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Do intracrine mechanisms regulate aromatase expression?☆

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1. Introduction

In recent years, considerable emphasis has been focused on the regulation of extragonadal estrogen biosynthesis, in particular that which occurs in adipose tissue and bone, and its importance in the well-being of the elderly [1]. The regulation of aromatase expression in normal adipose tissue from various body sites including the breast has been examined as a function of age [2], and significant changes in the regulation of the expression which occurs in adipose tissue proximal to a breast tumor [3] have been documented. This has led to the conclusion that tumorous epithelium of the breast, and/or macrophages recruited to the tumor site, produce factors such as PGE_2, TNFα and class I cytokines which regulate aromatase expression in the surrounding mesenchymal cells of the adipose tissue and of the tumor itself [4–6] (Fig. 1).

Since bone is a favourite site for breast cancer metastasis, attention has also focused on aromatase expression in osteosarcoma cell lines, in primary cultures of human fetal osteoblasts, as well as osteoclastic cell lines such as THP-1 [7,8]. We and others have observed that these cells exhibit high expression of aromatase activity which is regulated primarily by class I cytokines, IL-1β and TNFα. These observations have led to the conclusion that local estrogen production in bone cells plays an important role in the maintenance of bone mineralization and the prevention of osteoporosis. In an extension of these concepts we have advanced the hypothesis, also enunciated by Labrie et al. [9], that in post-menopausal women, and also in men, extragonadal estrogen biosynthesis in a number of sites, including adipose tissue, bone, various sites of the brain, vascular endothelial and smooth muscle cells, plays an important but hitherto largely unrecognized physiological and pathophysiological role in a paracrine, autocrine and indeed, intracrine, fashion [10]. The long-term health consequences of estrogen decline after the menopause include bone loss, urogenital aging, increased cardiovascular disease, and probably cognitive impairment culminating in dementia. The incidence and pattern of occurrence of all of these disease processes differ significantly between men and women, and cannot be explained by gender differences in circulating estrogen levels alone. For example, men have plasma estradiol levels in the postmenopausal range throughout their adult years, but rarely develop osteoporosis until very late in life. Hence our understanding of the peripheral metabolism of precursor steroids in the main estrogen target tissues appears fundamental to ascertaining the mechanisms underlying the development of diseases associated with the decline in circulating estrogen levels after menopause.

2. Sites of estrogen biosynthesis

While the ovaries are the principal source of systemic estrogen in the premenopausal non-pregnant woman, other sites of estrogen biosynthesis are present throughout the body and these become the major sources of estrogen beyond menopause. These sites include the mesenchymal cells of the adipose tissue and skin (reviewed in [1]) osteoblasts [11] and perhaps osteoclasts [12] in bone, possibly vascular endothelial [13] and aortic smooth muscle cells [14] as well as a number of sites in the brain including the medial preoptic/anterior hypothalamus, the medial basal hypothalamus and the amygdala [15]. These extragonadal

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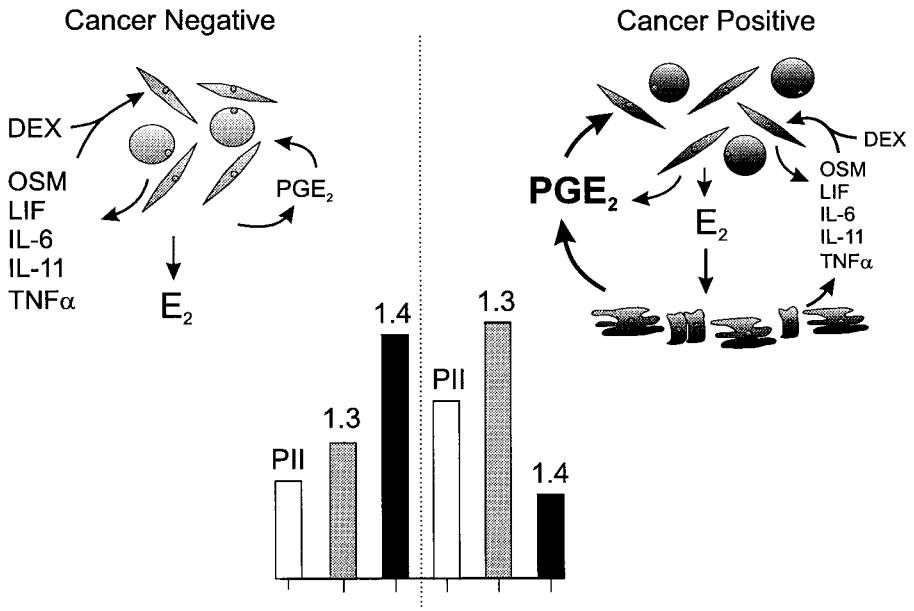

