Estrogen, cognition and female ageing

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Starting from fetal life, estrogens are crucial in determining central gender dimorphism, and an estrogen-induced synaptic plasticity is well evident during puberty and seasonal changes as well as during the ovarian cycle. Estrogens act on the central nervous system (CNS) both through genomic mechanisms, modulating synthesis, release and metabolism of neurotransmitters, neuropeptides and neurosteroids, and through non-genomic mechanisms, influencing electrical excitability, synaptic function and morphological features. Therefore, estrogen’s neuroactive effects are multifaceted and encompass a system that ranges from the chemical to the biochemical to the genomic mechanisms, protecting against a wide range of neurotoxic insults. Clinical evidences show that, during the climacteric period, estrogen withdrawal in the limbic system gives rise to modifications in mood, behaviour and cognition and that estrogen administration is able to improve mood and cognitive efficiency in post-menopause. Many biological mechanisms support the hypothesis that estrogens might protect against Alzheimer’s disease (AD) by influencing neurotransmission, increasing cerebral blood flow, modulating growth proteins associated with axonal elongation and blunting the neurotoxic effects of β-amyloid. On the contrary, clinical studies of estrogen replacement therapy (ERT) and cognitive function have reported controversial results, indicating a lack of efficacy of estrogens on cognition in post-menopausal women aged ≥65 years. These findings suggest the presence of a critical period for HRT-related neuroprotection and underlie the potential importance of early initiation of therapy for cognitive benefit. In this review, we shall first describe the multiple effects of steroids in the nervous system, which may be significant in the ageing process. A critical update of HRT use in women and a discussion of possible prospectives for steroid use are subsequently proposed.

Key words: ageing/CNS/cognition/estrogen/HRT

Introduction

One of the most exciting areas of research in women’s health over the past 10 years involves our growing appreciation that estrogens play important neurotrophic and neuroprotective roles during adulthood. This brings new meaning to the potential impact of the prolonged post-menopausal hypoestrogenic state on learning and memory and the potential increased vulnerability of ageing women to brain injury and neurodegenerative diseases. The increase in female life expectancy during the past century has meant that women now live one-third of their lives beyond cessation of their ovarian function. This evolution in demography has increased the need for the development of new therapeutical strategies to promote successful ageing, defined as low probability of disease, high cognitive and physical capacity and active engagement in life. Because changes in the ageing nervous system are subtle, it may be possible to reverse them and to improve cognitive performance by pharmacological treatments. The administration of steroids may be particularly promising in this regard: (i) they play an important role in the functioning of the central and peripheral nervous system (CNS and PNS); (ii) some steroids have neuroprotective effects; (iii) the levels of some neuroactive steroids markedly decrease with age; and (iv) unconjugated steroids easily cross the blood–brain barrier and rapidly accumulate throughout the brain.

Estrogen receptors (ERs) are found in both the hippocampus and the frontal lobes which subserve verbal memory, working memory and retrieval. It was reasonable to hypothesize that this hormone might play an important protective role against the deterioration in these cognitive functions that occur with normal ageing. It is indeed now well recognized that the functions of gonadal and adrenal steroid hormones go far beyond reproduction and that they regulate vital neuronal and glial functions by various mechanisms of action (Schumacher et al., 2000). In addition, some steroids, named ‘neurosteroids’, can be synthesized within the nervous system by both neurons and glial cells, and the stimulation of their synthesis offers new therapeutical possibilities (Baulieu, 1997).

Therefore, during the past two decades, investigators have attempted to determine whether the administration of estrogens to women at the time of or following menopause would protect
Sex steroids as pleiotropic factors in the nervous system from brain development to brain ageing

Sex steroid hormones play fundamental roles in the development and function of CNS. Estrogens, progestins and androgens are able to induce several effects in brain areas of the CNS, by binding with specific receptors. The action of sex hormones is not limited to the regulation of endocrine functions and mating behaviour: the identification of estrogen, progestin and androgen receptors outside their classical CNS regions, such as the pituitary and hypothalamus, justifies their role in controlling different brain functions (Genazzani et al., 1996).

These findings highlight the importance of sex steroids in the development and the regulation of the CNS. Marked differences are found in the structure and function of the brain of male and female animals and humans (Kruijver et al., 2000). Indeed, in humans and in rats, several areas of the brain show gender dimorphism, as indicated by differences in structure (such as different numbers of cells in specific areas). The different organization of brain areas in males and females appears to be largely dependent on the action of sex steroid hormones as demonstrated by the differential expression of steroid receptors in sexually dimorphic nuclei (Kruijver et al., 2001). These ‘organizational/developmental’ effects are permanent and are acting during development, mainly in the fetal–neonatal period when estrogens and aromatizable androgens modulate neuronal development and the formation of neuronal circuits. As a result, several areas of the CNS become sexually differentiated. This is also suggested by the evidence that the levels of circulating and locally produced steroids control the structure and activity of sexually dimorphic nuclei. Therefore, exogenous sex steroids could cause differences in the structure/function of specific brain areas with measurable clinical effects. Although abundant morphological and functional evidence exists for sex differences in brain development, much less is known regarding the underlying developmental mechanisms that direct these differences (Beyer, 1999). Because neurons are limited in their proliferative life and there is considerable programmed cell death after birth, the effect of steroids appears to involve the regulation of neural cell kinetics and apoptosis. Sexual dimorphism is also present in other cellular compartments such as the glia, and the sex-steroid-related developmental differences seen in both animals and humans are real and complex (Cooke et al., 1998); gender differences show up, among others, in dendritic structure, organization of the neuronal membrane, neuronal connectivity patterns, as well as in opiate receptor numbers (Pfaff, 1980).

