

Review Article

*Mechanisms of Disease***PRODUCTION AND ACTIONS
OF ESTROGENS**

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ESTROGENS have widespread biologic actions, and there are naturally occurring phytoestrogens that mimic some of the actions of endogenously produced estrogens. In this review, we will focus on new biochemical and molecular aspects of the action of estrogen, as well as the clinical and physiologic aspects.

**SYNTHESIS, TRANSPORT,
AND METABOLISM OF ESTROGENS****Synthesis of Estrogens**

The naturally occurring estrogens 17 β -estradiol (E₂), estrone (E₁), and estriol (E₃) are C₁₈ steroids derived from cholesterol (Fig. 1). After binding to lipoprotein receptors, cholesterol is taken up by steroidogenic cells, stored, and moved to the sites of steroid synthesis. This intracellular movement is facilitated by the cytoskeleton and by intracellular carrier proteins such as the sterol carrier protein-2.¹ Different steroids are formed by reduction of the number of carbon atoms from 27 to 18. The rate-limiting step in steroid production is the transfer of cholesterol from the cytosol to the inner membrane of the mitochondrion, where the cytochrome P450 enzymes that catalyze the cleavage of the side chain of cholesterol are located. The steroidogenic acute regulatory protein is an indispensable component in this transfer process.² Mutations of this protein result in a severe inability to synthesize steroids and are therefore potentially lethal.³

Aromatization is the last step in estrogen formation. This reaction is catalyzed by the P450 aroma-

tase monooxygenase enzyme complex that is present in the smooth endoplasmic reticulum and functions as a demethylase. In three consecutive hydroxylating reactions, estrone and estradiol are formed from their obligatory precursors androstenedione and testosterone, respectively (Fig. 1). The final hydroxylating step in aromatization does not require enzymatic action and is not product sensitive.

Several plant compounds have structural and functional similarities to estrogens and are therefore referred to as phytoestrogens (Fig. 1). Genistein and daidzein are isoflavonoids found in soybeans and clover. Green tea and various legumes contain the lignans enterolactone and enterodiol. Genistein inhibits steroidogenic enzymes⁴ as well as tyrosine kinase enzymes⁵ and may have antioxidant activity.⁶ Some epidemiologic data suggest that diets rich in phytoestrogens protect against breast cancer, prostate cancer, colon cancer, cardiovascular disease, and osteoporosis.⁷

Endogenous Sources of Estrogens

The primary sources of estradiol in women are the theca and granulosa cells of the ovaries and the luteinized derivatives of these cells. According to the "two-cell" theory of estrogen synthesis, the theca cells secrete androgens that diffuse to the granulosa cells to be aromatized to estrogens.⁸ There is, however, evidence that both of these types of cell may be able to form both androgens and estrogens.⁹ Estrone and estriol are primarily formed in the liver from estradiol.

Aromatase activity has also been detected in muscle,¹⁰ fat,¹¹ nervous tissue,¹² and the Leydig cells of the testes.¹³ During pregnancy, estriol is synthesized in the syncytiotrophoblast¹⁴ by aromatization of 16 α -hydroxyandrostenedione. The latter compound is derived from 16 α -hydroxyepiandrosterone sulfate, which is produced by the fetal liver and desulfated in the placenta. 16 α -Hydroxyepiandrosterone sulfate in turn is derived from dehydroepiandrosterone sulfate produced in the fetal adrenal gland. The combination of the fetal adrenal gland and liver and the placenta has been referred to as the "fetoplacental unit of steroid biosynthesis."

Puberty in girls is initiated by low-amplitude nocturnal pulses of gonadotropin that raise serum estradiol concentrations to 15 to 35 pg per milliliter (55 to 128 pmol per liter).¹⁵ During menstrual cycles, estradiol production varies cyclically, with the highest rates and serum concentrations in the preovulatory

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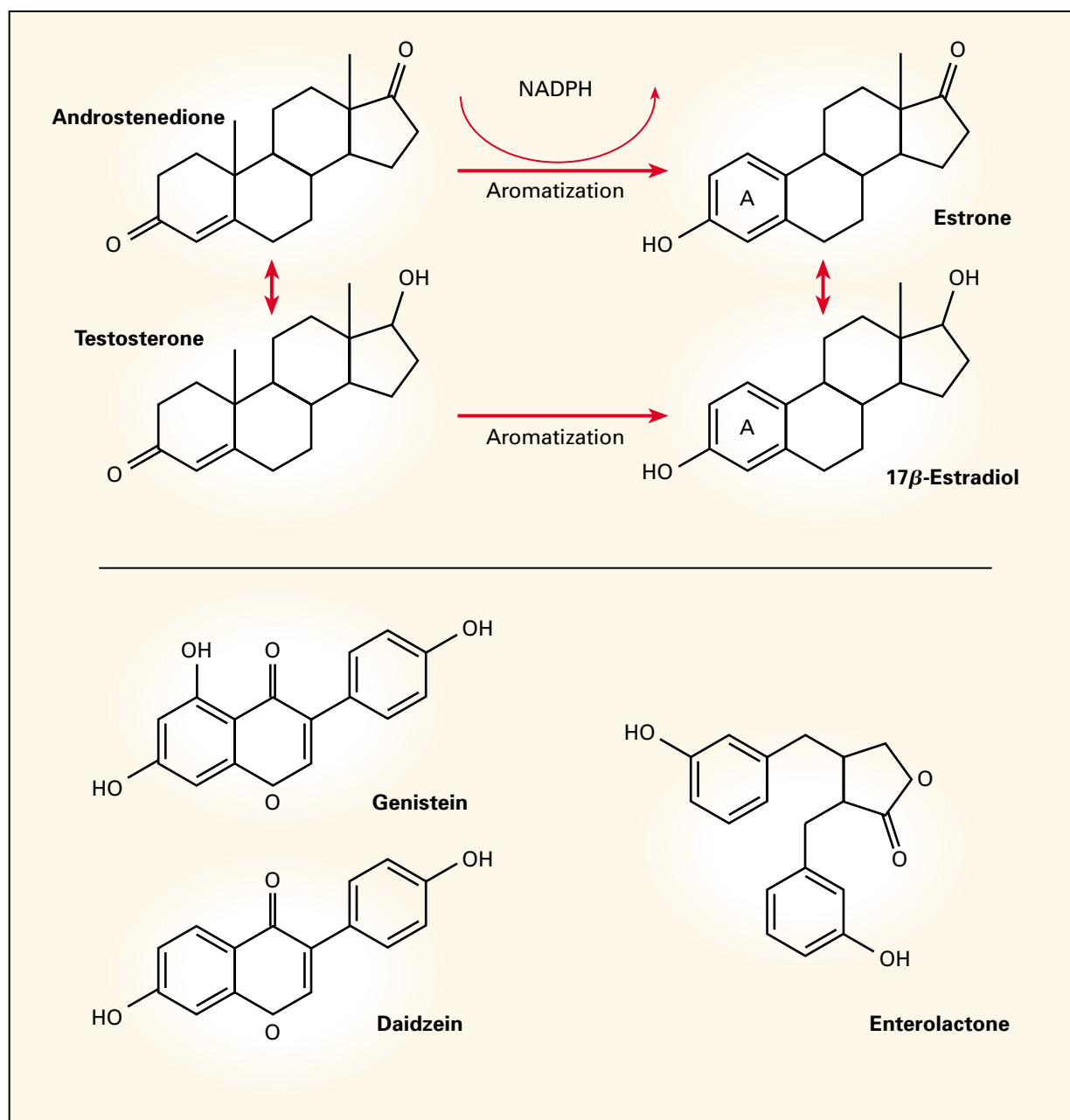


