Best Practice & Research Clinical Endocrinology and Metabolism
Vol. 16, No. 1, pp. 13–30, 2002
doi:10.1053/beem.2002.0177, available online at http://www.idealibrary.com on IDEAL®

BEST PRACTICE & RESEARCH

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Oestrogens and puberty

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Oestrogens induce the development of female reproductive tissues. Endogenous human oestrogens include oestradiol, oestrone and oestriol. Oestrogen signalling in target tissues is dependent on the tissue concentration of oestrogen and the interaction of oestrogen receptors with an array of cell-specific co-regulator proteins. The diverse mechanisms of oestrogen signalling are complex and incompletely understood. In puberty, oestrogen is derived from both gonadal and peripheral sources. Originally, oestrogen was only thought to drive feminization in females; now, oestrogen is known to be important for pubertal development of males as well. Oestrogen is required for normal maturation of the neuroendocrine–gonadal axis and bone in both sexes, and a variety of other tissues are also responsive to oestrogen. Abnormal puberty can be associated with either excessive or inadequate oestrogen production. Girls deficient in oestrogen should receive replacement in physiological doses. Aromatase inhibitors and anti-oestrogens may prove to be useful therapeutic tools in some types of abnormal puberty.

Key words: aromatase; endocrine disrupters; 17β-hydroxysteroid dehydrogenase; neuroendocrinology; receptors, oestrogen; signalling, oestrogen.

INTRODUCTION

Oestrogens play a critical role in female puberty and they are also important in many aspects of male puberty. Production of oestrogens occurs at substantial levels in utero, in early infancy and again during puberty (Figure 1). This chapter will begin with a review of the biosynthesis, physiology and signalling of oestrogens. Oestrogen action on neuroendocrine and peripheral target tissues will then be covered, followed by relevant topics in abnormal puberty and environmental oestrogens. The role of oestrogen in female puberty has recently been comprehensively reviewed.[1]

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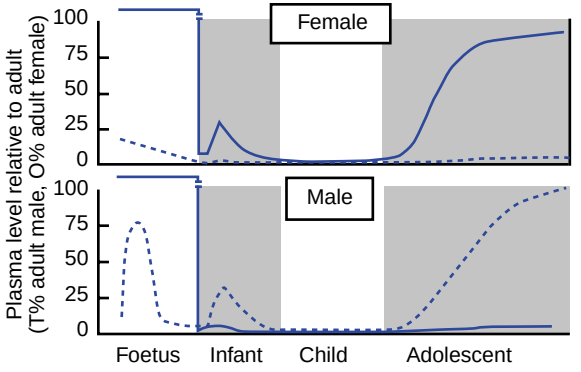

Figure 1. Sex steroid plasma levels during development are depicted as a percentage of adult normal values. T, testosterone; O, oestradiol; —— oestradiol; – – –, testosterone. Note the transient 'minipuberty' of early infancy, the nadir during childhood and the reinitiation of sex steroid production during puberty. (Source of data: Rosenfield.[1])

OESTROGENS: CHARACTERISTICS, BIOSYNTHESIS AND TRANSPORT

Characteristics of oestrogens

The word 'oestrogen' refers to a substance that promotes female reproductive development.[2] Oestrogenic activity has traditionally been quantified by bioassay, such as the ability to cause cornification of rat vaginal epithelium. Many structurally different compounds have oestrogenic activity. Natural biological oestrogens include human oestrogens, equine oestrogens, and plant-derived phyto-oestrogens. Synthetic oestrogens include pharmacological compounds such as ethinyl oestradiol, diethylstilboestrol, selective oestrogen receptor modulators (SERMs) and some industrial chemicals such as organochlorines and plasticizers. For more information on exogenous oestrogenic compounds see the section on 'synthetic oestrogens, environmental oestrogens and phyto-oestrogens' below.

Oestradiol is the most potent endogenous human oestrogen, with a high affinity for the oestrogen receptor (OR). Other endogenous oestrogens include oestrone, oestriol and catechol oestrogens. The relative dissociation constants measured using ORα are 0.13 nM for oestradiol, 0.3 nM for oestrone, and 1.4 nM for oestriol.[3] Some androgen metabolites, such as 3β-androstanediol and Δ^5-3β-androstenediol have weak oestrogenic activity.[3,4] For comparison, the dissociation constants for the potent synthetic oestrogen diethylstilboestrol are 0.04 nM (ORα) and 0.05 nM (ORβ), and for the phyto-oestrogen genistein they are 2.6 nM (ORα) and 0.3 nM (ORβ). Biological potency is determined not only by receptor affinity, but also by tissue concentration and half-life.