Brain plasticity is most apparent during early development with the formation of the nervous system, but it continues through puberty, reproduction and adult life (Keefe et al., 1994; Cooke et al., 1998). These ‘activational/neuroplasticity’ effects are transient and fluctuate considerably as the hormonal milieu changes, thus affecting almost potentially every aspect of brain physiology in different biological phases. Estrogen-induced synaptic plasticity is clearly seen during puberty and with seasonal changes as well as during the ovarian cycles. Estrogen appears to be important for the regulation and maintenance of network integrity of several brain areas related to cognition (Garcia-Segovia et al., 1994). In addition to changes in the cortex accompanying cognitive tasks, estrogen regulates the anatomy and connectivity of the hippocampus and associated structures (Polz et al., 1998; Genazzani et al., 2003). In post-menopause, neurotransmitters, neuropeptides and neurosteroids undergo important changes as a consequence of the failure of gonadal hormone production at a time when many CNS activities deteriorate, particularly those associated with hippocampal functions such as memory, attention, cognition and autonomic control (Genazzani et al., 2005). This neuroendocrinological ageing process represents a unique opportunity to investigate the actions of gonadal hormones on their specific receptors in the nervous system.

Mechanisms of action of estrogens in CNS

A comprehensive account of the molecular and cellular activities of estrogen on the CNS is beyond the scope of this article, and several recent reviews of the area are available (McEwen and Alves, 1999). However, in the attempt to describe the biological plausibility for the hypotheses that estrogens greatly affect aspects of cognitive function, the mechanisms that seem most relevant will be briefly described here.

ERs in CNS

Despite the long history of estrogen effects in the brain, the knowledge of estrogen action at the cellular and subcellular level is still scanty. It is beyond any question that steroids modulate gene transcription by interacting with nuclear receptors. The ligand-dependent regulation of gene expression is generally sensitive to transcriptional and translational inhibitors as well as to inhibitors of nuclear receptors. Two types of nuclear receptors are known: ERα and ERβ which are similar in their structural organization into domains but which have distinct differences in their binding affinities for different ligands and selective ER modulators (Gruber et al., 2002). Regarding the CNS, specific receptors for estrogen have been localized in the amygdala, hippocampus, cortex basal forebrain, cerebellum locus coeruleus midbrain rafe nuclei, glial cells and central grey matter, confirming an involvement of estrogen in controlling well-being, cognitive functions and memory processes in physiological as well as in pathological conditions (Sherwin, 1997). In these genomic mechanisms, steroids induce relatively long-term actions on neurons modulating the synthesis, release and metabolism of many neuropeptides and neuroactive transmitters and the expression of their receptors (Alonso-Soleis et al., 1996). ERα and ERβ are differently expressed throughout the rat brain, and there is anatomical evidence of distinct roles of each subtype. Hybridization and histochemical studies have shown that both receptors are present in the rat cortex, pituitary and hypothalamus (ERα mostly in the arcuate and ventromedial nuclei, whereas ERβ is mostly present in the paraventricular and ventromedial nuclei),
whereas the cerebellum expresses only ERα and the hippocampus expresses mostly ERβ (Keefe et al., 1994; Couse et al., 1997).

Moreover, sex steroids exert very rapid effects in the brain that cannot be attributed to genomic (i.e. transcriptional) mechanisms (Gruber et al., 2002). These ‘non-genomic or non-classical’ effects are probably to be mediated by receptors integrated or associated with the plasma membrane and by an activation of distinct intracellular signalling cascades (Falkenstein and Wehling, 2000; Kuppers et al., 2001). Various non-genomic estrogen effects includes (i) rapid actions on excitability of neuronal and pituitary cells, (ii) activation by estrogens of cyclic adenosin monophosphate (AMP) and mitogen-activated protein (MAP) kinase pathways that affect the activity of such targets as kainate and insulin-like growth factor-1 (IGF-1) receptors, (iii) modulation of G-protein coupling and effects on calcium currents, (iv) effects on calcium channels and calcium ion entry and (v) protection of neurons from damage by excitotoxins and free radicals (Kelly and Wagner, 1999; McEwen and Alves, 1999; Brinton, 2001; Kelly and Levin, 2001; Lee and McEwen, 2001; McEwen et al., 2001). Regarding the subcellular localization of ER, experimental data show that, in addition to the nuclear ERs, there is a predominant localization of ERs in proximity to the plasma membrane, including that of neurites and of the soma, dendritic spines and axon terminals (McEwen and Alves, 1999; Clarke et al., 2000; McEwen et al., 2001). These findings also imply that classical ERs may act within a cell in a dynamic way and suggest that ERs can be found in various subcellular structures. This is supported by the demonstrations that estrogen is capable of binding and interacting with proteins in the mitochondrial membranes and that ERs are associated with pre-synaptic structures, thereby controlling synaptic transmission (Wong et al., 1996; Kelly et al., 1999; Levin, 1999; McEwen and Alves, 1999).

In conclusion, estrogen effects in the brain include complex cellular mechanisms ranging from classical nuclear to non-classical membrane-mediated actions. Both ways of cell signalling may be activated separately, although there is good evidence that they are intertwined at several cellular instances and can affect each other reciprocally, producing synergistic effects (Kelly and Wagner, 1999) (Figure 1). Regarding the cellular and subcellular location of ERs, Beyer and colleagues proposed a highly dynamic intracellular mobility with classical ERs being located at nuclear sites but also extra-nuclear sites in different cell compartments including the plasma membrane (Panay et al., 1996; Ramirez et al., 2001; Adams et al., 2002; Behl, 2002; Beyer et al., 2003).

Neurotransmitters that mediate the effects of estrogens on mood and cognition: serotonin, norepinephrine and cholinergic systems

Sex differences and estrogen effects on the serotonergic, cholinergic, dopaminergic and noradrenergic systems may contribute to many aspects of brain function that are affected by ovarian hormones, including affective state, movement disorders and cognitive function, thus evoking a pivotal pathogenical role in neurodegenerative diseases (Phillips and Sherwin, 1992a).

A number of studies indicate gender-related differences in the central serotonergic system of the adult rat brain, with females displaying elevated serotonin [5-hydroxytryptamine (5-HT)] levels and greater synthesis and turnover compared with male rats. Higher concentrations of serotonin or 5-hydroxyindoleacetic acid (5-HIAA) have been reported in the female rat whole brain, forebrain, raphe, frontal cortex, hypothalamus and hippocampus compared with the male rat brain (Carlsson and Carlsson, 1988). The overall activity of 5-HT in the brain decreases with age (Meltzer et al., 1998).