Figure 1. Structure and Production of Endogenous Estrogens and Structure of Phytoestrogens.

Androstenedione and testosterone are the obligatory precursors of estrogens (top panel). The P450 aromatase monooxygenase enzyme complex catalyzes their conversion into estrogens. In this three-step process, three molecules of oxygen and three reducing equivalents from NADPH are used. In the liver, estradiol can be converted into estriol (not shown).

Genistein and daidzein are isoflavonoids, and enterolactone is a lignan (bottom panel). In isoflavonoids, the number and positions of the hydroxyl substituents determine the degree of steric homology with 17 β -estradiol and therefore the binding affinity for the estrogen receptor. The phenyl groups in isoflavonoids and lignans mediate the antioxidant capacity of these compounds.

TABLE 1. PRODUCTION RATES AND SERUM CONCENTRATIONS OF ESTROGENS IN THE MENSTRUAL CYCLE IN NORMAL WOMEN.*

PHASE	17 β -ESTRADIOL		ESTRONE		ESTRIOL	
	SERUM CONCENTRATION	DAILY PRODUCTION	SERUM CONCENTRATION	DAILY PRODUCTION	SERUM CONCENTRATION	DAILY PRODUCTION
	pg/ml	μ g	pg/ml	μ g	pg/ml	μ g
Follicular	40–200	60–150	30–100	50–100	3–11	6–23
Preovulatory	250–500	200–400	50–200	200–350	—	—
Luteal	100–150	150–300	50–115	120–250	6–16	12–30
Premenstrual	40–50	50–70	15–40	30–60	—	—
Postmenopausal	<20	5–25	15–80	30–80	3–11	5–22

*To convert values for serum estradiol to picomoles per liter, multiply by 3.67; to convert values for daily estradiol production to nanomoles, multiply by 3.67; to convert values for serum estrone to picomoles per liter, multiply by 3.70; to convert values for daily estrone production to nanomoles, multiply by 3.70; to convert values for serum estriol to picomoles per liter, multiply by 3.47; and to convert values for daily estriol production to nanomoles, multiply by 3.47. Data are from Baird and Fraser¹⁶ and Flood et al.¹⁷

phase^{16,17} (Table 1). Estradiol production and serum concentrations are lowest premenstrually. In the perimenopausal period, depletion of ovarian follicles leads to a steady decline in ovarian estradiol production, although serum estradiol concentrations vary considerably. In postmenopausal women, serum estradiol concentrations are often lower than 20 pg per milliliter (73 pmol per liter), and most of the estradiol is formed by extragonadal conversion of testosterone. Estrone is the predominant estrogen in these women (Table 1). The level of estrogen synthesis in extragonadal tissues increases as a function of age and body weight.

Little is known about the factors that regulate estrogen production in postmenopausal women, but in the reproductive period control is exerted by the gonadotropins. Genes responsive to follicle-stimulating hormone, for instance, govern the expression of steroidogenic enzymes.¹⁸ This trophic control is modified by paracrine factors. The insulin-like growth factors,¹⁹ for example, facilitate the action of follicle-stimulating hormone in follicular development, and the theca-derived growth factors or androgens reciprocally influence the action of follicle-stimulating hormone on granulosa cells. Androgen-receptor messenger RNA in granulosa cells is down-regulated by follicle-stimulating hormone. This inverse relation is part of the mechanism that determines which follicle will be the dominant estrogen-secreting follicle in a given menstrual cycle.²⁰ In peripheral tissues, the production of different estrogens and their interconversion depend on the local expression and activity of aromatase, 17 β -hydroxysteroid dehydrogenases, and estrone sulfatases. Polymorphisms in the genes coding for steroidogenic enzymes influence estrogen

production. Further evaluation of these polymorphisms with respect to the risk of cancer²¹ or the need for estrogen therapy²² may make possible a more individualized therapeutic approach in postmenopausal women.

Transport and Metabolism of Estrogens

In the serum, estradiol reversibly binds to sex-hormone-binding globulin,²³ a β -globulin, and binds with less affinity to albumin in a nonsaturable and nonstoichiometric manner (Fig. 2); about 2 to 3 percent is free. Estrogens are metabolized by sulfation or glucuronidation, and the conjugates are excreted into the bile or urine. Hydrolysis of these conjugates by the intestinal flora and subsequent reabsorption of the estrogen result in an enterohepatic circulation.