Fig. 1. Proposed regulation of aromatase gene expression in breast adipose tissue from cancer-free individuals and from those with breast cancer. In the former case, expression is stimulated primarily by class I cytokines or TNFα produced locally, in the presence of systemic glucocorticoids. As a consequence, promoter I.4-specific transcripts of aromatase predominate. In the latter case, PGE_2 produced by the tumorous epithelium, tumor derived fibroblasts, and/or macrophages recruited to the tumor site is the major factor stimulating aromatase expression, as evidenced by the predominance of promoter II and I.3-specific transcripts of aromatase.

sites of estrogen biosynthesis possess several fundamental features which differ from those of the ovaries. Principally, these sites are dependent on circulating precursor C_{19} steroids for estrogen biosynthesis. Although these extragonadal tissues have the capacity to convert C_{19} steroids to C_{18} steroids, unlike the ovaries they lack the ability to synthesise C_{19} precursors. Hence estrogen production in adipose, bone and brain is totally dependent on the availability of circulating C_{19} precursors. Another important feature is that the estrogen synthesized within these compartments, particularly bone, breast and brain, is probably only biologically active at a local tissue level in a paracrine or 'intracrine' fashion [9]. Thus the total amount of estrogen synthesized by these extragonadal sites may be small, but the local tissue concentrations achieved are probably quite high, and exert significant biological influence locally.

After menopause, the mesenchymal cells of the adipose tissue become the main source of estrogen [1,16]. Therefore in the post-reproductive years, the degree of a woman's estrogenization is mainly determined by the extent of her adiposity. This is of clinical importance since corpulent women are relatively protected against osteoporosis [17] and the incidence of Alzheimer's disease is lower in more corpulent postmenopausal women than in their slimmer counterparts (V. Henderson, personal communication). On the downside, obesity is positively correlated with breast cancer risk [18].

In the case of males, the Leydig cells and other cells of the testes produce estrogen [19]. Nevertheless, it has been estimated that at best the testes can account for 15% of circulating estrogens [20] and hence, in the male, local extraglandular production of estrogens is of physiological significance throughout adult life. For example, estrogen production in bone appears to be vital for the maintenance of bone mineralization and prevention of osteoporosis. This is supported by studies of men, either with a mutation of the gene encoding the aromatase enzyme [21,22] or a mutation of the estrogen receptor [23]. These individuals exhibit failure of epiphysial fusion, osteopenia and delayed bone age. Recently we have observed that male ArKO mice also exhibit alterations in bone histomorphometry characteristic of undermineralization [24]. In a similar fashion, it is reasonable to speculate that estrogen production in one or more brain sites has an influence on sexual behaviour, and as suggested by recent observational epidemiological studies, may have a role in the maintenance of cognitive function and the prevention of Alzheimer's disease (reviewed in [25]). In this context it is appropriate to reconsider why osteoporosis is more common in women than in men, and affects women at a younger age, in terms of fracture incidence. Similarly one may question why the incidence

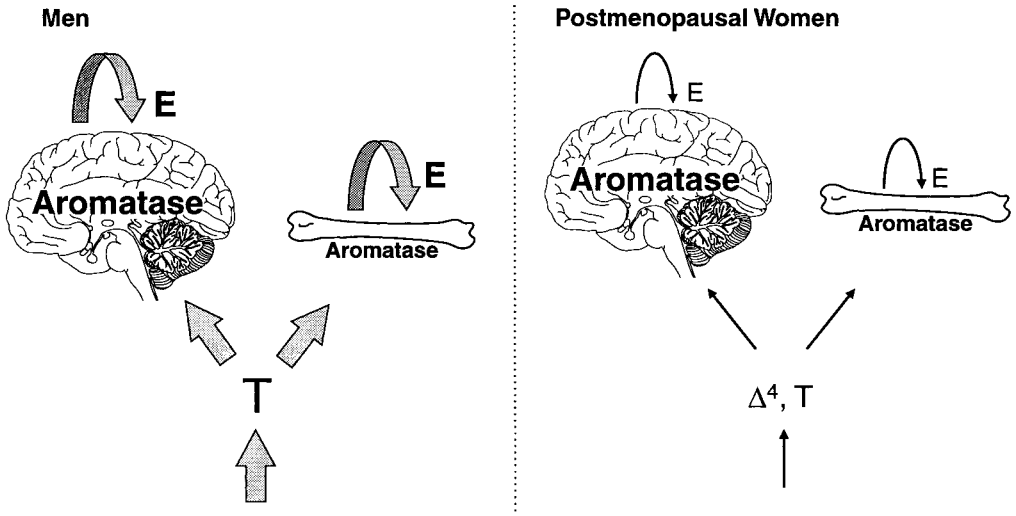

Fig. 2. Importance of circulating C_{19} steroids as precursors for extragonadal estrogen biosynthesis in men (left panel) and postmenopausal women (right panel).

of Alzheimer's disease is greater among women than among men.