Treatment of oophorectomized rats with estrogen, progesterone or both positively affects the serotonergic system of the female rat brain. The mechanisms by which estrogen exerts these neuro-modulatory effects are not known. CNS estrogen effects that may modulate mood include monoamine oxidase (MAO) inhibition at high levels, tryptophan displacement from plasma albumin-binding sites and effects on 5-hydroxytryptamine 2 (5-HT2) receptor

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**Figure 1.** Differential and integrated effects from genomic and non-genomic action of estrogens in central nervous system (CNS).
binding and down-regulation (Luine and Rhodes, 1983; Studd and Smith, 1994). When administered to oophorectomized monkeys, estrogens increased the synthesis of tryptophan hydroxylase and the expression of tryptophan hydroxylase mRNA in the dorsal raphe (Pecins-Thompson et al., 1996). Acute administration of estradiol (E2) to oophorectomized rats also increased the density of 5-HT2A receptors in brain areas that are thought to be involved in mood and cognition: the anterior frontal, anterior cingulate and pyriform cortices, the olfactory tubercle, the nucleus accumbens and the lateral dorsal raphe nucleus (Fink and Sumner, 1996). These findings suggest that estrogens may affect mood by increasing the concentration of 5-HT receptors in key areas of the brain.

Sex steroid hormones modulate the noradrenergic and dopaminergic systems at the hypothalamic level as well as at the extrahypothalamic regions of the brain controlling movement and behaviour in both animals and humans. Animal studies show an increase of norepinephrine (NE) turnover rates induced by estrogens during the pro-estrus period (Fink and Sumner, 1996). On the contrary, in castrated female rats, an impairment of cathecolaminergic neurons has been demonstrated, with an increase in noradrenaline and a decrease in dopamine release (Etgen and Karkanias, 1994). Estrogen administration decreases hypothalamic NE release, whereas an increase in dopaminergic neuronal activity with a parallel increase in dopamine release in the medio-basal hypothalamus has been shown (Herbison, 1998; Ansonoff and Etgen, 2000). Regarding the modulation of the different subtypes of adrenergic receptors, in vitro studies suggest that estrogens up-regulate α1-adrenergic and down-regulate β-adrenergic receptor activity (Honma and Wuttke, 1980). In an animal model, E2 and progesterone enhance noradrenaline release, leading to an increased excitability of ventromedial hypothalamic neuronal activity and to the expression of lordosis behaviour (Demling et al., 1985). Early studies show that NE turnover and content within the hypothalamic nuclei fluctuate in response to gonadal steroid manipulation, indicating that estrogen and progesterone may regulate the activity of NE neurons (Wise et al., 1981). Indeed, more recent studies in rats have shown an increase in NE release within the preoptic area on pro-estrus, in the progesterone receptor (PR) expression in NE neurons of the caudal brainstem and in the ability of estrogen to directly up-regulate PR gene expression in these neurons (Haywood et al., 1999). These observations support the hypothesis that changes in gonadal steroids may underlie the ability of estrogen and progesterone to alter NE transmission within the hypothalamus and elsewhere in the brain (Genazzani et al., 1997).

The basal forebrain contains cholinergic neurons that project to the cerebral cortex and hippocampus, where they play an important role in cognitive function. Normal ageing is associated with a reduction in cholinergic functional markers, such as choline acetyltransferase (ChAT), but a relative preservation of cholinergic cells and terminals (Decker, 1987). Studies in experimental animals have suggested that estrogens may influence brain function through effects on the cholinergic system. Receptors for gonadal hormones have been identified in the nuclei of the basal forebrain, the major source of cholinergic innervation to the cerebral cortex, hippocampus and hypothalamus (Toran-Allerand, 1996). Estrogen is known to provide trophic support to cholinergic cells and to regulate various markers of cholinergic function, including ChAT and acetylcholine release (McMillan et al., 1996). Studies of estrogen effects on the expression of cholinergic enzymes were among the first that pointed to non-reproductive actions of gonadal steroids. Experiments with ovariectomy plus estrogen replacement therapy (ERT) revealed an induction of ChAT, the rate-limiting enzyme for acetylcholine formation, within 6–24 h in the basal forebrain of female rats. In addition, estrogen treatment increased ChAT activity in projection areas of the basal forebrain 10 days after hormone injection, suggesting that estrogen-induced ChAT was transported from cell bodies to nerve endings in the cerebral cortex and hippocampus (Luine et al., 1980; Kuhl et al., 1999). Along with these effects, long-term ovariectomy caused a decline in the learned performance of active avoidance behaviour that was prevented by ERT (Singh et al., 1994).

Concerning the trophic effect of estrogens on CNS, in addition to the cholinergic system, it is interesting that cytoplasmic and intranuclear estrogen-binding sites colocalize and regulate the expression of neurotrophins such as nerve growth factor (NGF), its receptor trkA and brain-derived neurotrophic factor (BDNF) (Singh et al., 1994; Kuhl et al., 1999; Mufson et al., 2003; Scharfman and Machlusky, 2005).

**Estrogens and the opioid system and neuropeptide Y**

Several estrogens may modulate brain opioid peptide mRNA levels, opioid peptide levels, opioid receptor density and opioid receptor-mediated signal transduction (Simery et al., 1988; Sinchak et al., 2000; Craft et al., 2004). Estrogens may even attenuate the effects of endogenous and exogenous opioids by binding directly to opioid receptors (Schwarz and Pohl, 1994). In particular, our attention has been focused on β-endorphin (β-EP), the most important and biologically active endogenous opioid peptide, that exerts behavioural, analgesic, thermoregulatory and neuroendocrine properties. It has been demonstrated that ovariectomy in female rats induces a decrease in hippocampus, hypothalamus, pituitary and plasma β-EP content, the magnitude of which differs in each brain area analysed (Genazzani et al., 1988). It is probable that different brain tissues and plasma do not respond to gonadal steroid deprivation in the same way in terms of β-EP synthesis/release. Oral administration of estradiol valerate (EV), estrone sulphate (ES) and conjugated equine estrogens (CEE) in ovariectomized rats restores central β-EP levels to values similar or higher, at the maximum doses, to those observed in fertile rats. This positive stimuli is meaningful in the neurointermediate pituitary (Stomati et al., 2002). Aged female rats show reduced brain levels of β-EP, and the age-related decrease ranged from 40 ± 4% in the hippocampus to 83 ± 7% in the neurointermediate lobe with respect to fertile rats. Ageing may be associated with a reduction in brain β-EP production and storage, either directly by acting on opiateergic neurons or indirectly through the derangement of the neuroendocrine systems responsible for opiatenergic neuron control. Oral administration of CEE for 2 weeks in aged rats, at 0.1, 0.5 and 2 mg/kg/day, determines a dose-dependent increase in β-EP in hippocampus, in hypothalamus and in the neurointermediate lobe (Genazzani et al., 2004).

