Thus, in principle, it should be possible to develop selective aromatase modulators that block aromatase expression, for example, in breast, but allow unimpaired estrogen synthesis in other tissues such as bone.

STUDIES EMPLOYING MODELS of estrogen (E) insufficiency have revealed new and unexpected roles for E2 in both females and males (1). These models include mutations in humans of the aromatase gene, of which there are some ten cases documented, three of whom are men (2–4), and one case of a man with a mutation in the ERα (5). They also include mice with targeted disruptions of ERα and ERβ, the double ERα- and β-knockout mouse (6–9), and the aromatase knockout (ArKO) mouse (10–12). Recently described consequences of E deprivation revealed by these models challenge the traditional beliefs of gender specificity of sex steroid actions. For example, the lipid and carbohydrate phenotype of E insufficiency is not sexually dimorphic and appears to apply to both males and females (13, 14), as does the bone phenotype of undermineralization and failure of epiphyseal closure (2–4, 15). Even more dramatically, the roles of E2 in male germ cell development in mice (16) and humans (17), and efferent duct fluid transport in mice (18, 19), would indicate that in this local context E2 might be more appropriately defined as an androgen.

In premenopausal women, the ovaries are the principal source of E2, which functions as a circulating hormone to act on distal target tissues. However, in postmenopausal women, when the ovaries cease to produce estrogens, and in men, this is no longer the case. Under these circumstances, E2 is no longer solely an endocrine factor; instead it is produced in a number of extragonadal sites and acts locally at these sites as a paracrine or even intracrine factor (1, 20). These sites include the mesenchymal cells of adipose tissue, osteoblasts and chondrocytes of bone, the vascular endothelium and aortic smooth muscle cells, and numerous sites in the brain. Thus, circulating levels of estrogens in postmeno-

Abbreviation: ArKO, Aromatase knockout.

Aromatase and Its Gene

E biosynthesis is catalyzed by a microsomal member of the cytochrome P450 superfamily, namely aromatase cytochrome P450 (P450arom, the product of the CYP19 gene). The P450 gene superfamily is a very large one, containing (as of 1996) over 480 members in 74 families, of which cytochrome P450arom is the sole member of family 19 (21). This heme protein is responsible for binding of the C19 androgenic steroid substrate and catalyzing the series of reactions leading to formation of the phenolic A ring characteristic of estrogens.

The human CYP19 gene was cloned some years ago (22–24), when it was shown that the coding region spans 9 exons beginning with exon II. Upstream of exon II are a number of alternative first exons that are spliced into the 5’-untranslated region of the transcript in a tissue-specific fashion (Fig. 1). For example placental transcripts contain at their 5’-end a distal exon, L1. This is because placental expression is driven by a powerful distal promoter upstream of exon I.1 (25). Examination of the Human Genome Project data reveals that exon L1 is 89 kb upstream of exon II (Sebastian, S., and S. Bulun, personal communication). On the other hand, transcripts in ovaries and testes contain, at their 5’-end, genomic sequence that is immediately upstream of the translational start site. This is because expression of the gene in the gonads utilizes a proximal promoter, promoter II. By contrast, tran-
scripts in cells of mesenchymal origin such as adipose stromal cells and osteoblasts, contain yet another distal exon (I.4) located 20 kb downstream of exon I.1 (26). Adipose tissue transcripts also contain promoter II-specific exonic sequence, as do those present in endometriotic plaques (27), but promoter II-specific transcripts are undetectable in bone (28).

Splicing of these untranslated exons to form the mature transcript occurs at a common 3′-splice junction that is upstream of the translational start site. This means that although transcripts in different tissues have different 5′-termini, the coding region and thus the protein expressed in these various tissue sites is always the same. However, the promoter regions upstream of each of the several untranslated first exons have different cohorts of response elements, and so regulation of aromatase expression in each tissue is different. Thus the gonadal promoter (II) binds the transcription factors CREB and SF1, and so aromatase expression in gonads is regulated by cAMP and gonadotropins (29). In adipose tissue, as well as in endometriotic plaques, promoter II-mediated expression is stimulated by PGE2 (27, 30). On the other hand promoter I.4 is regulated by class I cytokines such as IL-6, IL-11, and oncostatin M, as well as by TNFα (31). Thus the regulation of E biosynthesis in each tissue site of expression is unique (reviewed in Ref. 31), and this leads to a complex physiological situation which makes, for example, interpretation of circulating E levels very difficult.

