

# Estrogen Actions in the Brain

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There is no debate over whether estrogen has functions in the brain. It is clear that estrogen affects the developing brain and centrally controlled reproductive functions, but there is much more to it than that. Estrogen also has effects in the brain that are not related to reproductive functions. Estrogen in the brain influences the cardiovascular system, mood, and behavior, as well as neuroprotection and neurodegeneration. But the mechanisms through which estrogen exerts its actions in these systems are less well understood. One recent development, which has contributed to a broader understanding of the role of estrogens in the brain, is the discovery of the second estrogen receptor, ER $\beta$ . Awareness of this receptor has changed our understanding of estrogen action in brain regions that are responsive to estrogen but lack estrogen receptor  $\alpha$  (ER $\alpha$ ). Regions such as the serotonergic neurons of the dorsal raphe nucleus, the GnRH neurons of the hypothalamus, and the sexually dimorphic supraoptic nucleus and paraventricular nucleus are being re-evaluated because of the exclusive presence of ER $\beta$  in these regions.

Important advances in our understanding of estrogen action in the brain have also come from the creation of knockout mice in which ER $\alpha$ , ER $\beta$ , or both receptors have been inactivated. These animals provide evidence of distinct roles for the two estrogen receptors in the brain. ER $\beta$ , in particular, has a role in development of the cortex during fetal life and in survival of hippocampal neurons in response to excitatory neurotoxins in adults. Despite this progress in the laboratory, debate is ongoing over the clinical benefits (or lack thereof) of hormone replacement therapy (HRT) in postmenopausal women. Because women react to estrogen deficiency in different ways, replacement with estradiol may, in fact, not be the optimal HRT. As a prospect in the future, we may look forward to replacement with selective ER $\alpha$  and ER $\beta$  agonists in protocols that are tailor made to suit the needs of individual women.

Estrogen influences development, plasticity, and survival of neurons (1-3). It is also an important regulator of expression of key enzymes involved in synthesis and turnover of classic neurotransmitters such as noradrenaline (4), dopamine (5), serotonin (6), and acetylcholine (7), and of transcription of several neuropeptides such as oxytocin, vasopressin, prolactin, and opioids (8-10). What now needs to be clearly defined is which estrogen receptor is regulating neuronal functions in which brain nuclei, and whether estrogen receptors can be selectively activated or inhibited to obtain more specific beneficial actions of estradiol. Through the use of in situ hybridization, the distribution of ER $\alpha$  and ER $\beta$  messenger RNAs (mRNAs) has been evaluated in the rodent brain [for review, see (11)]. Neurons expressing ER $\beta$  mRNA are more widely distributed throughout the brain than those containing ER $\alpha$ . Both receptors are expressed in the preoptic area and the bed nucleus of the stria terminalis, and throughout the lower brain stem, as well as in forebrain areas such as the cerebral cortex and hippocampus (12, 13). A comparison of the distribution of ER $\beta$  mRNA in rat and mouse brain (14) revealed that there are differences among species in the distribution

of ER $\beta$ . For example, in the mouse brain, ER $\beta$  mRNA is abundant in the suprachiasmatic nucleus, raphe nucleus, and entorhinal cortex, but is less abundant in these areas in the rat brain. In contrast, amounts of ER $\beta$  mRNA in the supraoptic nucleus and preoptic area are high in the rat and markedly lower in the mouse.

Immunohistochemical studies in rodent (15), monkey (16), and human (17, 18) brains have revealed neuronal populations that express both receptors and some that express only one of the two receptors. Neurons of the supraoptic nucleus, a sexually dimorphic nucleus that is larger in males than females, express ER $\beta$  but not ER $\alpha$ . In neurons in the anteroventral periventricular nucleus of the preoptic area (AVPV), which is larger in females than males, ER $\alpha$  is present but ER $\beta$  is not. From the distribution of the two estrogen receptors, it appears that because ER $\alpha$  is the predominant receptor subtype in the basal forebrain cholinergic neurons (19), estrogen probably acts through ER $\alpha$  to enhance cognitive functions by modulating production of acetylcholine. In the anterior dorsal raphe nucleus, ER $\beta$  is the only estrogen receptor expressed (20, 21), and it appears that estrogen increases the amount of mRNA encoding the serotonin receptor (5-HT 2A), tryptophan hydroxylase, and the serotonin transporter (22) through ER $\beta$ .

In the periphery, estrogen has well-documented effects on cytokines, activity of the transcription factor nuclear factor kappa B (NF- $\kappa$ B), growth, differentiation, and apoptosis. Estrogen receptors are ligand-activated transcription factors, so estrogen regulates transcription of many genes (23). If it also regulates cytokines, NF- $\kappa$ B activity, and apoptosis in the central nervous system (CNS), and modulates glutamate pathways, then it should affect neuronal survival, neurodegeneration, and the response of the CNS to ischemia and excitatory neurotoxins. If genes involved in synthesis, uptake, and degradation of catecholamines and serotonin are estrogen-regulated, then estrogen might affect mood and behavior. Why, then, is it so difficult to get clear-cut answers about the effects of estrogen depletion or estrogen replacement in the brain?