Oestrogen biosynthesis

01 Sources of oestrogen include direct secretion by the gonads, conversion of precursor steroids in peripheral tissues and desulphuration of conjugated oestrogens in the liver (Figure 2).[5,6] The gonads and adrenal glands secrete the prehormones for oestrogen biosynthesis, androstenedione and testosterone. In reproductive-age females the

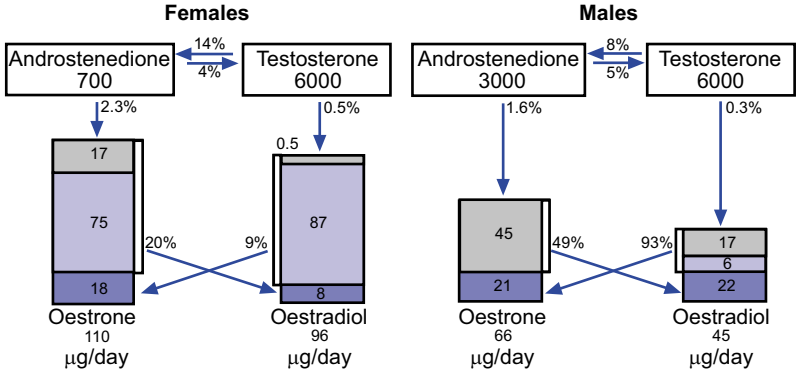

Figure 2. Sources of oestrone and oestradiol in men and premenopausal women. Oestrogen is derived from direct secretion by the gonad (□), aromatization of androgen (□), or conversion of an oestrogen precursor by 3β-HSD (■). Note that the testis secretes no oestrone and scant oestradiol. The percentage of substrate converted per day and the total approximate production in µg/day, are noted for each source. Female data were determined from blood oestrogens[6] and male data from urinary oestrogens.[5]

ovary is the major source of oestrogen, but peripheral tissues contribute as well. In males, some oestrogen is secreted by the testes but most is peripherally converted from prehormones. Conjugated oestrogens, principally oestrone sulphate, form a circulating reservoir of oestrogen that can be returned to the active pool by desulphuration in the liver.[7]

The biosynthesis of steroid hormones begins in the ovaries, testes and adrenal glands, where the side chain of cholesterol is cleaved to form pregnenolone (Figure 3). Androstenedione and testosterone are converted to oestrogens by specific 17β-hydroxysteroid dehydrogenase (17β-HSD) isoenzymes and aromatase in the gonads and in peripheral tissues.[8,9] Aromatase is expressed in fat, skin, osteoblasts, chondrocytes, vascular smooth muscle, endothelium and the central nervous system. The aromatase gene is unusual; tissues initiate transcription at different promoters, but the resulting protein is identical in each tissue. After splicing, the mRNAs differ only in the 5′ untranslated region. Tissues expressing aromatase may produce higher local oestrogen concentrations than tissues dependent on serum for oestrogen. Tissue oestradiol concentrations are also regulated by the rate of metabolism to weaker compounds. For example, progesterone antagonizes oestrogen activity by inducing 17β-HSD2 and 17β-HSD4, which convert oestradiol to oestrone.[10]

Oestrogen transport

Serum oestrogen appears to reach target tissues by simple diffusion from the vascular compartment. About 98% of plasma oestradiol is bound to albumin and sex hormone binding globulin (SHBG); the remaining unbound hormone forms the bioavailable pool. SHBG is an important determinant of oestradiol bioavailability because hormone that is bound to carrier protein is protected from metabolic clearance. Production of SHBG is increased by oestrogen and thyroid hormone and is decreased by androgen, insulin, glucocorticoid and growth hormone.[11]

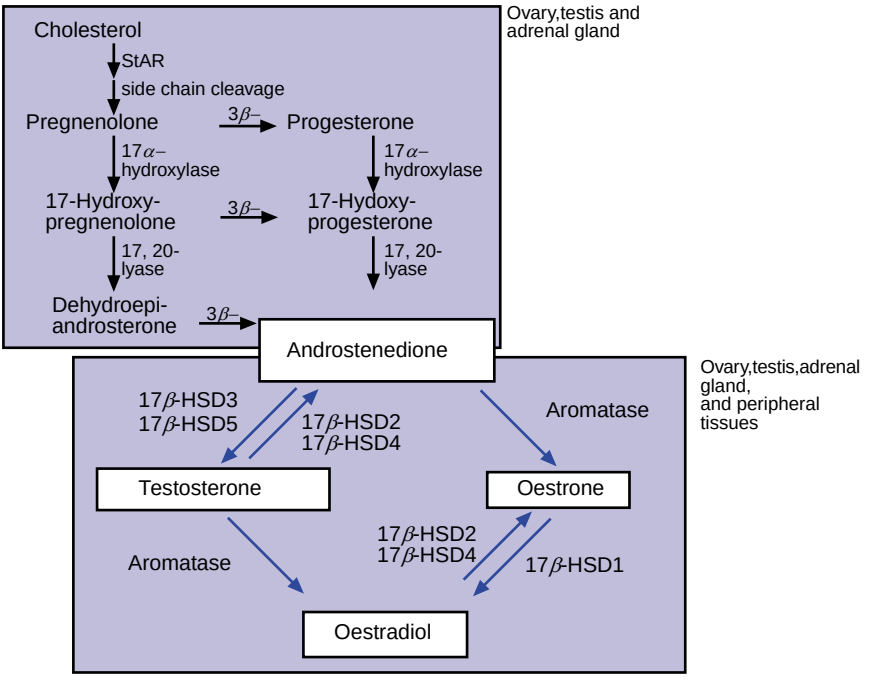

Figure 3. Oestradiol biosynthesis. Androstenedione is the preferred substrate for aromatase; testosterone is quantitatively less important. Although the majority of androstenedione is produced in the ovary, testis and adrenal gland as depicted, 3β-HSD in other organs such as the liver also contributes. 3β-, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase isoenzymes; StAR, steroidogenic acute regulatory protein.