Estrogens are also metabolized by hydroxylation and subsequent methylation to form catechol and methoxylated estrogens.²⁴ Hydroxylation of estrogens yields 2-hydroxyestrogens, 4-hydroxyestrogens, and 16 α -hydroxyestrogens (catechol estrogens), among which 4-hydroxyestrone and 16 α -hydroxyestradiol are considered carcinogenic. Methylation of the 2- and 4-hydroxyestrogens by catechol *O*-methyltransferase yields methoxylated estrogen metabolites.²⁵ Catechol estrogens bind to estrogen receptors and have weak estrogenic activity in animals, and they may inhibit catechol *O*-methyltransferase in the synaptic clefts between neurons. In addition, catechol estrogens are capable of continuous metabolic redox cycling, a process that yields quinone intermediates as metabolites. Because of the formation of free radicals in this process and the covalent binding of these intermediates to DNA, it has been proposed that estrogens have genotoxic activity.²⁶

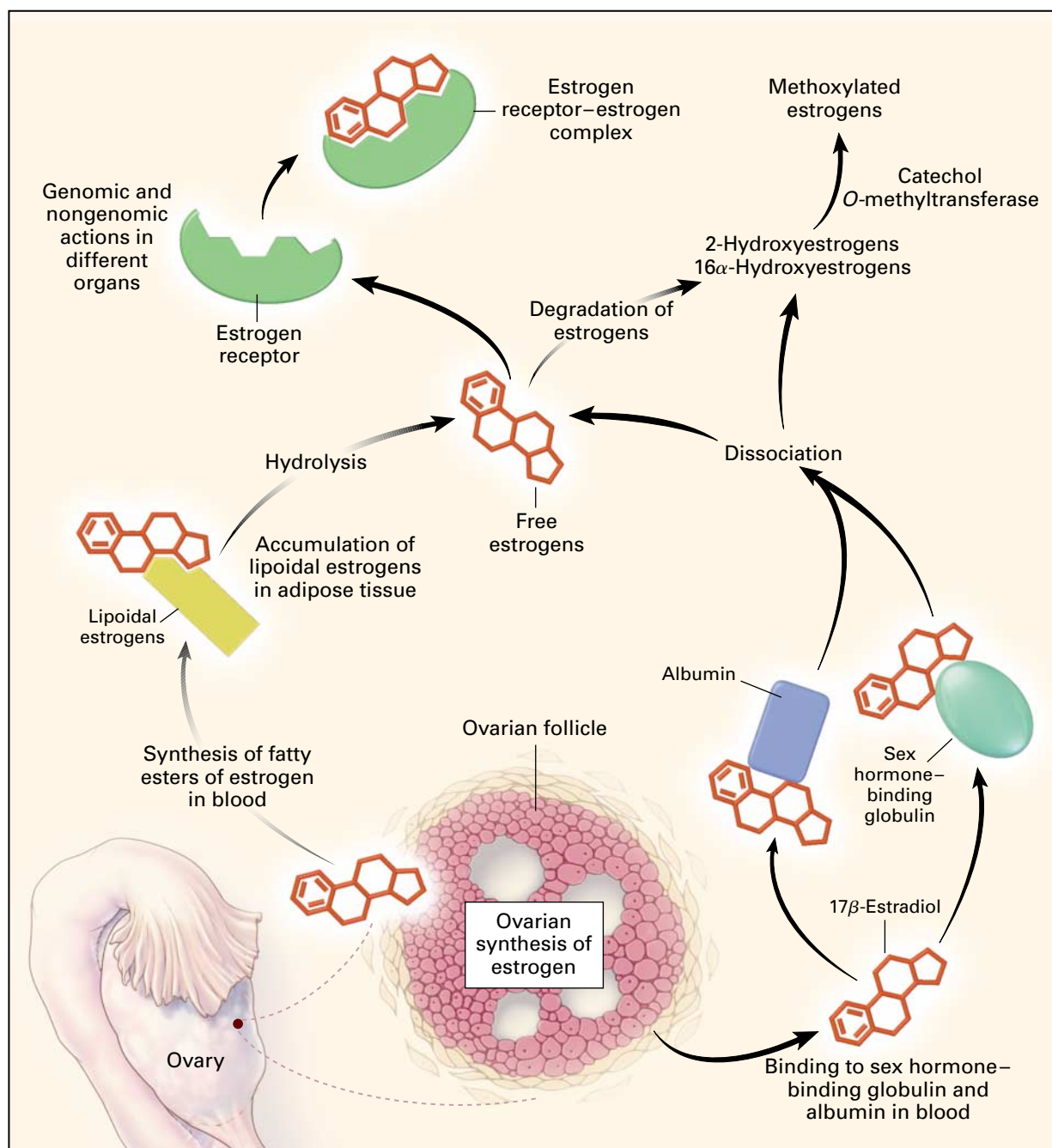


Figure 2. Ovarian Synthesis, Transport, and Metabolism of Estrogens.

After synthesis, mainly in the ovary, 17β -estradiol is secreted into the bloodstream, where it binds to sex-hormone-binding globulin and albumin. Free estrogens diffuse into target tissues to exert their specific genomic or nongenomic effects. Lipoidal estrogens are synthesized in the blood and presumably in other tissues but accumulate predominantly in fat. Enzymatic catabolism of estrogens yields the hydroxyestrogens and methoxyestrogens.

Lipoidal estrogens are fatty esters of estrogens that comprise a separate class of steroid hormones.²⁷ Although they are produced in various tissues *in vitro*, lipoidal estrogens are found predominantly in adipose tissue (Fig. 2). They are synthesized in blood, where they circulate and bind to lipoproteins. Overall, less than 10 percent of serum estradiol is associated with lipoproteins, mainly high-density lipoproteins,²⁸ but serum estradiol can be transferred to low-density lipoproteins by an unknown carrier mechanism. Lipoidal estrogens are more resistant to catabolism than free estrogens and are therefore cleared slowly. After lipoprotein-receptor-mediated uptake by cells and local hydrolysis, they may serve as a potentially relevant steroid reserve. Lipoidal estrogens also lower the concentration of estradiol necessary to inhibit oxidation of low-density lipoproteins *in vitro*.²⁹

MOLECULAR ACTIONS OF ESTROGENS

The specific nuclear actions of estrogens are determined by the structure of the hormone, the subtype or isoform of the estrogen receptor involved, the characteristics of the target gene promoter, and the balance of coactivators and corepressors that modulate the final transcriptional response to the complexes of estrogen and estrogen receptors.

