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Aging of Brain: Role of Estrogen

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Abstract The brain undergoes many structural and functional changes during aging. Some of these changes are regulated by estrogens which act mainly through their intracellular receptors, estrogen receptor ERα and ERβ. The expression of these receptors is regulated by several factors including their own ligand estrogen, and others such as growth hormone and thyroid hormone. The levels of these factors decrease during aging which in turn influence estrogen signaling leading to alterations in brain functions. In the present paper, we review the effects of aging on brain structure and function, and estrogen action and signaling during brain aging. The findings suggest key role of estrogen in the maintenance of brain functions during aging.

Keywords Estrogen · Aging · Estrogen receptorα · Estrogen receptorβ · Brain

Introduction

The brain undergoes many biochemical and structural changes involving both functional reorganization and compensation during aging. Majority of these changes are regulated by estrogen which is derived from either circulation or steroidogenesis in the brain [1–4]. Estrogen acts through nongenomic as well as genomic pathways. Whereas the nongenomic pathway is not well understood, the genomic pathway is mediated by intracellular receptors, estrogen receptor ERα and ERβ. The level of ERα and ERβ is influenced by a number of factors including age, cell density, growth hormone, thyroid hormone and gonadal steroids [5]. Consequently, the estrogen-mediated functions change in the brain leading to different diseases. Experimental studies using animal models and cell culture suggest that these diseases can be delayed or prevented if estrogen action is maintained. This article focuses on recent research findings in four areas—aging of brain, diverse actions of estrogen in the brain, effect of aging on estrogen signaling in brain and effect of estrogen during aging of brain.

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Brain aging

Structural changes

The most striking feature of aging brain is its shrinkage, weight loss and expansion of the ventricular volume. However, the age-related shrinkage of brain shows regional specificity [6]. The major factor responsible for age-dependent brain shrinkage is loss of white matter, which occurs due to damage of myelinated fibers and is closely correlated with the age-associated cognitive decline [7]. Other age-associated changes in the brain include increase in the number of microglia and astrocytes [8], reduction in dendritic arbors and dendritic spines of cortical pyramidal neurons [9–11]. Changes in dendrites include both shortening and fewer dendritic branches. Hippocampal circuits are also vulnerable to degeneration during normal aging and Alzheimer's disease (AD), though such effects show species specificity. Analysis of synapses in old rats

shows age-dependent reduction, while similar analyses in humans and monkeys indicate no loss of synapses in the hippocampus [12, 13].

Another inevitable consequence of brain aging includes neuronal death in neocortex and hippocampus, though the extent of neuronal loss during aging is much debated. The idea of significant neuronal loss during normal aging of human cortex evolved after examining the cortices of subjects between 18 and 95 years of age [14]. Data obtained from this and other similar type of studies suggested that most of the neocortical areas and hippocampal subfields lose 25–50% of their resident neurons in old age. However, this view has been modified after the development of relatively more accurate procedures for counting neurons [15, 16]. Careful applications of stereological techniques to several species including humans have led to the conclusion that the old brain shows no evidence of neuronal loss in the major areas of entorhinal cortex and CA1 region of hippocampus, which are involved in memory function. However, some age-related neuronal loss occurs in the hilus of dentate gyrus and subiculum [15].

Other structural changes occurring in the old brain include intracellular deposition of lipofuscin pigment (made up of peroxidized proteins and lipids), formation of neurofibrillary tangles, senile plaques, neuropil threads, granulovacuolar degeneration, hirano bodies and infarcts. However, except the lipofuscin pigment, all other deposits are considered as the hallmark of AD, though they also exist at lower density in the normal old brain [17].