OESTROGEN SIGNALLING

Overview

Oestrogen signalling is a complex process that is regulated at many levels.[12] When bound to oestrogen, the OR changes shape, dimerizes and interacts with DNA and other proteins. Classically, oestrogen enables the OR to bind DNA at a sequence known as an oestrogen response element (ORE). OREs are located upstream of genes that are regulated by oestrogen; binding of the OR to an ORE alters the transcription of oestrogen target genes. However, the reality of oestrogen signalling is more like a web of interrelated pathways than a simple linear path. Depending on the cell context, OR can activate transcription without oestrogen, OR can activate transcription from non-ORE sites and oestrogens can have direct cellular effects that are independent of gene regulation.

Oestrogen receptors

A member of the nuclear receptor superfamily, the OR is a high affinity receptor that is specific for oestrogen.[12] Two isoforms of OR have been studied in detail, ORα and ORβ (Figure 4). These proteins are encoded on different genes but are highly homologous in the DNA-binding (97%) and ligand-binding domains (60%). ORα mRNA

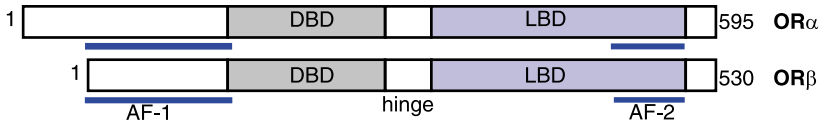

Figure 4. Domain structure of human ORα and ORβ. The DBDs share 97% homology, LBDs share 60% homology and other regions share less. DBD, DNA binding domain; LBD, ligand binding domain; AF-1, activation function-1; AF-2, activation function-2. (Source of data: from Hall et al.[12])

is strongly expressed in ovarian theca cells, uterus, breast, vagina, pituitary, prostate, testis and bone, while ORβ mRNA is found in granulosa cells, prostate, testis, spleen, bladder and lung.[3,13] Recently, additional ORβ isoforms have been described and these have been termed ORβcx and ORβ2.

The OR is believed to be transported to the nucleus immediately after synthesis. Unliganded OR in the nucleus is bound by heat shock proteins of the hsp90 chaperonin complex. Chaperone proteins influence receptor configuration, intracellular trafficking and receptor turnover. Oestrogen binding induces the OR to dissociate from the heat shock proteins, to dimerize and to interact with other nuclear proteins. Both homodimers and heterodimers of ORα and ORβ can be formed.

Regulation of OR activity occurs principally at the activation function domains, AF-1 and AF-2. AF-1, at the N-terminus, can be phosphorylated by a calcium-dependent mechanism involving mitogen activated protein (MAP) kinase. AF-2, in the C-terminus, interacts with nuclear coregulator proteins. X-ray crystallography has demonstrated that antagonist bound to ORα causes a change in the configuration of AF-2 such that the coactivator recognition groove is obliterated.[13] Thus, ligand structure can determine the interaction of OR with coregulator proteins. The spectrum of activities of ORα and ORβ differs. ORα and ORβ can have opposite actions at AP-1 and Sp1 sites.[15] Also, mice lacking ORβ demonstrate an excessive proliferative response to oestradiol in some tissues,[15] suggesting that one role of ORβ is to modulate ORα activity.

Transcription regulation by oestrogen receptors

Gene regulation by the OR occurs via several mechanisms. OR activity in the nucleus is largely dependent on coregulatory proteins recruited to the oestrogen–OR complex by the ligand-induced change in OR configuration. Over 19 different coactivators, and several corepressors, have been shown to interact with ORα.[17] Steroid receptor coactivators (SRCs) enhance transcriptional activation by multiple mechanisms, including recruitment of other coactivators such as the cyclic adenosine monophosphate response element binding protein (CREB) binding protein (CBP) to the complex.[18] Like SRCs, CBP has intrinsic histone acetyltransferase activity that alters chromatin structure to increase access to regulatory sequences and, hence, it interacts with multiple other coregulator proteins. ORs interact with many nuclear factors, including activator protein-1 (AP-1), Sp1, retinoid thyroid hormone, and orphan receptors.

OR can regulate transcription from non-ORE sites, such as the AP-1 site. In this case, OR does not bind to DNA, but is present as part of a multiple protein complex assembled near the transcription start site. OR regulation of transcription from AP-1 sites requires oestrogen, as well as the AP-1 transcription factors jun and fos.

Non-genomic oestrogen signalling

Oestrogen can also exert rapid effects from the cell membrane via mechanisms that do not involve genomic interactions.[19] Direct oestrogen action from the membrane is known to occur in brain, bone and the vascular system. Non-genomic oestrogen effects are probably mediated through a type of OR found at the cell membrane, although it is not yet clear what genes encode these receptors. Cytoplasmic signals involved include MAP kinase, inositol triphosphate (PI3) kinase, and guanine nucleotide regulatory (G) proteins.

PUBERTY: OESTROGEN PHYSIOLOGY

The minipuberty of the fetus and neonate

Gonadal hormones are present at early pubertal levels in the fetus and neonate (Figure 1). The fetus is exposed to high oestrogen concentration in utero because of the fetoplacental unit. Newborn girls show oestrogenization of the vagina and a palpable breast bud is present in one-third of full term neonates.[20] Menstrual bleeding and colostrum production sometimes occur as the baby is withdrawn from the oestrogenic environment.