Ligand-Dependent Activation of Estrogen Receptors — The Classic Pathway

The estrogen receptor, unattached to its ligand and loosely bound in its cytoplasmic or nuclear location, is attached to receptor-associated proteins. These proteins serve as chaperones that stabilize the receptor in an unactivated state or mask the DNA-binding domain of the receptor.³⁰ Other receptor-associated proteins may contribute to cross-talk between different signal pathways. The exact location of estrogen and other steroid-hormone receptors is not entirely clear. They are probably in an equilibrium distribution between the cytoplasm and the nucleus; this equilibrium is then shifted after ligand binding. As free estrogen diffuses into the cell, it binds to the ligand-binding domain of the receptor, which disso-

ciates from its cytoplasmic chaperones; the complex of estrogen and estrogen receptor then diffuses into the cell nucleus. These estrogen-estrogen receptor complexes bind to specific sequences of DNA called estrogen-response elements as homodimers or heterodimers.³¹ The estrogen-estrogen receptor complexes bind not only to the response elements but also to nuclear-receptor coactivators or repressors (Fig. 3). The exact mechanism of the nuclear translocation of estrogen-estrogen receptor complexes is not yet fully known, but it is known that the cytosolic protein caveolin-1 stimulates this translocation process through direct interaction with the receptor molecule.³²

Estrogens also regulate the transcription of genes that lack functional estrogen-response elements by modulating the activity of other transcription factors. The binding of the estrogen receptor to subunits of activating protein 1, for example, results in the formation of a transcription factor.³³ Estrogen receptors also interact with the nuclear factor κ B.³⁴

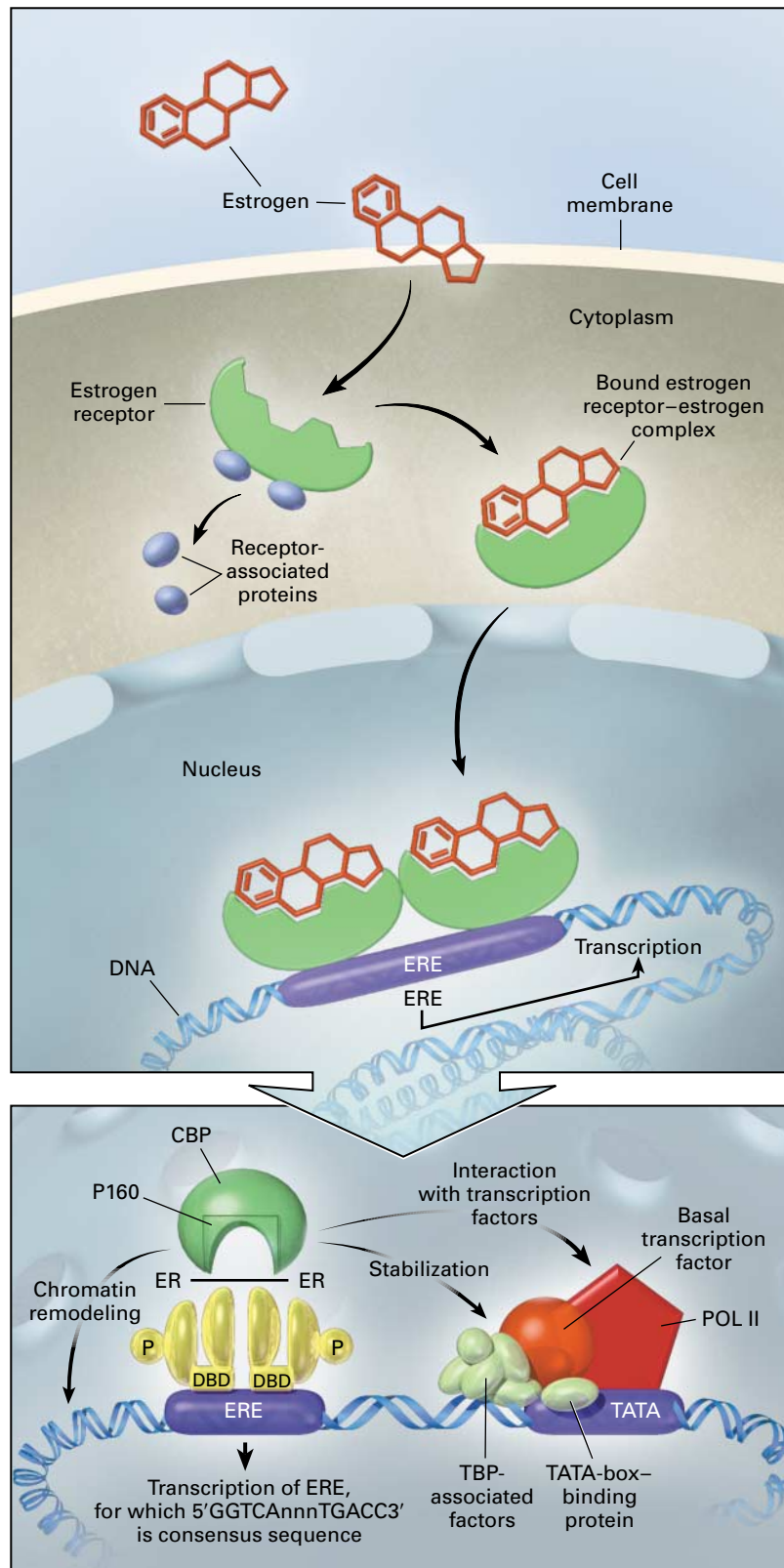
Estrogen Receptors α and β

Estrogen receptors are members of the nuclear hormone-receptor superfamily, which has approximately 150 recognized members. Estrogen receptors have several functional domains. The DNA-binding domain contains two zinc fingers that are involved in receptor binding and dimerization. The ligand-binding domain contains different sets of amino acids that bind to different ligands; this domain also interacts with coregulatory proteins. The N-terminal domain has a high degree of variability and normally contains a transactivation domain that can interact directly with factors of the transcriptional machinery. The C-terminal domain contributes to the transactivation capacity of the receptor.

