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Cellular and Molecular Basis of Estrogen's Neuroprotection

Potential Relevance for Alzheimer's Disease

Nibaldo C. Inestrosa,* María-Paz Marzolo, and Andrea B. Bonnefont

Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile

Abstract

Alzheimer's disease (AD) is one of the most common types of dementia among the aged population, with a higher prevalence in women. The reason for this latter observation remained unsolved for years, but recent studies have provided evidence that a lack of circulating estrogen in postmenopausal women could be a relevant factor.

Moreover, follow-up studies among postmenopausal women who had received estrogen-replacement therapy (ERT), suggested that they had a markedly reduced risk of developing AD. In addition, studies among older women who already had AD indeed confirmed that a decrease in estrogen levels was likely to be an important factor in triggering the pathogenesis of the disease.

In this review article, we will discuss the evidence suggesting that estrogen may have a protective role against AD, mainly through its action as: a trophic factor for cholinergic neurons, a modulator for the expression of apolipoprotein E (ApoE) in the brain, an antioxidant compound decreasing the neuronal damage caused by oxidative stress, and a promoter of the physiological nonamyloidogenic processing of the amyloid precursor protein (APP), decreasing the production of the amyloid-β-peptide (Aβ), a key factor in the pathogenesis of AD.

Index Entries: Neuroprotection; estrogen; cholinergic neurons; apolipoprotein E; APP processing; oxidative stress; Alzheimer's disease.

Introduction

Estrogen is a steroid hormone that acts through specific nuclear receptors, ER-α and the recently cloned ER-β (Kuiper et al., 1996; Mosselman et al., 1996), playing an important role in the normal development and differentiation of the brain, as well as in the production and maintenance of sexually dimorphic behavior throughout adult life (Breedlove, 1992;

*Author to whom all correspondence and reprint requests should be addressed.

Jones, 1988). However, the effects of gonadal steroids in the brain are not limited to the control of gonadal function and reproductive behavior. In fact, significant effects on axonal outgrowth, connectivity, and function have been described in a variety of brain regions not directly related to reproductive function (Wieland, 1992; Garcia-Segura et al., 1994; McEwen, 1988). Similarly, the action of estrogen on nonreproductive cognitive functions such as perceptual-spatial skills, learning, and memory has also been described (Breedlove, 1992; Van Harren et al., 1988; Williams and Meck, 1991). Cholinergic neurons, located in the basal forebrain, are involved in learning and memory processes and may be implicated in some of estrogen's effects (Gibbs, 1994).

The mechanism by which estrogen might exert a protective effect upon the aged brain is still unknown, but promotion of neurite outgrowth and synaptogenesis, protection against oxidative stress, and an increase in cholinergic activity, are some of its potential effects. Recent follow-up studies among postmenopausal women who received estrogen replacement therapy (ERT), suggested that they had a markedly reduced risk of developing Alzheimer's disease (AD). This disease is the most common cause of progressive cognitive decline and dementia in the aged population (Yankner, 1996), presenting a higher prevalence in women than men (Blass and Poirier, 1996). In this review article, we will discuss the evidence suggesting that estrogen may have a neuroprotective role and the relevance of this data regarding the AD.

Estrogen Receptors

The estrogen receptor is a member of the thyroid hormone/Vitamin D_3/retinoic acid steroid receptor superfamily and activates genes by its direct binding to specific regulatory elements in DNA (Evans, 1988). Besides direct transcriptional activation, the estrogen receptor also promotes genetic activation via second messengers and/or via trophic factors or its receptors. It is known that there are at least two estrogen receptors, ER-α and ER-β with similar affinity for estrogenic compounds and highly homologous (Kuiper et al., 1996; Mosselman et al., 1996). Nevertheless both receptors can act quite differently, depending on the particular ligand or antagonist binding to them. The tissue distribution of the receptors is different (Mosselman et al., 1996). Moreover, both receptors can dimerize (Pace et al., 1997), increasing the level of complexity to transcription activation in response to estrogen.

The estrogen receptor was the first neural steroid receptor to be recognized. The in vivo uptake of [^{3}H]- estradiol and its binding to the isolated hypothalamus cell nucleus revealed a steroidal specificity similar to that found in uterus (McEwen, 1981; McEwen et al., 1991). Since then, estrogen receptors have been detected in several brain areas including the hypophysis, preoptic area, amigdala, and the nucleus basalis of Meynert (Torand-Allerand et al., 1992; Miranda and Torand-Allerand, 1992). These receptors are located principally in neurons, although glial cells also express them in certain cerebral regions.