In the first few months of life the neuroendocrine–gonadal axis is transiently activated; gonadotrophin and oestrogen levels are similar to those in early puberty and respond to the gonadotropin releasing hormone (GnRH) agonist. Oestradiol levels are maximal at approximately 2–4 months of age and breast development may continue through early infancy. As the neuroendocrine–gonadal axis enters the quiescent state characteristic of childhood, oestradiol levels decline. Although there is evidence for the secretion of bioactive gonadotrophins at low levels during childhood, normally no sexual development results.[21]

Oestrogen secretion during normal puberty

Typical oestradiol serum levels in puberty are shown in Table 1. In early female puberty, gonadotrophin and oestradiol secretion are thought to be cyclical long before the onset of mature ovulatory cycles.[1] In prepubertal girls, the GnRH pulse generator is weak and, consequently, is sensitive to inhibition by small amounts of oestradiol. A model of cyclical oestradiol secretion in early puberty has been proposed. As hypothalamic GnRH pulses increase in amplitude and duration, gonadotrophin secretion increases, which leads to ovarian oestradiol production. Very small amounts of oestradiol are

Table 1. Typical normal ranges for oestradiol serum levels (pg/ml) in puberty.

	Females	Males
Prepubertal	< 10	< 10
Early puberty	10–60	$\leqslant 15$
Late puberty	15–75	10–40
Adult	25–250	10–50

To convert pg/ml to pmol/l, multiply by 3.75.
Source: Rosenfield[1] and Ghai & Rosenfield.[24]

sufficient to suppress both the GnRH pulse generator and gonadotrophin secretion, so follicle development and oestradiol secretion quickly wane. A period of quiescence occurs and then GnRH pulses regain sufficient strength to repeat the cycle.

As puberty progresses, auto-amplification phenomena augment hypothalamic–pituitary–gonadal dynamics at all levels of the axis. In girls, oestradiol output increases rapidly in the year approaching menarche.[22] During each cycle, follicle stimulating hormone (FSH) induces proliferation and growth of ovarian follicles. Luteinizing hormone (LH) induces theca cells to express steroid hormone biosynthetic enzymes, which produce androgenic substrates for oestrogen biosynthesis. Granulosa cells aromatize androgens secreted by theca cells to oestrogens. As the dominant follicle grows, oestradiol production increases. Preovulatory levels of oestradiol increase GnRH pulse size. Oestradiol also acts synergistically with progesterone to increase gonadotroph responsiveness to GnRH. When oestradiol levels rise above 200 pg/ml for 2–3 days, the negative feedback on GnRH and gonadotrophin release turns to positive feedback, leading to the ovulatory LH surge. After ovulation, oestradiol and gonadotrophin levels decline.

In boys, serum levels of oestradiol increase late in puberty. Gonadotrophins or GnRH agonists stimulate adult men to secrete oestradiol in quantities equivalent to follicular phase women. In early pubertal boys, LH or GnRH agonists stimulate secretion of testosterone but not oestradiol. Oestradiol secretion occurs only later, suggesting that aromatase is not inducible by gonadotrophins in early puberty.[23,24] Despite low serum levels of oestradiol, oestrogens produced locally may be important in the maturation of some tissues in boys.

TARGET TISSUE EFFECTS OF OESTROGENS

Models

Oestrogen biology in peripheral tissues has been studied extensively in non-human models, especially mouse. The ORα and ORβ genes have each been removed by homologous recombination to make OR knockout mice (α-ORKO and β-ORKO).[16] Combined ORα/β knockout mice were generated by breeding the α- and β-ORKO mice.[25] A second model is the aromatase knockout (ArKO) mouse, which cannot aromatize androgens to oestrogens.[26] Finally, several human patients have come to medical attention because of deficiency in the ORα or aromatase proteins.[27]

Hypothalamic–pituitary–gonadal axis

Oestrogen feedback on the hypothalamus and pituitary is not entirely understood. Oestradiol exerts both tonic negative and episodic positive feedback on the human female hypothalamic–pituitary axis, but probably only negative feedback on the male axis. The specific sites and mechanisms of action in the hypothalamus and pituitary are debated, because in vivo the GnRH neurone is not functionally separable from other hypothalamic cells or pituitary gonadotrophs and in vitro studies are contextually difficult to interpret. α-ORKO female mice have increased gonadotrophins with increased oestradiol levels; this may be due to reduced negative feedback on the hypothalamus, pituitary, or both. ORα and ORβ are both likely to be involved, because the combined knockout of both OR genes results in higher LH levels than the ORα knockout alone.[16]

ORs are expressed in the hypothalamus and the pituitary. GnRH neurones seem to have reduced levels of ORα mRNA relative to other hypothalamic cells, suggesting

that oestrogen effects on the GnRH neurone may be indirect, via neighbouring cells. However, GnRH cell lines in vitro express ORα, and sensitive techniques now find that both ORα and ORβ may be expressed in the GnRH neurone in vivo.[28,29] Both ORα and ORβ proteins[30] and mRNA[31] are expressed in pituitary cells including, but not limited to, gonadotrophs.

Negative regulation of GnRH secretion by oestradiol is likely to occur. The GnRH promoter sequence does not contain a full ORE, but contains a half site that binds OR. In vitro analysis of GnRH gene transcription using a luciferase reporter driven by the GnRH promoter demonstrates an 80% reduction in luciferase activity after oestradiol treatment.[32] In vitro studies have tested oestradiol effects on GnRH expression using either a hypothalamic cell line (GT1–7) that naturally expresses GnRH, or ovary cells transfected with the GnRH promoter regulating a luciferase reporter.[33,34] In both cases, oestradiol downregulated GnRH mRNA by approximately 50%, in an OR dependent manner. Thus, oestradiol seems to negatively regulate GnRH mRNA transcription by an OR dependent mechanism.