A decrease in plasma β-EP levels has been detected in postmenopausal women after surgical or spontaneous menopause, and it has been suggested to have a role in the mechanisms of hot flushes and sweat episodes (Aleem and McIntosh, 1985). The decrease in plasma β-EP levels has also been related to the pathogenesis of mood, behaviour and nociceptive disturbances occurring
in this period (Genazzani et al., 1984). Indeed, a positive role of HRT on vasomotor and subjective psychobehavioural symptoms may be mediated by effects on the opioidergic pathway (Linghtman et al., 1981). In fact, oral ERT subsequent to spontaneous or surgically induced menopause is followed by a significant increase in circulating β-EP levels (Adler, 1980).

The administration of transdermal E₂, independent of the type of progestin used, induces a significant increase in plasma β-EP levels to premenopausal values (Donouhe and Dorse, 1982). In addition, the lack of β-EP response to clonidine, a α₂-presynaptic receptor agonist, and to naloxone, an opiate receptor antagonist, in post-menopausal women suggests an impairment of adrenergic and opioidergic receptors in modulating β-EP release. HRT restores basal plasma β-EP levels to those present in fertile women as well as the response of β-EP to naloxone and clonidine tests (Genazzani et al., 1990; Stomati et al., 1997).

In addition to β-EP, the neuropeptide Y (NPY), which is modulated by gonadal steroids, regulates neuroendocrine functions by the pulsatile release of GnRH and gonadotrophins and regulates nutrient intake by influencing the appetite and satiety centre in the hypothalamus (Zarjevski et al., 1994). Estrogen is able to stimulate NPY synthesis and release in the hypothalamus. In castrated female rats, gonadal steroid deficiency reduces neurosecretion of NPY-producing neurons (Karla, 1996). Post-menopausal women show lower NPY plasma levels than young women (Milewicz et al., 2000a). Estrogens increase NPY content in the median eminence and the synthesis of NPY in arcuate nucleus, by inducing NPY gene expression. Indeed, recent findings demonstrate several interactions between NPY and β-EP neurons in the hypothalamus; thus, estrogens may indirectly exert modulatory effects on NPY when inducing β-EP release (Milewicz et al., 2000b).

**Estrogens and the pineal gland and melatonin system**

Melatonin is synthesized by the pineal gland, and both synthesis and secretion follow a circadian rhythm, with low rates of production and release during the day and high rates of production and release at night (Reiter, 1991). Several studies show an antioxidant property of melatonin (Reiter, 1998): the administration of a physiological dose of melatonin to female senescence-accelerated mice prevents the age-related oxidative DNA damage in the brain (Morioka et al., 1999). Moreover, melatonin administration in humans synchronizes endogenous rhythms to environmental cycles, favours a propensity to sleep, enhances LH and prolactin secretion (Lewy and Sack, 1997; Zhdanova and Wurtman, 1997) and reduces body temperature (Cagnacci, 1996). In hypertensive individuals, melatonin has been reported to reduce blood pressure (Birau et al., 1981) and more recently, in young men and women, to decrease blood pressure and internal carotid artery pulsatility index (PI) (Cagnacci et al., 1998; Cagnacci and Arangino, 2001). Several lines of evidence indicate that ageing influences the secretion and the biological responses to melatonin. Ageing reduces the circulating levels of the hormone and reduces the number of melatonin receptors in animals (Arent et al., 1983; Cagnacci et al., 1997). Since the identification of gonadal steroid receptors in the rat pineal gland, much evidence has suggested that melatonin synthesis may be modulated by the gonadal steroids (Okatani et al., 2000). In addition to environmental light, circulating levels of gonadal steroids also are able to influence the capacity of the pineal to synthesize and release melatonin (Sánchez et al., 2004). In female rats, estrogens regulate pineal melatonin synthesis and release by modulating the sensitivity of pinealocytes to adrenergic stimulation (Alonso et al., 1995). E₂ at physiological concentrations reduces the stimulation of melatonin synthesis evoked by the simultaneous activation of α₁- and β-adrenergic receptors in female rat pineal cells (Hernandez-Díaz et al., 2001).

However, in humans, the effect of gonadal steroid on pineal secretion of melatonin remains controversial: the effects of estrogen on pineal function vary markedly, depending on the dose of estrogen and the duration of estrogen administration (Cagnacci et al., 2001).