There are two subtypes of estrogen receptor and several isoforms and splice variants of each subtype. The first subtype, the classic estrogen receptor α ,³⁵ was first cloned in 1986; the second subtype, estrogen receptor β , was discovered more recently.³⁶ These two receptor subtypes vary in structure, and their encoding genes are on different chromosomes. The estro-

Figure 3 (facing page). Classic Pathway of Estrogen Signal Transduction.

When an estrogen molecule binds to an estrogen receptor (ER), the receptor dissociates from its cytoplasmic chaperones, the receptor-associated proteins. The hormone-receptor complex then moves to the nucleus, where it binds to DNA and initiates transcription. Transcription is catalyzed by RNA polymerase II (POL II) and requires the assembly of various proteins, including the TATA-box-binding protein (TBP) and other associated factors at a TATA box. Other transcription factors join thereafter, completing the preinitiation complex. Activated, phosphorylated (P) estrogen receptors interact with several proteins, such as the 160-kD steroid-receptor coactivator protein (P160) and p300-cyclic AMP response-element-binding protein (CBP). This complex binds to the estrogen-response element (ERE) through the DNA-binding domain (DBD) of the receptor and stimulates transcription; proposed mechanisms of stimulation include stabilization of the preinitiation complex, chromatin remodeling, and interaction with other transcription factors.



gen receptor α gene has been mapped to the long arm of chromosome 6, whereas the estrogen receptor β gene is located on band q22–24 of chromosome 14.

Although the DNA-binding domains of estrogen receptors α and β are very similar, the overall degree of homology of the receptors is low. This is particularly true for the ligand-binding domain, of which only 55 percent of the amino acid sequence is shared.³⁷ As a result, some ligands bind to the two receptors with different affinities (Table 2). For example, the short-acting 17 α -estradiol and the biologically weak estrone have a higher affinity for estrogen receptor α , but at least two phytoestrogens, genistein and coumestrol, bind with higher affinity to estrogen receptor β .³⁸ The selective estrogen-receptor modulator raloxifene binds with higher affinity to estrogen receptor α , whereas several environmental pollutants, such as the alkylphenols, have a higher affinity for estrogen receptor β .³⁹

The tissue distributions of estrogen receptor α and estrogen receptor β differ, although there is some overlap. Granulosa cells and developing spermatids contain mostly estrogen receptor β , and this subtype is present in several nonclassic target tissues, including the kidney, intestinal mucosa, lung parenchyma, bone marrow, bone, brain, endothelial cells, and prostate gland.⁴⁰ In contrast, endometrium, breast-cancer cells, and ovarian stroma contain mostly estrogen receptor α .

A man lacking functional estrogen receptors⁴¹ was found to have severe osteoporosis and reduced fertility. Both male and female mice in which the gene for estrogen receptor α is disrupted are infertile⁴²; femoral bone density is slightly decreased in the females and markedly decreased in the males. The protective effects of estrogen after carotid vascular injury were unaltered in estrogen receptor α -knockout mice.⁴³ Female mice in which the gene for estrogen receptor β is knocked out are infertile, and male mice with this defect have prostatic hyperplasia and loss of abdominal fat but are fertile.⁴⁴ In mice in which the genes for both estrogen receptors α and β are knocked out, the females have ovaries that contain seminiferous tubule-like structures that are filled with Sertoli-like cells, and the males are infertile because of a reduction in the number and motility of epididymal sperm — a phenotype similar to that in male estrogen receptor α -knockout mice.⁴⁵

Selective Estrogen-Receptor Modulators

The term “selective estrogen-receptor modulator” was introduced to define nonsteroidal ligands such as tamoxifen that antagonize the action of estrogen in some tissues, such as the breast, and mimic its action in others, such as the uterus. Among postmenopausal

TABLE 2. RELATIVE BINDING AFFINITIES OF DIFFERENT LIGANDS FOR ESTROGEN RECEPTOR α AND ESTROGEN RECEPTOR β .*

LIGAND	ESTROGEN RECEPTOR α	ESTROGEN RECEPTOR β
17 β -Estradiol†	100	100
17 α -Estradiol†	58	11
Estrinol†	14	21
Estrone†	60	37
4-Hydroxyestradiol†	13	7
2-Hydroxyestrone‡	2	0.2
Tamoxifen‡	4	3
Raloxifene‡	69	16
Genistein‡	4	87
Coumestrol‡	20	140
Daidzein‡	0.1	0.5
4-Octylphenol‡	0.02	0.07
Nonylphenol‡	0.05	0.09

*Values range from 0 to 100, with higher values indicating greater binding affinity.

†Data are from Kuiper et al.³⁸

‡Data are from Kuiper et al.³⁹

women, the agonist action of estrogen is desired in bone for the maintenance of density and in the cardiovascular system and brain for the maintenance of function, but not in the breast or endometrium. Raloxifene has estrogen-like effects on bone tissue and on serum lipid concentrations, but not on breast and endometrial tissue.⁴⁶ In the brain, however, raloxifene is more of an estrogen antagonist, because it increases the vasomotor symptoms of estrogen deficiency.

