Sources of estrogen and their importance

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Abstract

In premenopausal women, the ovaries are the principle source of estradiol, which functions as a circulating hormone to act on distal target tissues. However, in postmenopausal women when the ovaries cease to produce estrogen, and in men, this is no longer the case, because estradiol 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. These sites include the mesenchymal cells of adipose tissue including that of the breast, 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 postmenopausal women and in men are not the drivers of estrogen action, they are reactive rather than proactive. This is because in these cases circulating estrogen originates in the extragonadal sites where it acts locally, and if it escapes local metabolism then it enters the circulation. Therefore, circulating levels reflect rather than direct estrogen action in postmenopausal women and in men. Tissue-specific regulation of \( \text{CYP19} \) expression is achieved through the use of distinct promoters, each of which is regulated by different hormonal factors and second messenger signaling pathways. Thus, in the ovary, \( \text{CYP19} \) expression is regulated by \( \text{FSH} \) which acts through cyclic AMP via the proximal promoter II, whereas in placenta the distal promoter I.I regulates \( \text{CYP19} \) expression in response to retinoids. In adipose tissue and bone by contrast, another distal promoter—promoter I.4—drives \( \text{FSH} \) which acts through cyclic AMP via the proximal promoter II, whereas in placenta the distal promoter I.I regulates \( \text{CYP19} \) expression in response to retinoids.

1. Introduction

In premenopausal women, the ovaries are the principle source of estradiol, which functions as a circulating hormone to act on distal target tissues. However, in postmenopausal women when the ovaries cease to produce estrogen, and in men, this is no longer the case. Instead, estrogens are produced in a number of extragonadal sites. Although this has been in a general sense recognized, its significance is only now becoming appreciated, as will be explained further.

In postmenopausal women then, estradiol 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. These sites include the mesenchymal cells of adipose tissue including that of the breast, 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 postmenopausal women and in men are not the drivers of estrogen action, they are reactive rather than proactive. This is because in these cases circulating estrogen originates in the extragonadal sites where it acts locally, and if it escapes
local metabolism then it enters the circulation. Therefore, circulating levels reflect rather than direct estrogen action in postmenopausal women and in men [1–3].

2. Extragonadal estrogen biosynthesis

Extragonadal sites of estrogen biosynthesis possess several fundamental features that differ from those of the ovaries. First, the estrogen synthesized within these compartments acts predominantly at the local tissue level in a paracrine or intracrine fashion [4,5]. The total amount of estrogen 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 estrogen biosynthesis plays an important but hitherto largely unrecognized physiological and pathophysiological role.

The power of local estrogen biosynthesis is illustrated by the cases of men in whom aromatase expression in adipose tissue is greatly increased, whereas aromatase expression present in the testes is unaffected. This results in florid gynecomastia and short stature due to premature epiphyseal fusion [6,7]. 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 [7]. Another example relates to postmenopausal breast cancer [8]. It has been determined that the concentration of estradiol present in breast tumors of postmenopausal women is at least 20-fold greater than that present in the plasma. With aromatase inhibitor therapy, there is a precipitous drop in the intratumoral concentrations of estradiol and estrone together with a corresponding loss of intratumoral aromatase activity, indicating that this activity within the tumor and the surrounding breast adipose tissue is responsible for these high tissue concentrations [9].

In bone, aromatase is expressed primarily in osteoblasts and chondrocytes [10], and aromatase activity in cultured osteoblasts is comparable to that present in adipose stromal cells [11]. Thus, it appears that in bone also, local aromatase expression is the major source of estrogen responsible for the maintenance of mineralization, although this is extremely difficult to prove due to sampling problems. For both breast tumors and bone, therefore, it is likely that circulating estrogen levels have little impact on the relatively high endogenous tissue estrogen levels. Instead, the 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 estrogen levels in postmenopausal women and estrogen insufficiency in specific tissues.