Positive regulation of the preovulatory hypothalamic GnRH surge is mediated by oestrogen. A recent publication suggests that induction of the progesterone receptor may be a required step.[35]

Regulation of pituitary gonadotrophin secretion, including both negative and positive feedback, seems to be mediated directly by oestrogen. Direct positive feedback by ovarian factors on the pituitary was shown in classic experiments in female rhesus monkeys in which the pituitary was isolated from the hypothalamus. GnRH administered in an unvarying pulsatile fashion induced ovarian maturation, the LH surge and ovulation.[36] In males, estrogen mediate testosterone negative feedback at both the hypothalamican pituitory levels.[37] GnRH receptor expression on gonadotrophs is known to be regulated in complex fashion by oestradiol.[37,38]

Ovary

In addition to its role as the major source of oestrogen in females, the ovary is also an important target of oestrogen action.[39] ORα and ORβ are detectable by immunohistochemistry in the ovary, with ORα predominantly found in theca cells and stroma, and ORβ in granulosa cells. Although not much is known about oestrogen signalling in the human ovary, the α- and β-ORKO mice have abnormal ovarian function.[16] The α-ORKO females are completely infertile, with total absence of ovulatory follicles. α-ORKO germ cells and follicle progression appear to be histologically normal until the antral follicle stage, but no follicles proceed to ovulation. The β-ORKO females are fertile, although they ovulate less frequently than wild-type mice. In a dramatic departure from normal function, ovarian follicles of combined α/β-ORKO mice transdifferentiate post-natally into structures resembling the seminiferous tubules of the testis.[25]

Evidence to support a role for oestrogen in human ovarian function is less available. ORα and ORβ are expressed in the human ovary, most prominently in the granulosa cells. No OR mutant human females have been reported. Aromatase-deficient women demonstrate hypergonadotrophic hypogonadism, partially masculinized genitalia and polycystic ovaries. Studies of women with reduced oestrogen production on the basis of metabolic enzymatic defects (such as deficiency of 17α-hydroxylase, 17–20 lyase, or 3β-hydroxysteroid dehydrogenase), suggest that follicular 'expansion' as measured by antrum formation is possible in ovaries with severely diminished oestradiol levels.[39]

Testis

The reduced fertility of the male α-ORKO mouse implicates oestrogen in testicular function.[40] Excesses of oestrogen are known to reduce male fertility, but an oestrogen requirement for normal testicular function is a new finding. OR expression in Leydig cells has been controversial. Immunohistochemistry localizes ORα to Leydig cells and ducts, and ORβ to Leydig cells, Sertoli cells and seminiferous tubules. α-ORKO mice have defects in efferent ductule development, resulting in reduced fluid resorption and reduced sperm delivery to the epididymis. Despite the wider expression of ORβ in the testis, the β-ORKO male mouse is fertile and the testis is histologically normal. The α/β-ORKO mouse has a similar testicular phenotype to the α-ORKO mouse. The ArKO male is fertile, although sperm counts and overall fertility wane with time.

The single report of a man with ORα deficiency found that he had normal sperm density, but sperm viability of only 18%. Testicular volume was normal and sexual function was normal as well. Men with aromatase deficiency may have abnormal testicular function.[27] One had normal sexual development, testicular volume and nocturnal emissions, but a second was infertile with small testes, azoospermia and immotile sperm; however, his unaffected brother also suffered from azoospermia and infertility.

Uterus and vagina

Oestrogens are necessary for the development and sexual maturation of the female reproductive tract. Oestrogen receptors are expressed in the labia minora, prepuce and glans in females, but not in the homologous structures of males.[41] In utero, aromatase deficiency or anti-oestrogen exposure in female fetuses are associated with masculinization and ambiguous genitalia.[42] However, ORKO and ArKO mice have no obvious defect in genital tract differentiation.[16]

The female genital tract undergoes cyclical changes related to serum oestradiol levels. In the absence of oestrogen the stratified squamous epithelium of the vagina is thin, with only a single layer of cells over the basal layer. In response to oestrogen, epithelial proliferation occurs, leading to the formation of additional layers of cells. The cells also become thicker, with expanded cytoplasm and increased glycogen synthesis. Vaginal smears are an effective way to measure the degree of oestrogen effects on the vaginal epithelium. Prepubertal vaginal smears show virtually only suprabasal cells, intermediate cells predominate in early puberty and superficial cells are characteristic of mature vaginal epithelium. The endocervical mucous becomes viscous and elastic as oestrogens rise in the late follicular phase and returns to a thin, scanty secretion when oestrogen levels drop.

Endometrium also demonstrates cyclic changes in concert with oestrogen levels. In the follicular phase, the endometrial epithelium and stroma proliferate. The uterine glands increase in number and length. After ovulation, progesterone induces a further increase in endometrial thickness; uterine glands enlarge and secrete a glycogen-rich mucoid fluid, and the arterial bed becomes richer, in preparation for implantation. These changes reverse with withdrawal of oestrogen. Androgens and progesterone both antagonize oestrogen action on endometrium.[43]

Mammary gland

Oestrogen is required for breast development. Oestrogen stimulates the nipple to grow and darken in colour. The developmental processes of mammary duct branching and

adipose stromal growth are dependent on oestrogen. Lobulation of mammary ducts appears around menarche, when multiple blind saccular buds form by branching of the terminal ducts. Breast alveolar development occurs at the time of menarche; it is unclear whether this is due to increased oestrogen or progesterone levels. Breast stroma swells cyclically during each luteal phase. Full breast development normally only occurs during pregnancy under the influence of additional oestrogen, progesterone and prolactin.