The mechanism of the tissue selectivity of these compounds is complex. On both estrogen receptor α ⁴⁷ and estrogen receptor β ,⁴⁸ the conformation of the ligand-binding domain changes in different ways when estradiol, raloxifene, or genistein binds to it. Consequently, the conformation of a major transactivation domain is altered so that a different surface is exposed to the nuclear-receptor coactivators or repressors. Therefore, the transcriptional effects may vary. For example, tamoxifen and raloxifene serve as transcriptional activators at activating protein-1 sites when they form complexes with estrogen receptor β but suppress transcription when forming complexes with estrogen receptor α . Estradiol activates transcription when bound to estrogen receptor α but exerts opposite transcriptional effects when bound to estrogen receptor β .⁴⁹

Coactivators and Corepressors of Estrogen Receptors

Estrogen receptors interact with several coregulatory proteins that are intercalated between the activated receptor and the transcriptional machinery, as noted previously (Fig. 3). To form a transcription-initiation complex, the assembly of various factors, such as the TATA-box-binding protein and other associated factors at a TATA box, is required by RNA polymerase II. In contrast, nuclear-receptor coregulatory proteins interact with the receptor molecule to modulate its transcriptional capacity. These oligomeric complexes, which consist of estrogen receptors, transcription factors, and coactivator or corepressor proteins, are believed to provide stability and transcriptional specificity.

The 160-kD steroid-receptor coactivator protein⁵⁰ and the p300-cyclic AMP response-element-binding proteins⁵¹ appear to have the greatest capacity to increase the transcriptional activity of estrogen receptors. In contrast, corepressors suppress transcription after being tethered to a promoter by a DNA-bound receptor. The protein that acts as a corepressor of estrogen-receptor activity is a member of the latter class.⁵² This protein directly interacts with the agonist- or antagonist-occupied receptor but also competes with steroid-receptor coactivator proteins. This is an example of how the balance of corepressors and coactivators influences the transcriptional activity of activated estrogen receptors. It therefore seems likely that part of estrogen-receptor antagonism is a result of the recruitment of corepressors.⁵³

Estrogen-Response Elements and Estrogen-Response Units

The estrogen receptor is a transcription factor that, after being activated, establishes a direct nuclear interaction by binding to the estrogen-response elements of DNA, which confer estrogen inducibility on the gene. Estrogen-response elements are present in the regulatory regions of estrogen target genes (Fig. 3).

The sequence 5'GGTCAnnnTGACC3' from the *Xenopus laevis* vitellogenin gene (n denotes a random nucleotide) has been defined as the consensus estrogen-response element sequence.⁵⁴ It is a 13-bp inverted repeat with a spacing of three variable bases. However, only a small number of the most estrogen-inducible genes contain these consensus estrogen-response elements. In most cases, variant estrogen-response elements have been described. In the murine Bcl-x_i gene promoter, for instance, the sequence 5'GGT-CAnnnTGGCC3', which differs from the consensus sequence by 1 bp, mediates the inducibility of estrogen.⁵⁵ These variant sequences bind estrogen receptors with less affinity, depending on the flanking bases.⁵⁶

Furthermore, variant estrogen-response elements or even partial estrogen-response elements, often separated by many base pairs, can act in combination to confer estrogen responsiveness. These combinations are referred to as estrogen-response units. In the gene for human transforming growth factor α , for instance, the estrogen-responsive sequence is a 5'GGTCAnnnnTGCCC3' element that is separated by 20 bp from a 5'GGTGAnnnTAGCC3' element.⁵⁷

Alternative Pathways

Ligand-Independent Activation of Estrogen Receptors

Most nuclear receptors are phosphoproteins, and their function can be altered by changes in their phosphorylation in the absence of a hormonal ligand.⁵⁸ The activators of protein kinases, such as growth factors, can elicit estrogen-independent activation of the receptor molecule by inducing phosphorylation of the receptor at sites that differ according to the identity of the activator (Fig. 4). Phosphorylation occurs predominantly at specific serine or tyrosine residues and is catalyzed by enzymes such as receptor tyrosine kinase and mitogen-activated protein kinases.⁵⁹ Mitogen-activated protein kinases are composed of several serine-threonine kinases that are activated in response to various cell-growth signals and transduce extracellular signals to intracellular targets by way of membrane receptors.

In vitro results have proved the existence of such cross-talk between signal pathways in the case of the activation of estrogen-independent receptors by dopamine,⁶⁰ epidermal growth factor, transforming growth factor α ,⁶¹ insulin or insulin-like growth factor-1,⁶² heregulin,⁶³ and cyclic AMP.⁶⁴ Epidermal growth factor consistently induced markers of estrogenic action in vivo, such as the augmentation of progesterone messenger RNA transcripts, whereas it failed to have similar effects in estrogen-receptor-knockout mice.⁶⁵

Nonnuclear Actions of Estrogens

The traditional estrogen-signaling pathway involving nuclear interaction takes minutes or hours to increase protein synthesis by transcriptional activation. Estrogens have other effects that cannot be explained by a transcriptional mechanism because of their rapid onset. These effects are the result of direct estrogenic action on cell membranes and are mediated by cell-surface forms of estrogen receptor (Fig. 4). Although these receptors remain largely uncharacterized, they are thought to resemble their intracellular counterparts.⁶⁶ Examples of effects mediated by this alternative pathway are the short-term vasodilation of coronary arteries,⁶⁷ the rapid insulinotropic effect of estradiol on pancreatic beta cells,⁶⁸ and the rapid activation of growth-factor-related signaling pathways in neuronal cells.⁶⁹