Another fundamental feature of extragonadal estrogen biosynthesis is that it is dependent on an external source of C19 androgenic precursors, since these extragonadal sites are incapable of converting cholesterol to the C19 steroids [5,12]. Thus, circulating levels of C19 precursors become extremely important in terms of providing adequate substrate for estrogen biosynthesis in these sites.

3. Sources of C19 precursor for extragonadal estrogen formation

Testosterone circulates at concentrations which are an order of magnitude greater than those of estradiol in the blood of postmenopausal women (Table 1). An obvious implication of this realization is that androgens have an important role to play in female physiology. It is now recognized that much of the physiology of androgens is explicable in terms of the concept that testosterone functions as a circulating pro-hormone, which is converted in target tissues, on the one hand to 5α-dihydrotestosterone (DHT) and on the other hand to estradiol. The former is the principal ligand for androgen receptors, and the latter is the principal ligand for both estrogen receptor α and β isoforms. Therefore, in any consideration of the roles of testosterone in either women or men, it is necessary to discuss both of these pathways of testosterone metabolism.

The situation is complicated by the fact that, in postmenopausal women, only about 25% of circulating testosterone is derived by direct secretion from the ovaries. The rest is formed largely from circulating precursors derived either from the adrenal cortex or from the ovaries. These are principally androstenedione, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS). Whereas DHEAS is secreted entirely from the adrenals, androstenedione and DHEA are formed in both adrenals and ovaries. DHEA and DHEAS are present in the circulation in concentrations which are orders of magnitude greater than those of the active sex steroids (Table 1). Consequently, they form a large reservoir of precursor, which is available for conversion to testosterone and thus to estrogens in numerous peripheral tissue sites; it is also relatively insensitive, in the short term, to changes in secretion rates.

The fact that circulating testosterone levels in the postmenopausal woman are an order of magnitude greater than circulating estradiol levels (Table 1) suggests that circulating androgens might be more important for maintaining local estrogen levels in extragonadal sites than are circulating estrogens. Moreover, in men, circulating testosterone levels are

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean plasma steroid levels in men and in postmenopausal women</th>
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<tr>
<td></td>
<td>Women (nmol/l)</td>
</tr>
<tr>
<td>T</td>
<td>0.6</td>
</tr>
<tr>
<td>Δ⁴</td>
<td>2.5</td>
</tr>
<tr>
<td>E₁</td>
<td>0.10</td>
</tr>
<tr>
<td>E₂</td>
<td>0.04</td>
</tr>
<tr>
<td>DHEA</td>
<td>15</td>
</tr>
<tr>
<td>DHEAS</td>
<td>2500</td>
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</tbody>
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T: testosterone; E₁: estrone; E₂: estradiol; DHEA: dehydroepiandrosterone; DHEAS: dehydroepiandrosterone sulfate.
Fig. 1. ‘Intracrine’ interconversions between C19 and C18 steroids in steroid target cells. Quantitative aspects of the net flux will differ from cell type to cell type, but in general, the metabolism may not be reflected in changes in blood levels of active androgens or estrogens, but rather in their inactive metabolites, including reduced derivatives and conjugates. After Labrie et al. (2002), with permission.

an order of magnitude greater than those in postmenopausal women.

In this context, one may consider why osteoporosis is more common in women than in men and affects women at a younger age. Possibly, the uninterrupted sufficiency of circulating testosterone in men throughout life supports the local production of estradiol by aromatization of testosterone in estrogen-dependent tissues, and thus affords ongoing protection against the so-called estrogen 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 [13].