Neurochemical changes

There are several evidences suggesting that neurotransmitter systems are affected differentially by aging. The most consistent age-related change in the neurochemical system is the loss of glutamate receptors. A significant decrease in the mRNA level of glutamate receptor is reported in the rat cerebral cortex [18]. Among different glutamate receptors, *N*-methyl-D-aspartate (NMDA) receptor levels change in the prefrontal cortex of aged macaque monkeys and rats [19, 20]. In addition, the expression levels of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid and NMDA receptor subunit proteins, GluR2 and NR1 decrease significantly in neurons, suggesting corticocortical links between temporal and frontal cortices in aged monkeys. In vitro experiments also demonstrate an age-dependent decrease of glutamate receptor-dependent synaptic activation in prefrontal cortex layer 2/3 of pyramidal cells of the aged monkey [21]. However, so far there is no conclusive evidence for the age-dependent alteration in kainate (a glutamate receptor) and γ-amino butyric acid (GABA) receptors [6].

Besides different receptors, the levels of neurochemicals, their metabolites and presynaptic markers also show age-dependent changes. For example, the levels of metabolites of acetylcholine, dopamine (DA) and noradrenaline (NA) are reduced in the cerebral cortex of aged rats and monkeys [22]. The level of GABA also decreases in the old rat brain. In the rat hippocampus, the expression of presynaptic protein synaptophysin declines with age, and the degree of decline correlates with deficits in spatial memory [23]. The cholinergic and monoaminergic systems projecting from the basal forebrain and brainstem also show certain degree of functional impairment during aging [24]. Thus, age-related neurochemical alterations display region specificity that affects brain functions in old age.

Functional changes

Several age-dependent studies show impairments in gait control, sleeping cycle, learning and memory. However, Burke and Mackay [25] described the memory impairment with advancing age as a selective deficit rather than a general decline in all cognitive functions. The memory capabilities that depend on the hippocampal function (spatial memory) are particularly vulnerable with increasing age. The application of functional magnetic resonance imaging technique in aged humans demonstrated a decrease in the cortical activity [26]. Studies on spontaneous activities of cortical neurons indicate a reduced firing rate in old age [6]. However, the lack of age-related changes in the spontaneous neuronal firing rate in some areas of the hippocampus suggests that the loss of spontaneous neuronal activities may be restricted to specific circuits [27]. Apart from the decrease in firing rate, modifications also occur in the neuronal firing pattern in the area governing the circadian rhythm (suprachiasmatic nuclei) [6]. In the CA1 region, the significant loss of synapses matches with a decrease in the evoked synaptic potential and a reduction in the evoked GABA-mediated inhibitory postsynaptic potentials. However, there also occur compensatory changes for maintaining the magnitude of synaptic potential [28]. Such changes include an increase in the excitatory postsynaptic potential of NMDA receptor signaling in CA1 area of the aged rat. These compensatory alterations in synaptic function may account for relatively minimal age-related functional changes in the brain [29].

Estrogen action in brain

Numerous studies support the beneficial effect of estrogens on the function and viability of neurons and learning and memory processes [1–3]. Estrogen action in various regions of the brain influences reproductive process, higher cognitive functions, pain mechanism, fine motor skills, mood, temperature regulation, sleep and susceptibility to neurodegenerative disorders [30].

Actions on brain micromorphology

Estrogen significantly affects the microstructure of different brain regions [31–33]. The increase in synaptic spine and dendritic density, which depends on circulating estrogen levels, is correlated with the superior performance in behavioral and memory tests [34, 35]. The depletion of estrogen in adult female rats by removal of ovaries results in loss of spines from certain hippocampal cells, whereas the ovariectomized rats receiving exogenous estrogen show normal number of hippocampal spines. These changes are mediated through the estrogen dependent increase in NMDA receptor and its phosphorylation in rat hippocampal neurons [36, 37].