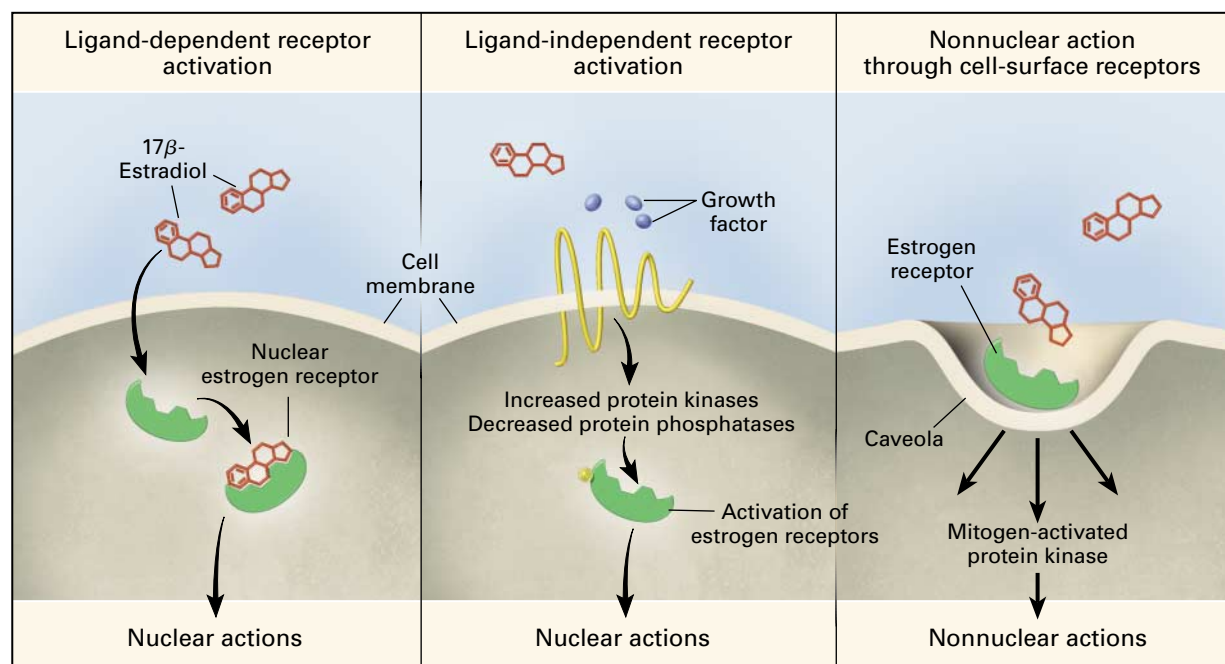


Figure 4. Ligand-Dependent and Ligand-Independent Estrogen-Receptor Activation.

The estrogen receptor can be activated by estrogen (left-hand panel) or independently of estrogen — for example, by growth factors that increase the activity of protein kinases that phosphorylate different sites on the receptor molecule. In this model (center panel), the unbound but activated receptor will then exert transcriptional effects. In the case of the nonnuclear estrogen-signaling pathway (right-hand panel), cell-membrane estrogen receptors are located in cell-membrane invaginations called caveolae. Their activity is linked to the mitogen-activated protein kinase pathway, resulting in a rapid, nonnuclear effect.

There is a direct link between estrogen cell-surface receptors and the mitogen-activated protein kinase signaling cascade (Fig. 4).⁷⁰ Coupling of the bound membrane estrogen receptor to the mitogen-activated protein kinase pathway has been demonstrated in osteoblasts, endothelial cells, neurons, and human breast-cancer cells.⁷¹

PHYSIOLOGIC ACTIONS OF ESTROGENS

Estrogens stimulate growth, blood flow, and water retention in sexual organs and are also involved in causing breast cancer and endometrial cancer. In the liver, estrogens increase lipoprotein receptors, resulting in a decrease in serum concentrations of low-density lipoprotein cholesterol.⁷² On the other hand, estrogens increase the potential for coagulation. In the gastrointestinal tract, estrogens may protect against colon cancer.⁷³ In aging skin, estrogens increase turgor and collagen production and reduce the depth of wrinkles⁷⁴ (Fig. 5).

Actions on Breast Tissue

The lobular units of the terminal ducts of the breast tissue of young women are highly responsive

to estrogen. In breast tissue, estrogens stimulate the growth and differentiation of the ductal epithelium, induce mitotic activity of ductal cylindric cells, and stimulate the growth of connective tissue.⁷⁶ Estrogens also exert histamine-like effects on the microcirculation of the breast. The density of estrogen receptors in breast tissue is highest in the follicular phase of the menstrual cycle and falls after ovulation.⁷⁷ Estrogens stimulate the growth of breast-cancer cells. In postmenopausal women with breast cancer, the tumor concentration of estradiol is high, because of *in situ* aromatization, despite the presence of low serum estradiol concentrations.⁷⁸

Actions on the Central Nervous System

The brain aromatization hypothesis proposes that sexual differentiation in the brain — that is, the ability of estrogen to cause a surge of gonadotropin secretion in women — is dependent on local conversion of androgens to estrogens.⁷⁹ The rate of aromatization of androgen to estrogen in the brain is low as compared with that in other tissues, but nevertheless, local estrogen production is believed to have important actions. One example of this is the synergistic