Bone

Bone growth and bone density accrual are regulated by oestrogen in both boys and [44,45] Oestrogen exerts a biphasic effect on long bone growth: at low concentrations it induces growth, but at higher concentrations growth is inhibited. About half of the growth stimulatory effect of oestrogen is due to activation of the growth hormone-insulin like growth factor axis by oestradiol.[46–49]

The inhibitory effect of oestrogen on growth is due to oestrogen-mediated epiphyseal closure at higher concentration. Men deficient in oestrogen signalling fail to close their epiphyses and continue to grow after sexual maturity is achieved.

One third of final bone mass is accrued during puberty. The men deficient in oestrogen signalling also suffer from severe osteoporosis; only those with aromatase deficiency respond to oestradiol treatment. Oestrogen is, therefore, critical to attaining normal bone density. Polymorphisms in the ORα gene have been reported to predict bone mineral density as well as adult height in the general population.[50]

Central nervous system

Fetal and neonatal sex steroids play an important role in central nervous system (CNS) development. In the adult brain, oestrogen contributes to the regulation of affect and mood, cognitive function, motor function, epileptic excitability and pain pathways.[51] ORα and ORβ mRNA are expressed widely in the CNS. ORα is found predominantly in the hypothalamic preoptic area, pituitary and amygdala, and ORβ is expressed in the olfactory bulbs, cerebellum and cerebral cortex.

Development of gender dimorphic regions of the brain may be regulated in part by oestrogen. The hypothalamic preoptic nucleus is larger in male rats[52], an effect that is due to a testosterone-induced reduction in apoptosis in this region.[53] However, administration of either oestrogen or testosterone to newborn female rats causes male-type enlargement of this nucleus, suggesting that oestrogen may mediate the effect. Testosterone is known to induce aromatase expression in the rat hypothalamus.[54,55] Conversely, oestrogen has been shown to induce androgen receptor expression in the rat forebrain.[56]

Gender dimorphic behaviours are more difficult to study. Male rats make better use of spatial cues to solve mazes; this may be due to testosterone-mediated upregulation of aromatase and ORs during hippocampus development.[57] Some studies report that boys are more adept than girls at visual–spatial tasks, but the relative contributions of environment versus gender programming are difficult to evaluate.[57] Visual–spatial ability is impaired in both hypogonadal boys and girls, suggesting that oestrogen may mediate this trait. Androgen acting through the androgen receptor seems to be important in male gender identity.[59]

Adipose tissue

Adipose tissue is a significant source of oestrogen as well as a target of oestrogen signalling. Some mouse strains develop increased fat stores when ovariectomized; this weight gain can be prevented by treatment with oestrogen. Prolonged treatment with anti-oestrogens can also increase fat stores. ArKO and α-ORKO mice develop strain-specific increased adiposity and body weight. β-ORKO mice, in contrast, have normal body weight and adipose stores. These data suggest that ORα plays an important metabolic role in adipocytes, but several genes are likely to be involved.

Girls have a greater percentage of body fat than boys, with fat distributed relatively more to the buttocks and thighs than the abdomen.[60] Androgenized girls, however, develop a more masculine distribution of adipose tissue. Serum levels of the adipocyte hormone leptin rise throughout puberty in girls, to reach higher levels than boys. It has been shown that oestrogen stimulates leptin secretion by omental fat in women, but not in men.

Vasculature

Oestrogen effects on the vasculature occur by both genomic and non-genomic pathways.[60] One important effect is the release of nitric oxide (NO) from endothelial cells, leading to vasodilation. Oestrogen added to human endothelial cell culture stimulates NO release within seconds and this is accompanied by an increase in intracellular calcium. Of great interest, a membrane-impermeant form of oestradiol was reported to stimulate NO release, and the effect was blocked by an OR inhibitor, suggesting the presence of ORs at the plasma membrane. A second vascular effect of oestrogen is reduction of smooth muscle proliferation, mediated by ORβ in the intima.

Pilosebaceous unit

Androgens are the primary steroid hormones involved in sexual hair growth; hair growth in ORKO and ArKO mice is normal. However, hypogonadal girls with Turner syndrome treated with depot oestradiol develop modest pubic hair growth, independent of adrenarche.[62] Oestrogens may upregulate the androgen receptor in hair as they have been shown to do in brain.[56] Immunohistochemistry demonstrates ORα in the nuclei of dermal papilla cells and topical oestradiol is reported to delay the transition from the telogen (resting) to the anagen (growing) phase.[63]

THE ROLES OF OESTROGEN IN ABNORMAL PUBERTY

Girls with insufficient oestrogen

Oestrogen deficiency occurs with primary ovarian failure or with hypogonadotrophic hypogonadism. Ovarian failure is most often caused by ovarian dysgenesis, in Turner syndrome, and other causes include steroidogenic defects, autoimmune ovarian injury, radiation, or chemotherapy. Central hypogonadism results from cerebral, hypothalamic or pituitary abnormalities. Congenital central hypogonadism can occur in combination with midline defects. Hypothalamic hypogonadism with anosmia, or Kallmann syndrome, occurs in females one-fifth as frequently as in males. Congenital pituitary resistance to GnRH has been described, in the form of GnRH receptor mutations. Acquired central hypogonadism can result from hypothalamic or pituitary

tumours, radiation or other CNS destructive processes, hyperprolactinaemia, anorexia nervosa, or starvation.