Neurotrophic action

In addition to affecting the microstructure of brain, estrogen regulates the level of neurotrophins such as nerve growth factor (NGF), which is essential for early development, differentiation and growth of neurons. Receptors for both estrogen and neurotrophins are located on same neurons in the rodent basal forebrain, hippocampus and cerebral cortex [38]. The functional significance of this co-localization is supported by the observation that estrogen increases the expression of $p75^{NGFR}$, a 75-kDa transmembrane protein which binds with low affinity to NGF and other neurotrophins. Estrogen also regulates the levels of brain-derived neurotrophic factor, insulin like growth factor-1 (IGF-1), transforming growth factor beta and related receptors TrkA and TrkB [39–41].

Neuroprotective action

Estrogen protects the neuronal damage through many ways. It reverses the effect of oxidative stress in neuronal cell culture by increasing the intracellular concentration of glutathione, a natural free radical scavenger. It modulates the activity of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase [42]. The antioxidant property of estrogen has been related primarily to its basic chemical property such as the presence of a hydroxyl group in steroid ring A of the estrogen molecule. Any modification or absence of this hydroxyl group leads to the loss of neuroprotective effect [43–45]. Estrogen replacement in young and middle aged rats after the removal of ovaries significantly decreases ischemic injury compared to vehicle-treated controls [46–48]. Such estrogen dependent protection against ischemia induced neuronal damage occurs by inhibiting the release of free calcium from intracellular stores and the influx of calcium from the extracellular space and thus preventing activation of apoptotic signaling [49]. Other mechanisms by which estrogen prevents the neuronal death involve inhibition of apoptosis by increasing the level of antiapoptotic proteins or repressing the level of pro-apoptotic proteins [50, 51]. Estrogen treatment also increases the clearance of amyloid β by microglia [52], protects against glucose deprivation [53], decreases the inflammatory reactions by blocking expression of pro-inflammatory factors [54], helps in laminin reorganization after injury and regulates the permeability of blood–brain barrier [55, 56].

Learning and memory

The variation in plasma concentration of estrogen during menstrual cycle is responsible for cyclic modulation of mood and cognitive activities. Systematic analysis of the impact of estrogen loss and replacement in human demonstrated that verbal memory declines with the loss of estrogen [57]. In Morris water maze test of rodents, retention/consolidation of spatial memory varies with alterations in estrous cycle and this hippocampus-dependent task is sexually dimorphic [58]. Although all studies do not show enhancement in estrogen dependent spatial memory tasks, results indicate that such enhancement may be limited to working versions of spatial memory [59–61]. The sexually dimorphic differences also exist in human cognitive functions, e.g., women excel in verbal memory, verbal fluency and fine motor skills, whereas men excel in visuospatial skills and gross motor coordination [62]. These cognitive changes may occur due to differences in the exposure of male and female brains to sex steroids during early development.

Modulation of neurotransmitter system

Serotonergic system

Estrogen regulates components of the serotonin system such as increase in the expression of tryptophan

hydroxylase, serotonin transporter mRNA, 5-HT_{1A} mRNA, 5-HT_{2A} mRNA and 5-HT_{2A} receptor binding [63–66] in dorsal and medial raphe of midbrain, amygdala, hypothalamus, hippocampus and many other brain areas of primates and rodents. Estrogen also causes a rapid decrease in the coupling of G proteins to 5-HT_{1A} receptor system, resulting in the reduction of inhibitory effect of 5-HT_{1A} agonists on lordosis behavior, hyperphagia, and oxytocin and corticotropin responses [67].

Dopaminergic system

Like serotonin system, estrogen influences the dopaminergic system involved in motor function, motivation, reward, cognition and hypothalamic–pituitary axis control [68, 69]. The level and turnover of DA fluctuate during the estrous cycle [70]. However, administration of estrogen following ovariectomy increases the release of DA [71, 72], and concentrations of D_1 and D_2 receptor in the striatum [73]. Estrogen inhibits the release of DA from the median eminence [74], but induces the release and turnover of striatal DA. Reuptake of DA increases in the rat preoptic-septal tissue, but decreases in the hypothalamus [75].