One of the reasons, as alluded to above, is the presence of two separate estrogen receptors with distinct cellular distributions and functions. As the physiological importance of ER $\beta$  becomes clearer (23), it also becomes evident that estrogen signaling is much more complex than was previously thought. Before the discovery of ER $\beta$  (24), it was thought that most actions of estrogen could be explained by the binding of estrogen to a single estrogen receptor (25). This specific binding resulted in an activated receptor that then could bind to estrogen response elements (ERE) in the promoters of target genes and thus regulate transcription of these genes. It is now known that there are two estrogen receptors and that interaction at EREs is only one of several ways through which gene transcription is controlled by estrogen [for review, see (22)]. Estrogen receptors can interact with several transcription factors and influence their interaction with DNA. However, ER $\alpha$  and ER $\beta$  can have opposite effects on some of these pathways (26). In addition, estrogen receptors are not exclusively nuclear. In the CNS, these receptors have been localized at synaptic membranes and dendritic spines (27). This localization may explain some of the rapid effects of estrogens that are unlikely to result from effects on transcription (3).

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There are two other confounding factors in the estrogen story. One is that there are at least two splice variants of ER $\beta$  in humans (28), ER $\beta$ 1 and ER $\beta$ cx. ER $\beta$ 1 is the estradiol-binding form of the receptor with affinity for estradiol similar to that of ER $\alpha$ . ER $\beta$ cx is a splice variant formed by incorporation of an alternative eighth exon, and it has no specific affinity for estradiol. When ER $\alpha$  and ER $\beta$ cx are co-expressed in cells, ER $\beta$ cx is, in fact, a dominant repressor of ER $\alpha$ . The second confounding factor is the specificity of ER $\alpha$  and ER $\beta$  for their interactions with co-activator proteins in the transcription machinery (29). Clearly, the mere presence of an estrogen receptor in a cell does not allow one to predict what effects estrogen will have in that cell. This information has to be complemented with information about splice variants of the receptor, their localization in the cell, and which co-activators and co-repressors are expressed in the cell.

Comparison of gene expression in *er $\beta$ <sup>-/-</sup>* and wild-type littermates on cDNA arrays confirmed that estrogen regulates the transcription of cytoskeletal and other genes that are required for neurite growth (30-33). In addition, genes that function in neuronal migration and axonal guidance, such as semaphorin G, syndecan 3, and reelin, are expressed in smaller amounts in *er $\beta$ <sup>-/-</sup>* than wild-type mice. Therefore, it appears that ER $\beta$  influences migration of neurons during development and neuronal survival throughout life.

Estrogen regulates expression of several growth factors and their receptors and, in turn, these peptides can modify the activity of estrogen receptors. Thus, nerve growth factor (NGF) regulates estrogen receptor action in the forebrain posttranslationally, possibly by the phosphorylation of regulatory domains in the receptor protein (34). Epidermal growth factor (EGF) can activate the unliganded estrogen receptor by mitogen-activated protein kinase (MAPK)-dependent phosphorylation (35). This participation of estrogen receptors in signal transduction pathways in the absence of ligands is a confounding factor for neuroendocrinologists struggling to understand estrogen signaling in the brain.

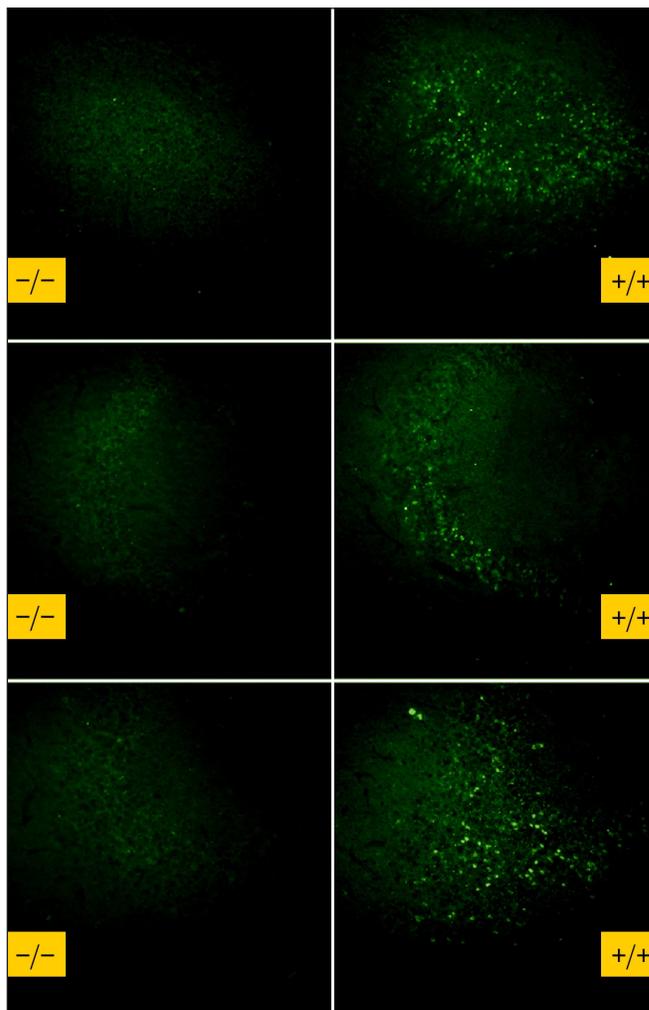
Estrogen enhances the growth and arborization of axons and dendrites in hypothalamic neurons grown in organotypic cultures (36), and also has growth-promoting effects in other brain areas (37-40). During neural development, estrogen also influences neurogenesis (41), apoptotic cell death (42), and migration of neuroblasts from the subventricular layer to their appropriate locations in the brain (43). When neural circuits are established, estrogen promotes synapse formation (44).