**Estrogens and neurosteroids**

Steroid hormones synthesized by the gonads and adrenal glands easily cross the blood–brain and the blood–nervous barriers and rapidly accumulate within the nervous tissues, except for their conjugated forms such as the steroid sulphates that do not easily enter the brain (Wang et al., 1997). Neurons and glial cells possess enzymes necessary for progesterone, testosterone and E₂ metabolism [aromatase, 5-alpha reductase (5a-R), mainly in neurons, and 3-alpha-hydroxysteroid dehydrogenase (3a-HSD), mainly in type 1 astrocytes] (Wang et al., 1997). The activities of these steroid-metabolizing enzymes are strongly influenced by the differentiation process of the precursor stem cells into terminally differentiated CNS cells. Neurons and glial cells co-ordinately metabolize steroid hormones, thus forming a functional unit; thus, both the endocrine glands and the local metabolism contribute to the pool of steroids present in the nervous tissues. Additionally, the age-dependent changes in circulating levels of steroid hormones may reflect changes in brain levels. Local aromatization of circulating androgens to E₂ in the brain plays an important role during neuroprotection and neuroregeneration. The activity rate of aromatase, an enzyme that converts androgens to estrogens, in rhesus monkeys has been measured throughout the brain, revealing the highest amount of activity occurring within specific regions of hypothalamic and amygdala, such as the preoptic area, bed nucleus stria terminalis and cortical amygdala. Aromatase activity under normal conditions is believed to be centralized to neural cell bodies and neuronal processes (axon terminals). However, increased aromatase expression and activity has been recognized in reactive astrogila in rat brain after induced injury to the brain. The increased expression of aromatase in injured brain areas (Garcia-Segura et al., 1994, 2001; Schumacher et al., 2003) suggests that this enzyme may be involved in the protection of nervous tissue by increasing local estrogen levels (Garcia-Segura et al., 1999). Estrogen formed by astrocytes may be released as a trophic factor for damaged neurons and may be involved in the compensatory restructuring of injured brain tissue (Peterson et al., 2001; Garcia-Segura et al., 2003). Thus, estrogen released by astroglia may affect synaptic function, selective regeneration of neuronal processes and local cerebral blood flow, contributing to facilitation of neuronal recovery and reduction of neuronal death. In addition, aromatase knockout mice showed an increased rate of apoptosis and cell loss in frontal cortex and reduced spatial working memory and short-term memory compared with the wild-type mice (Garcia-Segura et al., 2003). Because aromatase is expressed in the adult human brain, including the hippocampus (Sasano et al., 1988; Simpson and
Davis, 2001; Stoffel-Wagner, 2001), this enzyme may represent a new molecular target for the therapy or the prevention of neurodegenerative diseases, such as Parkinson’s disease and Alzheimer’s disease (AD), and other age-associated brain neurodegenerative disorders.

Although steroid-metabolizing enzymes allow the CNS to modify circulating steroids, the CNS is also able to synthesize steroids from cholesterol, at least in part, independently of peripheral steroidogenic gland secretion (Stoffel-Wagner et al., 1999), leading to the production of a series of potent steroidoid compounds. These brain-produced steroids have been named ‘neurosteroids’ and have been found to exert important regulatory actions on neurons and glial cells (Baulieu, 1991, 1997).

Several studies have shown that some psychological functions and symptoms such as depression, anxiety, irritability and affectivity can be related to the fluctuation of the synthesis and the release of the neurosteroids and in particular of allopregnanolone and dehydroepiandrosterone (DHEA). Allopregnanolone is a 3-α, 5-α reduced metabolite of progesterone, and the major sources of its circulating levels are the gonads and adrenal cortex, more than the CNS (Majewska, 1992; Mellon, 1994; Akwa and Baulieu, 1999; Baulieu et al., 2001). Allopregnanolone acts as an agonist on the γ-aminobutyric acidA (GABA_A) receptor, modulating stress, mood and behaviour with anxiolytic, sedative and antiepileptic effects (Wolf et al., 1997). Allopregnanolone brain levels increase during acute stress, pregnancy, antidepressant and anxiogenic drugs; in contrast, they decrease during chronic stress, parturition and depression. Fluctuating concentrations of central neurosteroids also modulate GABA_A receptor gene expression through genomic mechanisms, i.e. transcriptional induction. Ovarian steroids may produce changes in circulating allopregnanolone; in fact, female rats show significantly higher hippocampal allopregnanolone concentrations on the morning and afternoon of proestrus than at diestrous or estrous, with lowest levels at estrous (Schumacher et al., 1989; Palumbo et al., 1995; Genazzani et al., 1998, 2000; Serra et al., 2000). Moreover, it seems that allopregnanolone itself may influence gonadal function: the intracerebral injection of allopregnanolone in female rats inhibits the ovulatory process, whereas the injection of anti-allopregnanolone antiserum has been found to exert important regulatory actions on neurons and glial cells (Baulieu, 1991, 1997).

In ovariectomized rats, the administration of CEE, ES and EV reversed the ovariectomy-induced allopregnanolone changes in a dose-dependent fashion, therefore completely restoring their concentration. At higher doses (2 mg/kg/die), the estrogentic compounds induced significantly higher levels of allopregnanolone than in fertile rats. CEE induced higher allopregnanolone levels in hypothalamus, anterior pituitary and serum than did the other estrogentic molecules, and higher levels in the hippocampus than did EV alone (Stomati et al., 2002). In addition, in aged and hypoestrogenic rats, CEE treatment restores allopregnanolone content, reversing the effects of ageing on neurosteroidogenesis (Genazzani et al., 2004) (Figure 2). Experimental data showed that allopregnanolone serum levels are reduced in patients affected by both vascular dementia and AD, but the latter had a reduced response of allopregnanolone to corticotrophin release factor stimulation (Bernardi et al., 2000) (Figure 3). In addition, a comparative analysis of the concentration of several neurosteroids in various brain regions between aged Alzheimer’s patients and aged non-demented controls has shown a general trend towards lower levels of neurosteroids in the different brain regions of the patients compared with that of the controls (Laconi et al., 2001; Weill-Engerer et al., 2002).

These data indicate a major role for these compounds as neuroendocrine mediators of the effects of estrogens on the CNS, and the modulation exerted by HRT on allopregnanolone levels might be related to the anxiolytic and sedative effects of HRT in menopause women. Certainly, the evidence that gonadal steroids modulate neurosteroid levels opens new prospecs in the study of neuroendocrinological menopause-related changes.

![Figure 2. Allopregnanolone levels in the hippocampus and hypothalamus in cycling (cyc) (16 weeks old), ovariectomized (ovx) (16 weeks old) and aged (16 months old) female rats (white bars), in ovx rats treated with conjugated equine estrogens (CEE) (0.1, 0.5 and 2 mg/kg/day) (light grey bars) and in aged rats treated with CEE (0.1, 0.5, and 2 mg/kg/day) (dark grey bars). *P < 0.05 versus ovx controls; **P < 0.005 versus ovx controls; *P < 0.05 versus aged controls; **P < 0.005 versus aged controls (Genazzani et al., 2004).](https://academic.oup.com/humupd/article-abstract/13/2/175/661025)
Neuroprotective effects of estrogens with significance to cognition and female brain ageing

Most studies that investigate the potential neuroprotective actions of estrogen have been performed using experimental animal models and in vitro methodologies. The in vivo studies allow basic scientists to use experimental paradigms that mimic physiological conditions and clinical forms of brain trauma to elucidate differential mechanisms of estrogen action. The power of in vitro methods (explant cultures, primary dispersed cells or neuronal cell lines) lies in the fact that investigators can take advantage of more simple systems where direct and indirect actions of E₂ can be deciphered. These basic science studies complement clinical results and support the conclusion that E₂ can exert pivotal protective actions during adulthood (Table I). They reveal the breadth of mechanisms that estrogens use and uncover the interactive and complex nature of the cellular and molecular mechanisms that are involved.