As indicated previously, in postmenopausal women, the ovaries secrete about 25% of the circulating testosterone directly. The remainder is formed peripherally from androstenedione, DHEA and DHEAS (Fig. 1). The secretion of these steroids and their plasma concentrations, however, decrease markedly with advancing age [12]. Moreover, DHEA must first be converted to androstenedione prior to aromatization. Another major step is the reduction of the 17-keto group to the 17β-hydroxyl catalyzed by one or more members of the 17β-HSD family, which is essential for formation of the active estrogen, estradiol. 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. Thus, the metabolism of DHEAS is tissue-specific, and depends on the profile of metabolizing enzymes in each tissue [4]. An extremely important point is that this metabolism is not reflected in changes in blood levels of active androgens or estrogens, but rather in their inactive metabolites, which include reduced derivatives, and glucuronide and sulfate conjugates. Thus, DHEA is converted to active hormones in local sites without leakage of significant amounts of these active hormones into the circulation. This emphasizes once more the point that neither the levels of circulating estrogens nor of their precursors are likely to be of significance in assessing either the concentration of estrogen or the availability of precursor in a particular extragonadal site [4]. It has been estimated that in men as much as 30–40% of total androgens are synthesized in peripheral tissues from inactive adrenal precursors, whereas in postmenopausal women more than 75% are synthesized in this fashion. Moreover, although the circulating testosterone levels are much lower in women than in men, total androgen production in women may be as much as 70% of that in men.

4. Non-sexually dimorphic roles of androgens and estrogens

Studies employing models of estrogen insufficiency have revealed new and unexpected roles for estradiol in both females and males [13]. These models include mutations in humans of the aromatase gene, of which 10 cases are
documented, 3 of whom are men [14,15], and 1 case of a
man with a mutation in the estrogen receptor α (ERα) [16].
They also include mice with targeted disruptions of ERα and
ERβ; the double ERα- and ERβ-knockout mouse [17–20]
as well as the aromatase-knockout (ArKO) mouse [21–23].
Recently described consequences of estrogen deprivation
test the traditional beliefs of gender-specificity of sex
steroid actions. For example, the lipid and carbohydrate phe-
notype of estrogen insufficiency is not sexually dimorphic
and appears to apply to both males and females [23,24], as
does the bone phenotype of undermineralization and failure
of epiphyseal closure [25]. Even more dramatically, the
roles of estradiol in male germ cell development and efferent
duct fluid transport would indicate that, in this local context,
estriadiol might be more appropriately defined as an androgen
[26–28].

Thus, there is a growing appreciation that both andro-
gens 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 emerg-
ing knowledge of the evolution of steriodogenic genes on
the one hand those encoding steroid hormone receptors on
the other.

Largely from the work of Callard and co-workers, it is
now recognized that the biosynthesis of estrogens occurs
throughout the entire vertebrate phylum, including mam-
mals, birds, reptiles, amphibians, teleosts, and elasmobranch
fish as well as agnatha (hagfish and lampreys), and in proto-
cordates such as amphioxus [29,30]. Consistent with this,
phylogenetic analysis of steroid receptors in lower verte-
brates indicates that the first steroid receptor was an es-
trogen receptor, followed by a progestrone receptor [31].
No equivalents of the ‘classical’ steroid receptors have been
found in any species outside the vertebrates, although an or-
tholog 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 ver-
tebrates and one after [31]. Specific regulation of physio-
logical processes by androgens and corticoids are relatively
recent innovations that emerged after these duplications.
Thus, we might speculate that the role of C19 steroids only emerged later. On this basis, it is
estimated that the biosynthesis of estrogens in extragonadal sites of production
occurs in [32].