Estrogen as a Trophic Factor for Cholinergic Neurons

It has been shown that estrogen treatment significantly increased the neurite outgrowth of acetylcholinesterase-positive fibers from embryonic basal-forebrain tissues transplanted into the anterior chamber of the eye (Honjo et al., 1992), indicating that estrogen may have a direct trophic effect upon basal-forebrain cholinergic neurons. Consistent with this notion was the finding that estrogen was also able to induce or promote the formation of either dendrites in hippocampal neurons or dendritic specializations in PC12 cell neurites expressing both estrogen and neurotrophin receptors; these specializations were also able to induce interneural interactions (Lustig, 1994).

The cholinergic neurons located in the basal forebrain are particularly susceptible to degeneration in AD and have been implicated in age-

and disease-related cognitive decline (Whitehouse et al., 1981). The decreases in brain cholinergic parameters include: a decrease in the number and size of basal forebrain cholinergic neurons, decreases in high-affinity choline uptake, decreases in acetylcholine release, and reduced cholinergic synaptic transmission. A fall in choline acetyltransferase (ChAT) activity has also been described (Fisher et al., 1989). Areas of the basal forebrain not only possess estrogen receptors but also express high levels of aromatase cytochrome P-450, the enzyme responsible for the conversion of testosterone to 17-β-estradiol, the active form of estrogen (Miranda and Toran-Allerand, 1992; Evans, 1988). These findings point to a possible link between estrogen, aging, and neurodegenerative diseases (Whitehouse et al., 1981; Fisher et al., 1989). Indeed, there is evidence to suggest that ERT produces regionally selective effects on basal-forebrain cholinergic neurons that may help to protect against cognitive decline (Tang et al., 1996). These effects varied as a function of both the dose and duration of the treatment, and produced both short-term and long-term effects, each of which could contribute to the positive action of estrogen on learning and memory processes (Gibbs, 1997).

Estrogen and Nerve Growth Factor Receptors

In addition to a direct trophic effect, it is also possible that estrogen may cross-talk with other trophic systems. Recent studies have suggested that mechanisms involving an estrogen-nerve-growth factor (NGF) system in brain may play important roles, particularly with respect to NGF-responsive cholinergic neurons located in the basal-forebrain. Estrogen was shown to directly affect ChAT expression within specific basal forebrain cholinergic neurons, resulting in an increase in ChAT mRNA and protein levels and a corresponding rise in cholinergic activity (Luine, 1992). In turn, the increase in ChAT was accompanied by a decrease in the hippocampal levels of NGF mRNA and subsequently, a decrease in NGF release in the hippocampal formation of the cortex. The drop in NGF levels then led to a reduction in the number of functional NGF receptors located on basal-cholinergic neurons, a decrease in ChAT, and a decrease in cholinergic activity back to basal levels (Gibbs, 1994; Luine, 1992). This evidence therefore suggests that estrogen could significantly affect the expression of ChAT and NGF receptors in specific basal-forebrain cholinergic neurons and thus exert an effect on both cholinergic, and NGF-related systems.

In addition, estrogen was also found to regulate the expression of two neurotrophin receptor mRNAs in prototypic NGF peripheral neural targets, as well as to induce the expression of brain-derived neurotrophic factor (BDNF), a ligand of $p75^{NGFR}$ (Sohrabji et al., 1995). The BDNF gene contains a sequence similar to the canonical estrogen-response element found in estrogen-target genes. These findings again suggest that estrogen probably interacts (directly or indirectly) with pathways such as the NGF-related systems (Torand-Allerand et al., 1992).

This notion is also consistent with a report by Toran-Allerand and coworkers in which $p75^{NGFR}$-containing neurons in the adult basal forebrain were also shown to contain high-affinity estrogen-binding sites. In the medial septum and diagonal band of Broca, over 90% of the NGF-receptor-containing neurons detected, expressed both $p75^{NGFR}$-like immunoreactivity and trkA mRNA. NGF binding to trkA results in activation of tyrosine-kinase activity and autophosphorylation of the receptor (Kaplan et al., 1991a,b; Jing et al., 1992), a step that is essential for NGF-mediated effects. Moreover, this hypothesis was strengthened by the finding that NGF could increase the estrogen-receptor affinity for its ligand at the posttranscriptional level in trkA-positive cells from forebrain cortical explants (Miranda et al., 1996), as well as increase the $p75^{NGFR}$ levels in estrogen-responsive nonneuronal cells that expressed trkA. Hence, it was suggested that in nonneuronal cells, such as astrocytes, NGF could increase the cell's estrogen response, by modifying for

example the affinity of the estrogen receptor for its hormone (Stone et al., 1991). Finally, indirect evidence therefore suggests that estrogen interacts with NGF-related systems and that changes in circulating levels of estrogen could contribute to age-related changes in cholinergic activity as well as hippocampal levels of NGF (McEwen et al., 1972; 1975).