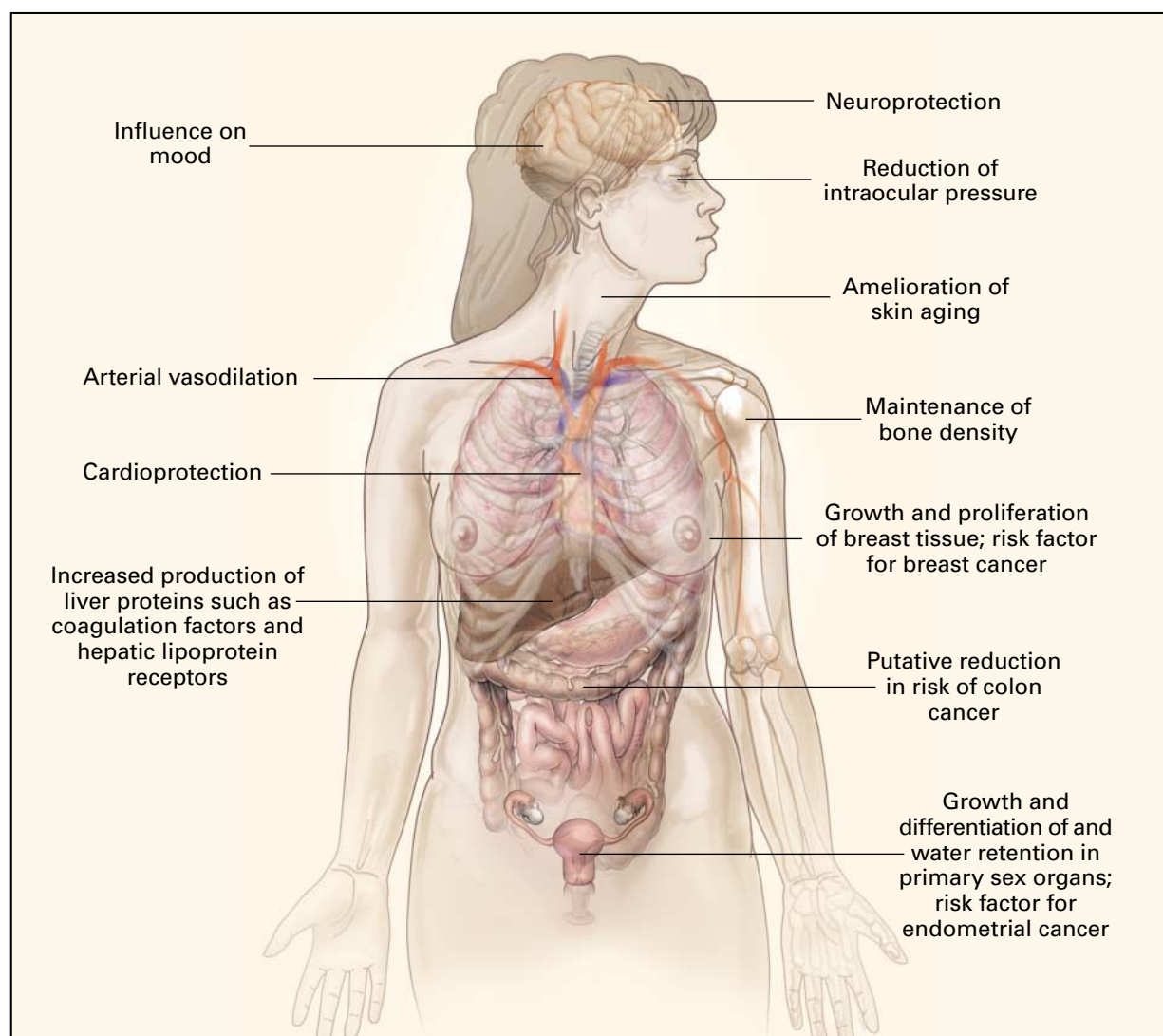


Figure 5. Effects of Estrogens in Different Organ Systems.

Estrogens have neuroprotective effects and reduce perimenopausal mood fluctuations in women. In the eye, estrogens lower intraocular pressure.⁷⁶ Estrogens are arterial vasodilators and may have cardioprotective actions. In the liver, estrogens stimulate the uptake of serum lipoproteins as well as the production of coagulation factors. Estrogens also prevent and reverse osteoporosis and increase cell viability in various tissues. In addition, estrogens stimulate the growth of endometrial and breast tumors. Estrogens may protect against colon cancer, since colon cancer appears to be less likely to develop in postmenopausal women who are receiving estrogen-replacement therapy than in women who are not receiving this therapy. When applied topically, estrogens increase skin turgor and collagen production and reduce the depth of wrinkles.

action of estrogens with neurotrophins that is reflected in reciprocal receptor regulation or coupled signaling pathways.⁸⁰

In later life, estrogens are thought to have neuroprotective actions. In brain tissue from adult rats, estrogens induce synaptic and dendritic remodeling⁸¹ and cause glial activation.⁸² In neurons of the hip-

pocampus, an area involved in memory, estrogens increase the density of *N*-methyl-D-aspartate receptors and increase neuronal sensitivity to input mediated by these receptors.⁸³

In cultured human neuroblastoma cells, estrogens have neuroprotective effects⁸⁴ and reduce the generation of beta-amyloid peptides.⁸⁵ Some epidemio-

logic data suggest that in postmenopausal women, estrogen deficiency is associated with a decline in cognitive function and an increased risk of Alzheimer's disease.⁸⁶ However, in a randomized trial, estrogen administration had no beneficial effect in women with established Alzheimer's disease.⁸⁷

Vascular Effects

Estrogens are thought to be natural vasoprotective agents. Estrogen receptors have been detected in smooth-muscle cells of coronary arteries⁸⁸ and endothelial cells in various sites.⁸⁹ Estrogens cause short-term vasodilation by increasing the formation and release of nitric oxide and prostacyclin in endothelial cells.⁶⁷ They also reduce vascular smooth-muscle tone by opening specific calcium channels through a mechanism that is dependent on cyclic guanosine monophosphate.⁹⁰ A protective role of estrogens against atherosclerosis is suggested by the finding that estrogen treatment reduced the progression of coronary-artery atherosclerosis in oophorectomized monkeys. There was, however, no effect on preexisting plaques.⁹¹ On the cellular level, estrogens inhibit apoptosis of endothelial cells⁹² and promote their angiogenic activity in vitro.⁹³

Despite these findings, one of the key questions in women's health — whether estrogen treatment in the postmenopausal period prevents atherosclerosis — remains controversial.⁹⁴ The favorable findings of epidemiologic studies have to be balanced by the lack of benefit of estrogen for secondary protection against cardiovascular disease in the Heart and Estrogen/Progestin Replacement Study.⁹⁵

Effects on Bone

Both osteoclasts⁹⁶ and osteoblasts⁹⁷ express estrogen receptors and are direct targets for estrogens, but overall, estrogens are classified as antiresorptive agents. Estrogens directly inhibit the function of osteoclasts. In oophorectomized mice, estrogen deficiency increased the production of interleukin-6, interleukin-1, and tumor necrosis factor in osteoblasts and other bone-derived stromal cells. These factors indirectly stimulate osteoclast differentiation.⁹⁸ In bone extracts from postmenopausal women with osteoporosis, the concentrations of interleukin-6 and interleukin-1 mRNA were also high.⁹⁹ Estrogen deficiency is known to accelerate bone loss and increase susceptibility to fractures. Estrogen therapy diminishes bone loss¹⁰⁰ and reduces the risk of fracture in women with osteoporosis and in those without this condition for the duration of therapy.^{101,102}

CONCLUSIONS

It is now clear that estrogens act by multiple mechanisms in many different tissues. Agents with estrogenic activity in a limited number of estrogen-target

tissues are already available. In the future, it should be possible to target particular actions of estrogen with increasing specificity and therefore with more benefits and fewer unwanted effects.

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