Girls require oestrogen for sexual maturation; congenital complete hypogonadism precludes natural puberty. Acquired hypogonadism may result in failure to enter puberty, arrested puberty, or secondary amenorrhea, depending on the timing of oestrogen deficiency relative to the onset of puberty. Girls with hypogonadism require hormone replacement to promote breast development and accrual of bone mass and to prevent psychological maladjustment and poor self image.

The dose of oestrogen replacement is important because excessive oestrogen restricts height potential. Oestrogen effects on long bone growth are biphasic: growth stimulation is optimal at approximately 4 μg/day of oestradiol or ethinyl oestradiol.[64,65] A dose of 1.0 mg of depot oestradiol/month during mid-puberty induces feminization without compromising adult height potential.[62] Adult oestrogen replacement doses are inhibitory to growth.

Girls with oestrogen excess

Precocious thelarche, premature isolated breast enlargement, is the mildest form of oestrogen excess. These girls have slightly higher serum oestradiol levels than age-matched controls, although the measurements are within the normal prepubertal range. They may have increased FSH secretion.[21,66] Premature thelarche has been reported in girls eating food contaminated with oestrogens and in girls on soy formula.[67,68] Abnormally large breast growth in puberty is known as virginal hypertrophy of the breast, or gigantomastia. Although the endocrine basis for breast enlargement has not been formally studied, these girls are reported to respond to tamoxifen.[69]

Female precocious puberty may be thought of as inappropriate oestrogen secretion for age. Complete precocious puberty is due to early activation of the neuroendocrine-gonadal axis; incomplete is due to gonadotrophin–independent oestrogen secretion. Hypothyroidism can cause sexual precocity with the unusual features of growth arrest and galactorrhea. The mechanism may be related to thyrotropin releasing hormone (TRH)-stimulated hyperprolactinaemia sensitizing the ovary to gonadotrophins. In McCune–Albright syndrome (MAS), precocious puberty in combination with café-au-lait spots and polyostotic fibrous dysplasia, the sexual precocity is independent of gonadotrophins – luteinized ovarian cysts secrete oestrogen autonomously. The aromatase excess syndrome has been reported to cause feminization of both sexes.[70] Aberrant oestradiol formation in endometrial stroma has been incriminated in the pathogenesis of endometriosis.[71]

Slowly progressive complete precocious puberty, characterized by mildly accelerated growth with proportionately advanced bone age, is associated with oestradiol levels of approximately 10–20 pg/ml. These girls do not have a reduction in predicted adult height and can be observed without treatment.[72] Oestradiol levels greater than about 20 pg/ml in sexual precocity are associated with reduced height potential, necessitating treatment. If oestradiol levels exceed 75 pg/ml, an oestrogen-secreting tumour should be suspected.

Girls with substantial excess oestrogen require treatment to prevent short stature premature sexual development. In fact, high dose oestrogen is occasionally used to ce final height in girls with tall stature. Central precocity can be treated with GnRH analogues, which shut off the hypothalamic–pituitary–gonadal axis. However, gonadotrophin-independent precocity, such as occurs in MAS, does not respond to GnRH analogues. These girls can be treated with aromatase inhibitors to reduce

peripheral oestrogen production.[73] Alternatively, the SERM tamoxifen has been used in a girl with MAS that was resistant to aromatase inhibitor.[74]

Boys with insufficient oestrogen

Oestrogen contributes to the male growth spurt[45] and to spermatogenesis.[40] Insufficient oestrogen signalling in boys with hypogonadism, oestrogen receptor abnormalities or aromatase deficiency also results in a reduction in final bone mass and failure to close the epiphyses. Boys with aromatase deficiency respond to oestrogen treatment by increasing bone mass and fusing epiphyses. Conversely, aromatase inhibitors have been suggested as treatment for constitutional delay of puberty, since they allow masculinization to proceed without epiphyseal fusion. A placebo-controlled study demonstrated an increase in predicted adult height in boys with constitutional delay of puberty after treatment with testosterone and an aromatase inhibitor.[75]

Boys with oestrogen excess

Oestrogen excess in boys can result from either a frank excess of oestrogen secretion, or a relative excess of oestrogenic over androgenic signalling.[76] Gynaecomastia is the most prominent physical evidence of excess oestrogen. Androgens antagonize oestrogen tissue activity; genetic males with androgen insensitivity are feminized with normal male oestrogen levels. Oestrogen excess results from increased testicular or adrenal oestrogen secretion or from peripheral aromatization. Testicular secretion of oestradiol is stimulated by human chorionic gonadotrophin (hCG) or LH; this is the mechanism of feminization in hCG-secreting tumours such as dysgerminoma and bronchogenic cancer. Oestradiol may be directly secreted by adrenal or sertoli cell tumours.