Nonsexually Dimorphic Roles of Androgens and Estrogens

As indicated above, there is a growing appreciation that both androgens and estrogens have general metabolic roles that are not directly involved in reproductive processes and apply to a greater or lesser extent to both sexes. This is perhaps more readily understood when placed in the context of the emerging knowledge of the evolution of steroidogenic genes on the one hand and those encoding steroid hormone receptors on the other. Largely from the work of Callard and her colleagues, it is now recognized that the biosynthesis of estrogens occurs throughout the entire vertebrate phylum, including mammals, birds, reptiles, amphibians, teleosts, and elasmobranch fish as well as agnatha (hagfish and lampreys) and in protochordates such as amphioxus (32, 33). To our knowledge, E biosynthesis has not been reported in nonchordate animal phyla. Consistent with this, phylogenetic analysis of steroid receptors in lower vertebrates indicates that the first steroid receptor was an ER, followed by a PR (34). No equivalents of the classical steroid receptors have been found in any species outside the vertebrates, although an ortholog of the estrogen-related receptor (ERR) is present in Drosophila, namely the ecdysone receptor. Genome mapping and phylogenetic analysis indicate that the full complement of mammalian steroid receptors evolved
from these ancient receptors by two large-scale genomic expansions; one before the advent of jawed vertebrates and one after (34). Specific regulation of physiological processes by androgens and corticoids are relatively recent innovations that emerged after these duplications. Thus, we might speculate that the role of C19 steroids was in the first instance merely to serve as a precursor for the estrogenic steroids and that specific physiological roles for C19 steroids only emerged later. On this basis, it is reasonable to expect that estrogens should play important physiological roles in males as they do in females. It is also consistent with the knowledge that at least in placental mammals the female phenotype is the default phenotype and that the difference between maleness and femaleness is not an absolute one, but rather is governed by a subtle balance of the ratios of estrogenic vs. androgenic actions.

The Concept of Local Estrogen Biosynthesis

Extragonadal sites of E biosynthesis possess several fundamental features that differ from those of the ovaries. The first important point is that the E synthesized within these compartments acts predominantly at the local tissue level in a paracrine or intracrine fashion (35, 36). Thus, the total amount of E synthesized by these extragonadal sites may be small, but the local tissue concentrations achieved are probably high and exert biological influence locally. As a consequence, extragonadal E biosynthesis plays an important but hitherto largely unrecognized, physiological, and pathophysiological role.

The power of local E biosynthesis is illustrated by the cases of boys and men in whom aromatase expression in adipose tissue, and possibly also in bone, is greatly increased whereas that present in the testes is unaffected. This results in florid gynecomastia and short stature due to premature epiphyseal fusion (37, 38). This condition is a consequence of chromosomal rearrangements that result in the insertion of a constitutive promoter upstream of the start of translation of the aromatase gene (38).

Another example relates to postmenopausal breast cancer. It has been determined that the concentration of E2 present in breast tumors of postmenopausal women is at least 20-fold greater than that present in the plasma (39, 40). With aromatase inhibitor therapy, there is a precipitous drop in the intratumoral concentrations of E2 and estrone, together with a corresponding loss of intratumoral aromatase activity, indicative that it is this activity within the tumor and the surrounding breast adipose tissue that is responsible for these high tissue concentrations (41).

An interesting example is that of endometriotic plaques, which frequently express high levels of aromatase activity and promoter II-specific transcripts whose expression is stimulated by PGE2 (27, 42). In the case reported in (42), treatment with an aromatase inhibitor led to a dramatic improvement in the condition of a postmenopausal woman with severe endometriosis.

In bone, aromatase is expressed primarily in osteoblasts and chondrocytes (43), and aromatase activity in cultured osteoblasts is comparable to that present in adipose stromal cells (28). Thus, it appears that in bone also, local aromatase expression is the major source of E2 responsible for the maintenance of mineralization, although this is extremely difficult to prove due to sampling problems. Hence for both breast tumors and bone, as well as for endometriotic plaques, it is likely that circulating E levels have little impact on the relatively high endogenous tissue E levels. This is probably true for other extragonadal sites of E formation also, such as brain. Thus, circulating levels merely reflect the sum of local formation in its various sites. This is a fundamental concept for the interpretation of relationships between circulating E levels in postmenopausal women and E insufficiency in specific tissues.

The second important point is that E production in these extragonadal sites is dependent on an external source of C19 androgenic precursors because these extragonadal tissues are incapable of converting cholesterol to the C19 steroids (35, 36). As a consequence, circulating levels of T and androstenedione as well as DHEA and DHEAS become extremely important in terms of providing adequate substrate for E biosynthesis in these sites.

It should be pointed out that in the postmenopausal woman, circulating T levels are an order of magnitude greater than circulating E2 levels. This by itself suggests that circulating androgens might be more important for maintaining local E levels in extragonadal sites than are circulating estrogens. Moreover in men, circulating T levels are an order of magnitude greater than those in postmenopausal women. In postmenopausal women, the ovaries secrete 25–35% of the circulating T. The remainder is formed peripherally from androstenedione and DHEA produced in the ovaries, and from androstenedione, DHEA and DHEAS secreted by the adrenals. However the secretion of these steroids and their plasma concentrations decrease markedly with advancing age (20). Moreover, DHEA must first be converted to androstenedione before aromatization. Another major step is the reduction of the 17-keto group to 17β-hydroxyl catalyzed by one or more members of the 17β-HSD family, which is essential for formation of the active E, namely E2. The distribution of these enzymes in various extragonadal sites of aromatization has not yet been fully established, although reductive and/or oxidative members are expressed in many tissues.