Basic science

Several experimental data show that estrogen treatment can protect against a wide range of neurotoxic insults, including free radical generators, excitotoxicity, β-amyloid-induced toxicity and ischaemia (Dubal and Kashon, 1998; Behl and Holsboer, 1999; Brinton et al., 2000; Green and Simpkins, 2000). Estrogen neuroprotective effects are multifaced and encompass systems that range from the chemical to the biochemical to the genomic mechanisms. It is not yet clear whether there is one unifying neuroprotective cascade induced by estrogen or whether estrogen induces multiple mechanisms that are selectively neuroprotective against different neurotoxins or whether it is a combination of both. The hippocampus is a brain region that is involved in episodic, declarative, contextual and spatial learning and memory as well as in serving as a component in the control of autonomic and vegetative functions (Jacobson and Sapolsky, 1991; Eichenbaum and Otto, 1992; Phillips and LeDoux, 1992). The hippocampus is vulnerable to damage by stroke and susceptible to damage during ageing and repeated stress. Estrogen effects on memory have been reported in animal models and in studies on humans. The memories affected are ones in which the hippocampus plays a role along with the basal forebrain cholinergic system and other neurochemical systems. Rather than one estrogen-regulated process, many types of estrogen actions on different neurochemical and neuroanatomical substrates are likely to underlie the actions of estrogens on cognition and other aspects of behaviour such as mood, pain perception and nociception (McEwen, 2002).

One of the best-known processes regulated by ovarian hormones is the cyclic formation and breakdown of excitatory synapses in the hippocampus (Woolley and McEwen, 1992, 1993). Estrogen treatment increases dendritic spine density on CA1 pyramidal neurons in the hippocampus within 24–72 h of acute administration.

Table I. Potential mechanisms involved in neuroprotective effects of estrogens

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<th>Mechanism</th>
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<td>Modulation of neuropeptides, neurotransmitters and neurosteroids synthesis and activity (Baulieu 1991, 1997; McEwen and Alves, 1999; Baulieu et al., 2001; Genazzani et al., 2003, 2005)</td>
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<td>Reduced cell apoptosis (Nilsen et al., 1999)</td>
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<td>Modulation of neuronal growth and synaptic plasticity (Woolley and McEwen, 1992; Woolley and McEwen, 1993; Gould et al., 1990)</td>
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<td>Modulation of mitochondrial activity (Nilsen and Brinton, 2003)</td>
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<td>Antioxidant properties (Kelly and Wagner, 1999)</td>
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<td>Modulation of brain immune system (Mor et al., 1999)</td>
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<td>Reduced formation of β-amyloid (Greenfield et al., 2002)</td>
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<td>Induction of tau protein (Behl and Holsboer, 1999; Brinton et al., 2000)</td>
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<td>Modulation of the “extrasynaptic volume transmission” (Nicholson and Sykova, 1998; Sykova, 2001; Sykova et al., 2002)</td>
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(Gould et al., 1990). When progesterone is administered after E₂ priming, spine density increases during the first 6–8 h, followed by a rapid return to low baseline levels. Memory performance in rats is strictly temporally related to the hormonal status: 10-μg injection of E₂ is able to improve memory retention until 72 h (Sandstrom and Williams, 2001).

Maintenance of intracellular calcium homeostasis may also be a component of estrogen neuroprotection. Loss of calcium homeostasis correlated to glutamate excitotoxicity is implicated in several brain disorders including stroke, epileptic seizure and the pathogenesis of AD (Mattson, 1992; White and Reynolds, 1996). Glutamate excitotoxicity results from energy depletion, overactivation of glutamate receptors, excessive calcium influx and oxidative stress. Hippocampal neurons pretreated with estrogens and then exposed to excitotoxic glutamate respond with an attenuated rise in intracellular calcium and increased cellular survival. Mitochondria are major targets of estrogen action on calcium homeostasis: it has been demonstrated that E₂ treatment in intact neurons potentiates an increase of mitochondrial sequestration of calcium induced by excitotoxic glutamate through the activation of MAPK. In addition, estrogen effects stabilize mitochondrial membrane potentials, prevent ATP depletion and reduce the generation of oxygen free radicals (Mattson et al., 1997; Wang et al., 2001; Nilsen and Brinton, 2002). These actions of estrogens reduce the production and the actions of free radicals in causing cell damage and promoting cell death through apoptosis.

Another mechanism potentially involved in neuroprotective estrogen effects is the regulation of the Bcl-2 family of genes (Nilsen and Brinton, 2003a). Estrogen treatment up-regulates expression of Bcl-2 immunoreactivity (Brinton et al., 1997). Multiple signalling pathways involved in estrogen regulation of Bcl-2 family genes have been identified: Bcl-2 is elevated with E₂ treatment in an Era⁺/ERb⁻, but not in an Era⁻/ERb⁺, neuronal cell line, and the synchronous activation of MAPK/ERK and Akt signalling pathways has been proposed (Nilsen and Brinton, 2003b). In addition, Brinton and colleagues have proposed a unified model of estrogen-induced neuroprotection that incorporates both novel mechanisms and several existing estrogen-inducible pathways based on the fine regulation of intracellular and mitochondrial calcium homeostasis (Nilsen et al., 2000).