Cholinergic system

Administration of estrogen to ovariectomized rats increases the activity of choline acetyl transferase (ChAT) in the basal forebrain, and two of its projection areas, CA1 region of hippocampus and frontal cortex. ChAT is involved in the synthesis of acetylcholine [76]. In the noradrenergic system, both α- and β-adrenergic receptors are upregulated by 17β-estradiol in ovariectomized female rats [75, 77]. However, β-adrenergic receptors are eventually downregulated due to a hormone dependent increase in noradrenergic activity. The synaptic uptake of NA decreases when estrogen is administered alone [78], but increases when estrogen is followed by progesterone in rats [77].

Effect of aging on estrogen signaling in brain

Estrogen mediates numerous responses via three distinct types of signaling, namely genomic, nongenomic and ligand-independent pathways.

Genomic pathway

The classical genomic pathway involves signaling through intracellular receptors, ERα (NR3A1) and ERβ (NR3A2). Both ERα and ERβ are ligand-activated transcription factors belonging to the nuclear receptor superfamily of steroid receptors [79]. In the absence of ligand (estrogens), ERs are sequestered in a multiprotein inhibitory complex within the nuclei. A recent study shows the localization of ERβ exclusively in the mitochondria of target cells [80]. The binding of ligand induces conformational changes in ERs such as homo- or hetero-dimerization of receptors and high affinity binding to specific estrogen responsive elements (EREs) located as *cis*-acting enhancers within the regulatory regions of target genes. The DNA-bound receptors contact general transcription apparatus either directly or indirectly via coregulators, cointegrators and other proteins having histone modification activities. It is generally accepted that the ER–coactivator interaction stabilizes the formation of transcription pre-initiation complex and facilitates the remodeling of chromatin at ERE. Depending upon the cell and promoter context, the DNA-bound receptor exerts either positive or negative effects on the expression of downstream target genes [1, 79, 81].

Estrogen receptorα and ERβ can also modulate the expression of target genes that do not have ERE in their promoter regions. ERE independent pathway implies the interaction of liganded ERs with other transcription factors such as Fos and Jun proteins at AP1 binding sites and Sp1 transcription factor in GC-rich promoter sequences [82, 83]. These actions of ERs depend on ligand, cell and receptor subtype [84]. Repression of interleukin-6 (IL-6) gene by estrogen involves the interaction of ERs with two transcription factors, nuclear factor κB (NF-κB) and CCAAT/enhancer binding protein β. The interaction of ERα with *c-rel* subunit of NF-κB prevents the binding of NF-κB to IL-6 promoter resulting in the repression of IL-6 expression [84].

Nongenomic pathway

Nongenomic actions of estrogens are mediated by the binding of hormone to either a subpopulation of classical ERs, which are located at the plasma membrane [85, 86] and exist as functional dimers when activated by estrogens [87] or novel membrane ERs such as ER-X [88]. Nongenomic pathway involves the activation of various protein kinase cascades such as src, ras, MEK and MAPK [89, 90].

Ligand-independent pathway

In addition to ligand mediated activation, ER functions can be modulated by ligand-independent pathway

through extracellular signals. Signaling through peptide growth factors such as epidermal growth factor, IGF-1 and cAMP activates ER and target gene transcription. ERα and ERβ also act as the target of growth factor dependent phosphorylation which occurs through MAPK signaling pathway [3], after phosphorylation both ERα and ERβ interact with different coactivators for activation of target genes [91].

Signal transduction pathways also connect the nongenomic actions of estrogens to genomic responses [89]. The nongenomic pathway stimulates a second messenger system, which phosphorylates various cellular substrates including transcriptional regulators like cAMP response element (CRE) binding protein (CREB) by protein kinase A or serum response factor (SRF)–Elk-1 complex by MAPK/ERK [89]. These transcription regulatory proteins CREB and SRF–Elk-1 bind to DNA regulatory regions namely CRE and serum response element, respectively. The resulting cascades are capable of regulating non-ERE-containing genes. Thus, stimulation of second messenger system can regulate both ER dependent and ligand-independent genomic actions, independent to each other.