3. Precursor availability

A key factor in the gender difference in the incidence of these diseases appears to be the availability of precursor C_{19} steroids for aromatization to estrogens in extragonadal sites, a concept also advanced by Labrie et al. [26]. In post-menopausal women the principal source of C_{19} steroid production is the adrenal cortex which elaborates androstenedione, dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS). However, the secretion of these steroids and their plasma concentrations decrease markedly with advancing age [27]. Moreover, DHEA must first be converted to androstenedione prior to aromatization. Another major step is the reduction of the 17-keto group to 17β-hydroxyl, catalyzed by 17β-HSD type I, which is essential for formation of the active estrogen, estradiol. The distribution of this enzyme in the various extragonadal sites of aromatization has not yet been fully established, although it is expressed in tumorous breast epithelium [28] and in bone [29]. It should be noted in this context that there is a recent report that 17β-HSD type III, which converts androstenedione to testosterone, is present in visceral fat [30], together with 17β-HSD type II.

In the male circulation, in contrast, the levels of testosterone are at least an order of magnitude greater than those circulating in the plasma of postmenopausal women (10–30 v 0.5 nmol/l), while the levels of androstenedione are rather similar (~2.5 nmol/l). Since the levels of circulating testosterone in the male are similar to the K_m of aromatase (20–30 nmol/l), it is likely that circulating testosterone can be converted efficiently in extragonadal sites to give rise to local concentrations of estradiol sufficient to transactivate both estrogen receptors (α and β) ($K_D \sim 1$ nmol/l). Moreover, although testosterone levels in the plasma of men decrease with advancing years, this decrease is small compared to the decrease in the circulating levels of adrenal C_{19} steroids. Consequently, compared with women, males maintain a high circulating level of the active precursor testosterone throughout life, which is available for conversion to the active estrogen, estradiol, in extragonadal sites. Not only is the level of circulating testosterone in men much greater than that in women, but it is also two orders of magnitude greater than the mean levels of circulating estradiol in postmenopausal women (less than 130 pmol/l) and in men (~25–130 pmol/l). Given that most of this circulating estradiol is probably bound to sex hormone binding globulin, it is unlikely to have a major impact on transactivation of the estrogen receptor, compared to estrogen produced locally as a consequence of conversion of circulating testosterone. Thus, 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 contribute to the maintenance of cognitive function and prevention of Alzheimer's disease in men (Fig. 2).

Currently, there is considerable interest in the use of testosterone as a component of hormone replacement therapy (HRT) for post-menopausal women, but its

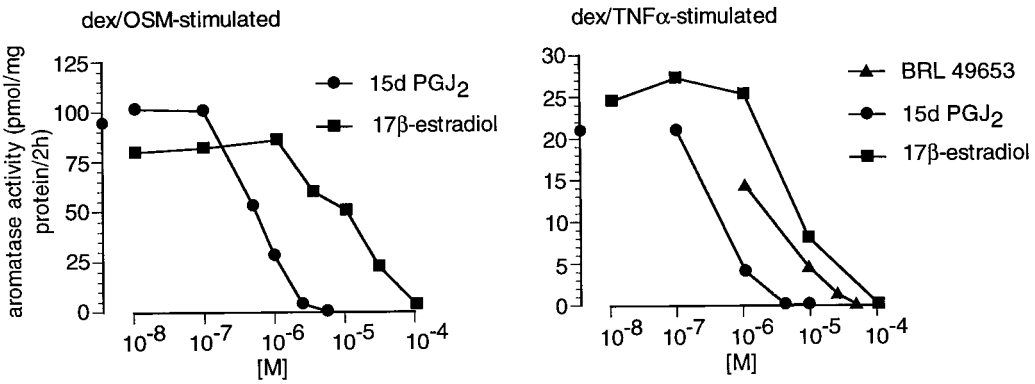

Fig. 3. Inhibition of aromatase activity of human adipose stromal cells by the PPARγ ligands BRL 49653, and 15-deoxy-$\Delta^{12,14}$-PGJ_2, in the presence of TNFα plus dexamethasone, or else oncostatin M plus dexamethasone. The inhibitory action of estradiol is also shown.

use is mostly limited to those women who complain of loss of sexual interest and libido. However, there is increasing evidence that postmenopausal testosterone replacement is effective in both the prevention and treatment of osteoporosis [31,32]. Thus, the present discussion suggests a broader role for the use of testosterone in HRT, namely as a circulating precursor for local synthesis of estrogen in target tissues where the latter acts in an autocrine and paracrine fashion.