The immune system exerts a series of important actions in the brain. First, it maintains homeostasis, the silent cleaning and refurbishing of the brain. The brain is constantly assaulted by free radicals, metabolic waste, trauma, infectious disease and so on. While the immune response is fundamental to the maintenance of CNS homeostasis, if excessive or not regulated, immune reactions can damage brain cells even destroying neural processes (Bechmann et al., 1999; Silva et al., 2001). There are ‘speed bumps’ or immune checkpoint proteins that must be negotiated before the immune reaction can be enacted. Estrogens may modulate cellular and humoral immune responses. Particularly, estrogen regulation of the brain immune system maintains the immune response under control. This regulation of checkpoint proteins, such as the Fas/Fas-ligand as well as the CD40–CD40 ligand systems, is strengthened by estrogen. Amongst the various CNS cell types, including astroglia, estrogens have been shown to regulate microglial cells at several levels, specifically modulating microglial expression of cytokines and growth factors. Given the relevance of the actions of estrogens on immune-brain interactions, it may be expected that, in the absence of estrogen, less restricted immune responses may hasten clinical brain disorders (Mor et al., 1999).

Glia cells are involved in age-correlated brain modifications, and specific neuroanatomical changes may be observed. For instance, modifications of extrasynaptic ‘volume’ transmission can be seen in aged brains because of the loss of extracellular matrix and of narrower intercellular clefts. Progressive loss of the orientation and number of glial processes (anisotropy) and replacement of neurons by hypertrophy and proliferation of glial processes are also seen with ageing (gliosis). Deposition of macromolecules (e.g. amyloid) can be seen with ageing, as well, and are a typical feature of pathological conditions such as AD. Structural and functional modifications contribute to the reduction of diffusion of neuroactive substances in the extracellular space, therefore, leading to the progressive decline of synaptic and extrasynaptic transmission and of synaptic plasticity, ultimately helping to explain reduced brain performance (Nicholson and Sykova, 1998; Chvatal and Sykova, 2000; Sykova, 2001; Sykova et al., 2002).

In addition to the abundant experimental data (see above) demonstrating the positive effect on cholinergic activity, estrogen also influences two key proteins implicated in AD pathology: tau and β-amyloid. Estrogen induces tau formation, a process that coincides with the enhanced growth of axons and dendrites. Estrogen also acts to reduce the formation of β-amyloid, blunting its neurotoxic effects. The large amyloid precursor protein, encoded by a normal chromosome 21 gene, can be proteolytically processed at alternative sites. At physiological concentrations, E₂ leads to degradation products that are unable to accumulate as β-amyloid (McEwen et al., 1997; Greenfield et al., 2002).

Clinical studies

In addition to the abundant cellular and molecular evidences demonstrating a critical role for sex steroids in modulating and preserving brain function, epidemiological data support the notion that HRT will reduce the likelihood of two common and debilitating conditions linked with menopause and ageing, namely depression and dementia. Although it is not commonly appreciated, dementia is typically accompanied by mood dysregulation. Both depression and anxiety can affect cognitive performance in older persons, and likewise, mood dysregulation and stress mimic and enhance the likelihood of dementia (Henderson, 2000). This clinically recognized relationship between mood and cognition is another example of the brain as a target of sex steroids. Convergent evidence for effects of estrogen on cognitive function comes from studies that have examined cognition in relation to menstrual cycle phase, biomarkers of lifelong estrogen exposure, menopausal symptoms, ER polymorphisms, neuroimaging studies and circulating hormone levels (Phillips and Sherwin, 1992a,b; Berman et al., 1997; Carlson and Sherwin, 1998; Jacobs et al., 1998; Yaffe et al., 2000; Maki et al., 2002). Several observational and longitudinal studies of healthy community-dwelling women suggest that women who use HRT (either unopposed estrogens or estrogens plus progesterone) may perform better on a broad spectrum of cognitive skills or may outperform non-users on more discrete memory measures. Estrogen users showed significantly higher scores on verbal memory, verbal fluency and visual memory and higher scores on the Modified Mini-Mental State Examination (3MS) compared with age-matched non-users (Kampen and Sherwin, 2001).
In a well-characterized ageing cohort in Baltimore, women receiving estrogen performed significantly better than women who had never used estrogen on the Benton Visual Retention Test, a task that measures short-term non-verbal memory and drawing skills (Kawas et al., 1997). In this study, estrogen users seemed resistant to age-associated declines in the test scores. Because CEE 0.625 mg was the most popular drug and dose used to treat post-menopausal women at the time these studies were undertaken, most women who participated were taking CEE 0.625 mg.

More recent randomized controlled trials (RCTs) of estrogens and cognition have better characterized post-menopausal subjects. Several of the RCT studies imply that women given estrogen outperformed placebo-treated women on various psychometric measures, including choice reaction time, attention and concentration, distract ability, verbal memory and abstract reasoning (Henderson, 2000). Sherwin has argued persuasively that estrogen-induced improvement is most apparent on tasks assessing the recall of verbal information, such as recalling details from a paragraph-length narrative (Sherwin, 2003). In general, the magnitude of an estrogen effect in healthy women appears to be modest, but in some circumstances, differences appear to be large enough to be clinically meaningful.

Compared with observational and longitudinal studies, RCTs provide stronger evidence of an estrogen effect on cognition: although the preponderance of findings shows that estrogen users performed better on cognitive tests and experienced less deterioration in aspects of cognition with increasing age than the non-users, findings from longitudinal and observational studies are much more inconsistent than those from the RCTs. Different hypotheses have been argued to explain these differences: the selection bias, because the observational and longitudinal study encompasses self-selected woman, and usually, women taking HRT have better education and higher socioeconomic status. Thus, it is difficult to sort out, in these studies, the effects of genetics and environment from the effects of estrogen.

In addition, generally, data from estrogen-alone users and estrogen plus progestin users have been analysed together because the estrogen group and their scores on cognitive tests were compared with the non-users, constituting a failure to acknowledge that progestins, themselves, have psychoactive properties.