Brain aging and ERs

The level of ERα and ERβ is determined by a balance between its synthesis and degradation during aging of the brain. The binding of ER to its cognate ligand varies in specific regions of the brain of young and old rats [92]. Aged female rats show decreased binding of 17β-estradiol in preoptic area, but no difference is found in amygdala, medial basal hypothalamus and pituitary [93]. In contrast, other groups have reported decreased binding in hypothalamus, preoptic area and pituitary of old female rats [94, 95]. Such discrepancy in results needs to be resolved by additional approaches. Further complexity in the interpretation of these results was added after the discovery of ERβ which has almost similar affinity of binding with 17β-estradiol as ERα.

In situ hybridization studies using ERα specific probes demonstrate little or no change in ERα mRNA expression in the preoptic area and hypothalamus of old rats [96–99]. Further, immunohistochemical studies show that the number of cells expressing ERα protein does not change in the median preoptic nucleus (MPN) of adult and old female rats [100]. However, the number of cells expressing ERα protein increases in anteroventral periventricular nucleus (AVPV) but decreases in ventromedial nucleus of old rats [101]. A recent report from our laboratory indicates that ERα protein level does not vary with age, but shows sex dependent differences in the cerebral cortex of AKR mice [102].

The expression of ERβ mRNA shows no effect of age in MPN, paraventricular or periventricular preoptic nuclei [99], but decreases significantly in supraoptic nucleus. The immunoreactivity of ERβ also shows no change in principal nucleus of the bed nucleus of the stria terminalis, but increases in AVPV of old rats [103]. We have recently reported an age-dependent decrease in the level of ERβ protein in the cerebral cortex of AKR mice [102].

Estrogen effects in aging brain

In addition to age-related changes in ER expression, the response to estrogen varies with age. As described earlier, experiments using laboratory animals and cell culture suggest beneficial effect of estrogen treatment on the brain; however, almost all of these studies involve young or middle aged animals. Studies using senescent laboratory animals suggest that estrogen treatment may or may not have the same effect in old brain as in adult [104–107]. Estrogen treatment in the gonadectomized aged rats has been shown to be responsible for the reversal of hippocampus related memory impairment, blocking of long-term depression, decreased cytosolic calcineurin activity [58, 108], increased level of growth associated protein-43 and choline acetyltransferase [109].

Estrogen is also involved in the modulation of expression of amyloid precursor protein (APP) associated with AD in old brain. Of the various APP isoforms (APP770, APP751 and APP695), the APP695 is predominantly found in the brain and its level remains high under non-pathological conditions. Experimental evidences suggest that the level of APP695 is upregulated by estrogen treatment in old female mouse cerebral cortex [110–112], suggesting that estrogen treatment in old age may shift the APP load in non-pathological condition.

In contrast to these beneficial effects, estrogen treatment in aged rats fails to induce an increase in spine number but has an impact on the molecular nature of CA1 axospinous synapses through enhancement of synaptic NR1 and NR2B expression, suggesting that estrogen can restore a partial youthful NMDA receptor profile in aged rats [113]. Similarly, Jezierski and Sohrabji [114] reported that aged forebrain is unresponsive to estrogen dependent neurotrophin expression. Estrogen treatment reduces the permeability of blood–brain barrier in the olfactory bulb of

young but not old rats, and increases the permeability in the hippocampus of old females compared with age-matched untreated animals, suggesting that the hormonal decline leads to increased permeability of the blood–brain barrier, which is further exacerbated by estrogen treatment in specific regions [56].

The comparison of estrogen replacement effects between young adult and reproductively senescent animals suggests that estrogen replacement is beneficial when given to ''surgically menopausal'' (ovariectomized) animals. However, estrogen replacement appears to be deleterious for the acyclic reproductively senescent animals, where target organs such as the brain have been in a prolonged estrogen-deficient state [115].