Studies with the brains of *er $\beta$ <sup>-/-</sup>* mice showed that in both males and females, there is a generalized neuronal hypocellularity, or decreased number of neurons, that causes the most severe deficit in the somatosensory cortex. In the limbic system, but not in the cortex, *er $\beta$ <sup>-/-</sup>* mice show increased proliferation of astroglia. As *er $\beta$ <sup>-/-</sup>* mice age, the number of neurons in the brain continues to decrease, and by two years of age, neuronal cell bodies throughout the brain have degenerated (45). This is particularly evident in the substantia nigra, a region where loss of dopaminergic neurons leads to Parkinson's disease in humans.

Estrogen influences cognition in both laboratory animals and women. Memory impairment can be reversed by estrogen administration in aging women (46, 47), in women following natural or surgically induced menopause, and in women suffering from dementia associated with Alzheimer's disease. The usefulness of estrogen in protection against degenerative diseases of aging in women is highly debated. Some (46, 48), but not all (49, 50), epidemiological studies suggest that estrogen replacement therapy decreases the likelihood of developing Alzheimer's disease in postmenopausal women. It is not clear that estrogen replacement is of value in reversing,

slowing, or preventing neurodegeneration.

Estrogen exerts its effects on cognition by augmenting the activity of the *N*-methyl-D-aspartate (NMDA) glutamate receptors in the hippocampus (51, 52). Stimulation of the NMDA receptor causes a rise in the concentration of intracellular calcium ([Ca<sup>2+</sup>]<sub>i</sub>), which induces long-term potentiation and storage of information (53). Unfortunately, the drawback of this effect is that overstimulation of these receptors, for example, with the excitatory neurotransmitter kainic acid, causes prolonged increase in [Ca<sup>2+</sup>]<sub>i</sub> followed by neuronal death (54, 55). The role of the two estrogen receptors in the functions of the hippocampus still need clarification, but studies on *er $\alpha$ <sup>-/-</sup>* and *er $\beta$ <sup>-/-</sup>* mice suggest that both ER $\alpha$  and ER $\beta$  are needed for learning. In the absence of ER $\beta$ , spatial learning is impaired (56) and treatment with kainic acid causes marked apoptosis in the hippocampus at doses that do not affect wild-type littermates (Fig. 1). In the absence



**Fig. 1.** Increased sensitivity of the brains of *er $\beta$ <sup>-/-</sup>* mice to kainic acid. Kainic acid dissolved in saline was administered by intraperitoneal injection to 2-month-old *er $\beta$ <sup>-/-</sup>* mice and their wild-type littermates. The single dose of kainic acid used (10 mg/kg/day) did not cause severe seizures as do higher doses of this drug. Four days after dosing, brains were removed and examined by immunohistochemistry for apoptosis with the terminal-deoxynucleotidyl-transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay kit (Boehringer Mannheim).

of ER $\alpha$ , estradiol either completely blocks or delays learning acquisition in the spatial water maze task (57). Not only do both estrogen receptors appear necessary for learning, but ER $\beta$  protects cells against calcium-induced apoptosis. Although estrogen is only one of many factors influencing the complex pathways involved in memory and apoptosis in the brain, the overall action of estrogen on these two endpoints may result from a balance between the activities of ER $\alpha$  and ER $\beta$ .

There is a firm scientific basis for the reported beneficial effects of estrogen in the CNS. But because of the complex nature of estrogen signaling in the brain, it is not surprising that epidemiological data on the benefits of HRT deliver a mixed message. A great deal of work is needed to fully understand estrogen action in the brain. Central to this effort must be clarification of the roles of the two estrogen receptors ER $\alpha$  and ER $\beta$  in regulating neurotransmitter systems, brain cytokines, oxidative stress,  $\beta$ -amyloid deposition, apoptosis, glial cell function, and neuronal imprinting. The natural hormone estradiol is a rather nonspecific drug, so development of selective ligands for ER $\alpha$  and ER $\beta$  will permit a sharper dissection of the functions of the two receptors and provide insight into when agonists or antagonists of either receptor would provide the optimal therapeutic intervention.

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