The epidemiological data on the neuroprotective effects of estrogen-based therapy were reviewed by LeBlanc et al. (2001): women who were symptomatic from the menopause had improvement in verbal memory, vigilance, reasoning and motor speed when given HRT. The same meta-analysis of observational studies examining HRT and cognitive function also suggests a significant reduction in the risk of AD among women who have ever used HRT. In particular, the strongest evidence for an association between HRT and AD comes from two cohort studies: the Manhattan Study of Aging (Tang et al., 1996) and the Baltimore Longitudinal Study of Aging (Kawas et al., 1997). These two prospective cohort studies that reported a significantly reduced risk of AD in estrogen users are particularly compelling because they avoid both recall and prescribing-practice bias. In an Italian Longitudinal Study on Aging, ERT was associated with a reduced prevalence of AD in 2816 women [odds ratio (OR), 0.24; 95% CI, 0.07–0.77] (Baldereschi et al., 1998). Analysis of observational data from the Cache County Study (Zandi et al., 2002) suggested a reduction in the risk of AD for past HRT users for 3–10 years. In the same study, the ‘excess’ risk of AD when compared with age-equivalent men disappeared among women who received HRT for >10 years. However, like the longitudinal studies on estrogen use and cognition, these studies on ERT and the risk for AD show possible biases that suggest caution in their interpretation. Nevertheless, their findings should be considered consistent in suggesting a protective effect of ERT with regard to the development of AD.

A major controversy has been launched during the past 2 years concerning the use of HRT in post-menopausal women (Craig et al., 2005; Sherwin, 2005). Recent findings from the Women’s Health Initiative Memory Study (WHIMS) indicated an increased risk in post-menopausal women treated long-term with CEE–medroxyprogesterone acetate (MPA) for diagnosis of probable dementia and mild cognitive impairment compared with placebo. In addition, in the same study, post-menopausal women aged ≥65 years recruited in the WHIMS, estrogen plus progestin did not improve cognitive function when compared with placebo. Therefore, findings from the estrogen-alone arm of WHIMS indicate that women aged ≥65 years treated with CEE had a slightly lower average cognitive function compared with women assigned to placebo. Moreover, in the same study, the incidence of dementia was higher in women receiving CEE alone compared with the placebo group, but this negative trend did not reach statistical significance (Rapp et al., 2003; Shumaker et al., 2003, 2004; Espeland et al., 2004).

The discrepancy between the earlier, smaller RCTs and the WHIMS became apparent, and results from the WHIMS are attributed to the critical issue of the study design: the WHIMS findings do not address the possible role of HRT initiated before the age of 65 years. Group differences in pre-existing risk factors (hypertension, obesity and diabetes) partly explained the increased rates of adverse vascular effects among women taking HRT (Wassertheil-Smoller et al., 2003). Moreover, WHIMS conclusions, like those of the earlier reports of WHIMS study, are related to the specific strength formulation of hormone therapy and not to different formulations or routes of administration. The oral HT used in WHIMS was either unopposed CEE or continuous combined CEE and MPA. Previous reports suggest that MPA counteracts the beneficial effects of estrogen (Nilsen and Brinton, 2003a,b).

The most persuasive explanation for the failure of WHIMS to find a beneficial effect of estrogen on cognition is that the women were too old at the time treatment was initiated for it to have had any protective effect. There are some suggestions that estrogens may be more protective against AD when used by younger post-menopausal women or when initiated at an earlier age.

The analysis of observational data from Cache County Study, suggested an increased risk of AD in older current HRT users, might be viewed as predicting the WHIMS finding of dementia for women initiating after 65 years, with benefit for older women with more typical pattern of past use at a younger age. These issues support the assumption of the presence of a critical period for HRT and related neuroprotection, already raised in the WHIMS accompanying editorial by Schneider (2004). Evidence to support the idea of a window of effectiveness for the initiation of HRT to protect against cognitive ageing is also raised in the studies of Henderson et al. (2003) and Matthews et al. (1999). Similarly, evidence from animal studies indicates that the neuroprotective effects of estrogen and neuroprotection are based on a model of early therapy initiation after the menopause. Ageing may affect the expression of ERs and ERs’ coactivators such as growth factors, neuromodulators and neurotransmitters: for instance, young adult...
rats responded to estrogen with an increased expression of BDNF, which is important for the maintenance of plasticity in the ageing brain, whereas estrogen administered to senescent rats decreased BDNF expression in the olfactory bulb and basal forebrain, suggesting that there is a general decline in hormonal responsiveness of trophin receptors in older, reproductive senescent animals compared with younger animals (Cardona-Gomez et al., 2001; Jezierski and Sohrabji, 2001; Adams et al., 2002). Thus, the effects of estrogen in the brain of young animals might be not predictive of the effects of the same molecules in an aged brain. To test this hypothesis in women, MacLennan et al. presented the results of a pilot study (REMEMBER study) examining the timing of initiation of HRT on later cognitive function in a population-based study of 428 women. Early initiators of HRT performed better than late initiators on the 3MS and were faster than non-users on the Trail Making Test Part A (MacLennan et al., 2006). However, only the results from a more representative population-based study will address more consistent data on the ‘critical window hypothesis’ for HRT.

Conclusion and perspectives

The overall analysis of studies on estrogens and cognitive function from basic science to clinical applications provides some answers for their discrepant findings and suggests new research directions. Convergent evidences suggest that estrogen treatment has positive effects on aspects of memory and cognition when it is administered to naturally menopausal women shortly after the cessation of their menstrual cycles or immediately following surgical menopause. Data from literature suggest that estrogen treatment to older women has scant beneficial or even detrimental effects on cognitive ageing. Thus, the variability of HRT effects across cognitive domains and in relation to age and timing of initiation has yet to be carefully studied. A ‘critical period’ shortly after menopause, when HRT needs to be prescribed to protect cognitive function, has been suggested, but no definitive data demonstrate it. In addition, whether this critical period is related to decreased responsivity of neurons to estrogen with increasing age or to the inability of the hormone to reverse brain dysfunction which may have occurred ≥10 years is not known. Furthermore, understanding how estrogens exert trophic and protective actions should lead to its use as an important therapeutic agent in the maintenance of normal neural function during ageing and after injury. However, results using young animal models may be not predictive of the effects of the same molecules in the aged human brain. Additional studies are needed to understand whether discrepancies between basic science, observational studies and clinical trials reflect the study design, timing of HRT initiation or unsolved insight into estrogen actions.

Acknowledgements

This work was partially supported by a grant from the Fondazione Cassa di Risparmio di San Miniato, San Miniato, Italy.

References


Submitted on June 6, 2006; accepted on August 2, 2006