Estrogen biosynthesis from C19 steroids is catalyzed
by the enzyme aromatase cytochrome P450. Aromatase is
encoded by the CYP19 gene which maps to chromosome
15q21.2 in humans. The structure and hormonal regula-
tion of CYP19 is complex, the gene spans 120 kb with a
coding region of 30 kb comprising nine translated exons
[7,32]. A number of untranslated exons I each associated
with a unique promoter exist upstream of exon II. These
are spliced into a common site in the 5′-untranslated re-
gion, ensuring that the coding region and hence protein
is identical in every tissue site of expression. Tissue-specific
regulation of CYP19 expression is achieved through the use
of these distinct promoters, each of which is regulated by
different hormonal factors and second messenger signaling
pathways. Thus, in the ovary, CYP19 expression is regulated
by FSH which acts through cyclic AMP via the proximal
promoter II, whereas in placenta the distal promoter I.1 regu-
lates CYP19 expression in response to retinoids. In adi-
pose tissue and bone by contrast, another distal promoter—
 promoter I.4—drives CYP19 expression under the control
of glucocorticoids, class 1 cytokines and TNFs (reviewed
in [32]).
The importance of this unique aspect of the tissue-specific
regulation of aromatase expression lies in the fact that the
low circulating levels of estrogens which are observed in
postmenopausal women have no bearing on the concentra-
tions of estrogen reached in extragonadal sites of production
due to local synthesis, as stated earlier. The significance of
this paradigm shift cannot be underestimated, namely that
the estrogen which is responsible for breast cancer devel-
oment, for the maintenance of bone mineralization and for
the maintenance of cognitive function is not circulating es-
trogen but rather that which is produced locally at these spe-
cific sites within the breast, bone and brain. These concepts
are now being accepted [2] and lead to the following
considerations.

As stated earlier, in adipose tissue including that of breast,
and in bone, a distal promoter namely promoter I.4 drives
CYP19 expression under the control of glucocorticoids,
class 1 cytokines of TNFs. However, in breast adipose
tissue of breast cancer patients, aromatase activity and CYP19
expression are elevated [33]. This occurs in response to
tumor-derived factors such as prostaglandin E2 [34] pro-
duced by breast tumor fibroblasts and epithelium as well as
infiltrating macrophages. This increased CYP19 expression
we showed previously is associated with a switch in pro-
moter usage from the normal adipose-specific promoter I.4 to
the cyclic AMP responsive promoter, promoter II [33,35].
Since these two promoters are regulated by different cohorts
of transcription factors and coactivators, it follows that the
differential regulation of CYP19 expression via alternative
promoters in disease-free and cancerous breast adipose
tissue may permit the development of selective aromatase
modulators (SAMS) that target the aberrant overexpression of aromatase in cancerous breast, whilst sparing estrogen synthesis in other sites such as normal adipose tissue, bone and brain. A more complete understanding of the mecha-
isms regulating CYP19 transcription from promoter II in breast adipose tissue therefore, is an essential prerequisite for the development of such SAMS.

Why is the development of SAMS important from a ther-
apeutic standpoint? This stems from the fact that the local source of estrogen in a breast tumor and surrounding tissue provides the drive for growth of estrogen receptor positive tumors and is the target of anti-estrogen adjuvant ther-
apies in postmenopausal women [36]. Recently, several highly specific, high affinity inhibitors of the catalytic activity of aromatase have been developed, notably the Novartis compound letrozole and the AstraZeneca compound arom-
ide. In a number of trials including the recently highly publicized large-scale trial (the ATAC trial) [37–39], these compounds have been shown to be superior to tamoxifen as adjuvant therapy for breast cancer and are likely to re-
place tamoxifen in a number of modalities of treatment.

Since these compounds inhibit aromatase globally, that is in all sites of synthesis, they have the potential to adversely affect bone mineralization, accumulation of fat in the liver, cognitive function and hot flushes [2,36]. While this may not be a major problem in treatment of elderly women with advanced disease, for young postmenopausal women with early onset disease or in the chemo-prevention setting, these problems could reach serious proportions.

Clearly if one could selectively inhibit aromatase expres-
sion within the breast, but spare the other sites of local ex-
pression, then one could offer a clear therapeutic advantage, particularly to these categories of patients. The fact that the proximal promoter II drives aromatase expression in breast tissue in the presence of a tumor, uniquely allows for this possibility, since the ovaries are no longer expression aro-
matase and the potential to adversely affect bone mineralization, accumulation of fat in the liver, cognitive function and hot flushes [2,36]. While this may not be a major problem in treatment of elderly women with advanced disease, for young postmenopausal women with early onset disease or in the chemo-prevention setting, these problems could reach serious proportions.

Hence factors that regulate aromatase expression in breast mesenchymal and tumor cells via promoter II are potential targets for such drug development.

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References


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