Recent studies in non-human primates suggest that aged female monkeys (equivalent to perimenopausal women) are just as responsive as young monkeys with respect to estrogen-induced increase in CA1 spine number [116]. A behavioral analysis demonstrated significant estrogen-induced enhancement of cognitive function in aged ovariectomized rhesus monkeys [117]. The estrogen-treated group showed enhanced performance in hippocampus-dependent task (delayed non-matching to sample, DNMS) as well as the delayed response task (a prefrontal task that is sensitive to aging). Although the effects of estrogen on DNMS were moderate, estrogen treatment dramatically affected the delayed response performance, restoring it to that of young monkeys [117]. This indicates that in primates the prefrontal cortex might be at least as responsive to estrogen as the hippocampus, implicating a much broader array of cognitive functions than suggested by the rat hippocampal data.

Like the aged laboratory animals, results from clinical studies characterizing the cognition-enhancing and neuroprotective efficacy of estrogen in old age have revealed conflicting results. Earlier clinical investigations indicate that as compared to young women, postmenopausal women are more vulnerable to neurodegenerative diseases such as AD, Parkinson's disease, stroke and memory dysfunction and estrogen replacement therapy (ERT) not only reduces the risk to AD but increases the verbal memory [104]. Using positron emission tomography, Rasgon et al. [118] reported that estrogen replacement may preserve regional cerebral metabolism and protect against metabolic decline in postmenopausal women, especially in the posterior cingulate cortex, and thus estrogen enhances the chance of neuronal survival. Another study showed differences between estrogen users and non-users in the cerebral blood flow in the hippocampus, parahippocampal gyrus and temporal lobe. These studies suggest that at least some areas of the brain involved in memory circuits and sensitive to AD are responsive to ERT in old females [119]. ERT in postmenopausal women also increases the choline containing compounds in parietal and hippocampus regions, indicating increased neuronal/glial membrane turnover and suggesting that neuroprotective effects of estrogen may involve modulation of cell integrity [120].

However, recent intervention trial (Women's Health Initiative) concluded that the replacement of estrogen and other hormones prescribed to postmenopausal women does not improve global cognitive impairment and dementia [121]. The intervention trial also found that 65–79 years old women with 10–20 years postmenopausal state are less responsive to estrogen replacement than perimenopausal women in their early 1950s [122]. Postmenopausal women of 65 years and above, and free of probable dementia and treated with estrogen and progesterone had a negative impact on verbal memory and a trend to a positive impact on figural memory over time compared with placebo, but other cognitive domains were not affected. Both effects on memory were evident only after long-term therapy [122]. In another double-blind experiment, hysterectomized women (age 58–75 years) receiving placebo, estradiol or estradiol/progesterone treatment failed to show any beneficial effects in any of the cognitive tests (out of nine parameters) [123]. Therefore, this study does not support the notion that treatment with sex hormones has beneficial effects on cognition in old women. Taken together, these reports suggest a ''short period of opportunity'' as a function of age and duration of estrogen depletion, after which replacement is less effective. In fact, such age-associated alterations in response to estrogen might be a crucial factor for the failure of estrogen replacement to protect against cognitive impairment.

Conclusions

Aging is associated with alterations in brain structure and function. Estrogen action in brain influences many anatomical and neurochemical processes that go beyond their traditional role. So far the information about age-dependent changes in the membrane ER(s) is lacking, while very little is known about the changes occurring in nuclear ERs. Changes in ERs depend upon receptor subtypes and brain regions with the likely net outcome of a differential response to estrogen in the aging brain. Experimental evidences obtained from laboratory animals suggest that these effects may be of particular importance in the context

of aging when circulating estrogen level decreases. However, the effect of estrogen supplementation in old females is not as beneficial as in adults, at least in the case of cognitive impairment, indicating the importance of detailed knowledge about age-dependent changes in estrogen signaling pathway and fidelity of other downstream interacting molecules.

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