4. Regulation of aromatase expression

We have suggested previously [1] that aromatase is a marker of the undifferentiated adipose mesenchymal cell phenotype. In support of this, the factors which stimulate expression in adipose tissue of cancer-free individuals are factors which 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 . Moreover all of these factors act via promoter I.4 of the aromatase gene and require glucocorticoids as a co-stimulator (reviewed in [1]). Adipocyte differentiation is driven by transcription factors such as C/EBPα and β and also PPARγ [33], and while involving the down-regulation of aromatase expression, the differentiation process also involves the upregulation of markers such as lipoprotein lipase, the insulin receptor and GLUT4. These actions are antagonized by TNFα which is also expressed in adipocytes [34]. Significantly in this context, mice lacking TNFα function are protected from obesity-induced insulin-resistance [35]. These considerations suggest that factors which stimulate adipocyte differentiation such as ligands of the PPARγ receptor e.g. BRL 49653 and 15-deoxy-$\Delta^{12,14}$-PGJ_2 would inhibit aromatase expression and this has proven to be the case (Fig. 3). They also indicate that individuals with insulin-resistance have higher levels of aromatase expression in their adipose, and therefore are at greater risk of developing breast cancer. While the former has not been shown, there is epidemiological evidence to support the latter contention [36].

Further light on the role of estrogens in adipose tissue metabolism has come from our recent studies employing the ArKO knock-out mouse in which there is a redistribution of adipose tissue with diminished subcutaneous and increased visceral fat deposits (Table 1). To investigate the mechanism whereby the subcutaneous fat depots are decreased, we examined the actions of estrogen on the differentiation of 3T3 L1 cells, and observed that estradiol mimics the effects of troglitazone in this context. So an important issue which arises is to determine the mechanism whereby estradiol elicits this response and in particular to determine whether estradiol or a downstream metabolite is an endogenous ligand of PPARγ. These results would also lead us to predict that estrogen would mimic thiazolidinediones in inhibiting aromatase expression in adipose stromal cells, and in preliminary results we have found that estradiol in high concentrations does this (Fig. 3). So there appears to be an important homeostatic mechanism operating in these cells to regulate the levels of estrogen biosynthesis in the context of adipocyte differentiation.

Table 1
Body mass and visceral fat content of female ArKO mice and wild-type littermates

		Wild-type	ArKO
Body mass (g)	10–12 weeks	19.3±0.4	21.9±0.1
	4–5 months	21.7±0.3	27.0±3.3
Gonadal fat mass (mg)	10–12 weeks	162.5±13.2	269.6±86.9
	4–5 months	280.0±84.7	490.0±96.0

5. Clinical considerations

An important issue pertaining to the role of estrogen in the development of breast cancer in post-menopausal women is the relationship between HRT and breast cancer risk. A collaborative analysis of a large body of the available epidemiological data which address this issue found that despite the influence of estrogen produced locally on the development of breast cancer, systemic administration of estrogens plus progestins to post-menopausal women leads to at most a 1.35 fold increase in breast cancer risk [37]. The reason for this small effect may have been revealed by the studies discussed here. Locally produced estrogen within the breast stimulates breast cancer development and is regulated by the mechanisms which have been discussed. The resulting intratumoral estradiol concentrations are an order of magnitude higher than in the circulating plasma [38]. Thus the increase in circulating estrogen as a consequence of HRT may have little influence on intratumoral levels. The action of locally-produced estrogen is largely paracrine in nature, and mediated via the classical estrogen receptor(s) or else via DNA adduct formation by quinone intermediates [39]. Additionally, estrogens may have an autocrine or 'intracrine' action on adipose cells themselves whereby estradiol or a downstream metabolite generated within the cell site of synthesis may act by an alternative mechanism, possibly involving PPARγ, to inhibit aromatase expression.

In conclusion, we believe that the results of recent studies reveal the significance of extra-gonadal estrogen production in the physiology and pathophysiology of the elderly, in particular its importance in the maintenance of bone mineralization, and its role in the development of breast cancer. Local estrogen production may also play a role in the prevention of cardiovascular disease and in the maintenance of cognitive function. These studies not only throw light on the role of extragonadal estrogens in the health and disease processes of the elderly, but may also lead to new and hitherto unexpected modalities of therapy. This is already apparent from the observation that tumor-derived PGE_2 is a major factor stimulating local aromatase expression in the breast fat of cancer patients, which leads to the consideration that prostaglandin synthesis inhibitors such as aspirin and ibuprofin would be beneficial in breast cancer prevention or treatment [6], and indeed there is an epidemiological study which supports this [40]. Similarly the observation that PPARγ ligands, namely the thiazolidinediones, inhibit aromatose expression would suggest that these compounds, which are now available in the USA for the treatment of insulin-resistant diabetes, would also be beneficial in breast cancer prevention.

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