In this context, it is appropriate to consider why osteoporosis is more common in women than in men and affects women at a younger age in terms of fracture incidence. We have suggested that uninterrupted sufficiency of circulating T in men throughout life supports the local production of E2 by aromatization of T in E-dependent tissues, and thus affords ongoing protection against the so-called E deficiency diseases. This appears to be important in terms of protecting the bones of men against mineral loss and may also contribute to the maintenance of cognitive function and prevention of Alzheimer’s disease (1).

Selective Aromatase Modulators

In adipose tissue, it has been suggested previously (31) that aromatase is a marker of the undifferentiated mesenchymal cell phenotype. In support of this, the factors that stimulate aromatase expression in adipose tissue are ones
that either inhibit or reverse the differentiated phenotype of adipocytes, namely class I cytokines such as IL-6, oncostatin M and IL-11, or else TNFα (44–46). All of these agents act via the mesenchymal promoter I.4 of the aromatase gene, and require glucocorticoids as co-stimulators (reviewed in Ref. 31). These considerations suggest that factors that stimulate adipocyte differentiation such as ligands of the PPARγ receptor, e.g. troglitazone, rosiglitazone, and 15-deoxy-Δ12,14-PGJ2 would inhibit aromatase expression in adipose tissue, and this has proven to be the case (47).

As indicated previously, when a breast tumor is present, aromatase activity within the tumor and surrounding adipose tissue is such that intratumoral E2 levels are at least an order of magnitude greater than those in circulating plasma of postmenopausal women (this may be one reason why taking hormone replacement therapy carries little increased risk of breast cancer). This is because the tumor produces factors that stimulate aromatase expression locally. This stimulation is associated with switching of the aromatase gene promoter from I.4 to promoter II, the gonadal-type promoter (48–51) (Fig. 2). This appears to be because the tumor-derived factors include PGE2 (46, 52), which stimulates adenylate cyclase in adipose stromal cells, and promoter II is regulated by cAMP. It was found that indeed PGE2 is a powerful stimulator of aromatase expression in these cells via promoter II (30). Moreover, expression of the CYP19 gene was correlated with COX-1 and COX-2 expression in human breast cancer and normal tissue specimens (53). A case-control study published some years ago indicated that daily use of nonsteroidal antiinflammatory drugs such as ibuprofen reduced the incidence of breast tumors by up to 40% (54). More recently it has been shown that the COX-2 inhibitor, celecoxib, has strong chemopreventive activity against mammary carcinoma in rats (55). From the considerations presented above, it appears likely that inhibition of aromatase expression selectively in breast tissue would play an important role in this chemopreventive action of cyclooxygenase inhibitors.

Third generation aromatase inhibitors are finding utility in the treatment of E-dependent diseases such as breast cancer and more recently endometriosis (42). However, these have the disadvantage that they inhibit aromatase activity in a global fashion and thus could have a detrimental impact at sites where E is required for normal function, such as the maintenance of bone mineralization and possibly the prevention of hepatic steatosis (4) and loss of cognitive function. The concept of selective aromatase modulators is made possible by three considerations presented here. Firstly, in postmenopausal women and in men, E is not a significant circulating hormone but rather acts at a local level at sites where it is produced, in a paracrine or even intracrine fashion. Secondly, aromatase expression in these different tissue sites of expression is regulated by the use of tissue-specific promoters. Thirdly, the various tissue-specific aromatase promoters employ different signaling pathways and thus different cohorts of transcription factors. Thus it is possible to envision tissue-specific inhibition of aromatase expression in a similar fashion to the concept of tissue-specific regulation of E action (the concept of selective ER modulators). Specifically, drugs that target promoter II-driven expression of

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**Fig. 2.** Proposed regulation of aromatase gene expression in breast adipose tissue from cancer-free individuals and from those with breast cancer. In the former case, expression is stimulated primarily by class I cytokines and TNFα produced locally, in the presence of systemic glucocorticoids. As a consequence, promoter I.4-specific transcripts of aromatase predominate. In the latter case, aromatase expression is increased, and PGE2 produced by the tumorous epithelium, tumor derived fibroblasts, and/or macrophages recruited to the tumor site, is a major factor stimulating aromatase expression, as evidenced by the predominance of promoter II-specific transcripts of aromatase.
aromatase would be most useful because, in postmenopausal women, this promoter would appear to be exclusively used in tumor-containing breast tissue (and in endometriotic plaques) (27), and thus bone in particular, which does not express promoter-II specific transcripts (28), would be spared.

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