Feminization also occurs when mildly increased gonadotrophin-driven oestradiol secretion is combined with testosterone deficiency, such as in Klinefelter syndrome, enzymatic defects in testosterone synthesis and secondary testicular failure. Peripheral aromatase is responsible for gynaecomastia in the setting of increased androstenedione levels from adrenal enzyme deficiency or cirrhosis. Medications including oestrogens, gonadotrophins and anti-androgens can cause relative oestrogen excess in developing boys. Some researchers have hypothesized that exposure to environmental oestrogens may be a cause of idiopathic gynaecomastia or decreased sperm count.

Physiological gynaecomastia in pubertal boys usually resolves by late puberty and is not pathological.[76] A significant increase in serum oestradiol and the oestradiol-to-testosterone ratio occurs with the onset of gynoecomastia, but levels remain within the normal range.[77] Anti-estrogen therapy has been reported to be helpful.[78]

SYNTHETIC OESTROGENS, ENVIRONMENTAL OESTROGENS AND PHYTO-OESTROGENS

Exogenous oestrogens with biological activity include synthetic therapeutic oestrogens, environmental oestrogens, and phyto-oestrogens. Synthetic oestrogens, such as diethylstilboestrol (DES) and SERMs, have variable affinity for the OR; DES binds avidly, but most SERMs have a lower affinity. SERMs mediate variable effects in a tissue specific manner depending on the cell context.[79] The chemical structure of the SERM determines the configuration of the OR, resulting in a spectrum of activities from agonist to antagonist, depending on which coregulators are available for recruitment in

the target cell. Raloxifene is an oestradiol agonist in bone but an antagonist in uterus and breast; tamoxifen is oestrogenic in bone and uterus but anti-oestrogenic in breast.

Environmental oestrogens, known as 'endocrine disrupters', are industrial chemicals found in the environment that bind to ORs and can activate oestrogenic pathways in animal models. Environmental oestrogens include organochlorines such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs), plasticizers such as Bisphenol A and pharmaceutical agents that make their way into the environment such as DES, which was used widely to improve meat quality in cattle for decades.[80] A heated public health issue is the relation to endocrine disrupters of a possible increase in cryptorchidism, a testicular cancer and a decline in male fertility due to reduced sperm counts. Exposure to environmental oestrogens may be related, although causality has not been demonstrated.[81,82]

Phyto-oestrogens, or plant oestrogens, are a chemically diverse group of oestrogenic compounds. The most studied are the flavonoids, which include isoflavones such as genistein and flavones such as luteolin. Soybean plants utilize flavonoids to recruit *Rhizobium* bacteria into a symbiotic relationship for nitrogen fixation.[80] ORβ appears to be the major mediator of genistein action.[3] Soy, licorice, red clover, thyme, turmeric, hops and verbena all contain compounds that can bind to ORα and have oestrogenic activity in an in vitro breast cancer bioassay.[83] Little is known about the public health impact of endocrine disrupters or phyto-oestrogens on human puberty.

SUMMARY

Oestrogens are compounds that bind and activate oestrogen receptors. Signalling by oestrogen is complicated and incompletely understood. Oestrogen tissue concentration depends not only on serum levels, but also on tissue specific expression of aromatase and 17β-HSD isoenzymes. Once oestrogen reaches the target cell, the potency and character of the response is determined largely by the cellular context. The presence of coregulator proteins dictates which genes will be regulated and whether transcription will be increased or decreased. Oestrogen also elicits rapid, non-genomic effects by mechanisms that are not yet entirely clear.

In puberty, the best understood oestrogen effects are feminization of developing girls. However, oestrogens are now recognized to have less obvious, but physiologically important, roles in other aspects of male and female puberty. In both boys and girls, oestrogen is required for normal function of the neuroendocrine–gonadal axis and also for skeletal maturation. Oestrogen has direct effects on the gonads in both sexes and may play a role in gender dimorphic CNS development. Adipose tissue, vascular tissue and the pilosebaceous unit also respond to oestrogen signals.

Abnormal puberty can be associated with changes in oestrogen physiology. Girls with insufficient oestrogen can now be treated with physiological oestrogen replacement regimens without compromising final adult height. Aromatase inhibitors or anti-oestrogens are potential treatment modalities for delayed puberty in boys and incomplete precocious puberty in both sexes. The explosion of information in the biological sciences in recent years will open doors for further understanding and treatment of oestrogen-related disorders.

Practice points

- oestrogen effects on growth are biphasic: early pubertal female amounts stimulate growth, larger doses inhibit growth and mediate premature epiphyseal fusion in both boys and girls. Corollaries of this concept are:
- very low doses of oestrogen should be used to initiate hormone replacement in girls if growth is to be optimized
- girls with premature breast development due to minor degrees of oestrogen excess (premature thelarche or slowly progressive precocious puberty) do not require treatment to suppress oestrogen excess
- girls and boys with rapidly progressive precocious puberty require treatment to preserve height potential
- anti-oestrogen or aromatase inhibitor therapy may be indicated to preserve height potential in gonadotrophin-independent precocious puberty in girls or boys

Research agenda

- the development of very low dose oestradiol delivery systems suitable for induction of puberty in short, hypogonadal girls is needed
- clarification of the role of aromatase inhibitors and anti-oestrogens in the management of gynaecomastia and gigantomastia and in the management of constitutional delay of puberty in boys is required
- the development of SERMs that do not inhibit bone growth is needed

Acknowledgements

These studies were supported in part by USPHS grants T32-DK07011 (LCA) and RR-00055 (RLR).

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