Estrogen Modulation of Apolipoprotein E Expression

In normal humans and in a primate model, the plasma levels of apolipoprotein E (ApoE) were found to be inversely correlated to the estrogen concentration in blood (Kushwaha et al., 1991; Sacks and Walsh, 1994). Part of this effect is probably caused by the induction, by estrogen, of the expression of LDL-receptor (LDL-R) in the liver, increasing the catabolism of the ApoE-associated lipoproteins (Windler et al., 1980). ApoE is a 34-kDa component of various lipoproteins, including chylomicrons, very-low-density lipoproteins (VLDL), and a subset of high-density lipoproteins (HDL) that regulate plasma-lipid transport and clearance by acting as ligand for lipoprotein receptors such as the LDL-R and the LDL-receptor-related protein (LRP) (Mahley, 1989). ApoE mRNA is present in abundance in the brain (Elshourbagy et al., 1985) where at least four other ApoE receptors are expressed: LDL-R (Pitas et al., 1987), LRP (Bu et al., 1994; Ishiguro et al., 1995; Narita et al., 1997), VLDL-R (Christie et al., 1996), and the recently cloned ApoE-R2 (Kim et al., 1996).

ApoE has been implicated in the transport of cholesterol and phospholipids for the repair, growth, and maintenance of membranes that occur during development or after injury (Mahley, 1989; Boyles et al., 1989; Poirier et al., 1991; 1993). In vitro ApoE is a potent neurite-outgrowth agent as part of a lipoprotein complex (Mahley, 1989; Bellosta et al., 1995). Recently, ApoE knockout mice were shown to fail to induce compensatory synaptogenesis in response to hippocampal differentiation (Masliah et al., 1995). ApoE synthesis has been shown to occur in astrocytes both in vivo and in vitro (Stone et al., 1991; Poirier et al., 1991; Pitas et al., 1987) and also in microglia (Stone et al., 1991; Nakai et al., 1996), where ApoE is secreted and associated to a HDL-like particles, that are the lipoprotein forms produced in brain (Pitas et al., 1987; Puttfarcken et al., 1997), which implies that local ApoE synthesis could form part of the neurotrophic and protective role of glial cells in the brain (Banker, 1980; Carpenter et al., 1994; Elkabes et al, 1996).

Recently it has been shown that the administration of estrogen increased the expression of ApoE mRNA in mice brain (Srivastava et al., 1996) and that in vivo, ApoE expression both at the mRNA and protein level, was increased in the CA1 region of the hippocampus in correlation with an increasing synaptic density, when estrogen levels were elevated (during the rat proestrus period) (Stone et al; 1991). This estrogen-induced expression occurred in both astrocytes and microglia and could be reproduced in mixed cell cultures of glial cells, suggesting that heterotypic cell interactions may be necessary for the brain estrogen response (Stone et al., 1991). Moreover, astrocytes obtained from the cerebral cortex of neonatal rats and cultured cells in vitro also produce 17-β-estradiol and express aromatase mRNA (Zwain et al., 1997).

ApoE is present in the senile plaques of AD brains (Namba et al., 1991). Of the various ApoE isoforms, E2, E3, and E4 differ by a single unit of net charge (Weisgraber, 1994), and ApoE4 has been associated with AD as a risk factor for the sporadic and late-onset familial forms of the disease (Corder et al., 1993; 1995; Poirier et al., 1993) as well as with an increased Aβ-peptide deposition in senile plaques (Rebeck et al., 1993; Schmechel et al., 1993). Expression of the *apo*E4 allele is also correlated with the AD susceptibility in as far as that ApoE levels are reduced in the hippocampus and cortical areas in AD subjects, in relation to the *apo*E4 gene dose (Bertrand et al., 1995; Yamada et al., 1995). Furthermore, a reduction in ChAT in AD patients has also been shown to be correlated with an increased *apo*E4 allele copy number (Soininen and

Riekkinen, 1996; Poirier, 1994). Some studies have found that ApoE4 is an inhibitor of neurite-outgrowth in culture (Bellosta et al., 1995; Nathan et al., 1994), yet this result is in discord with a recent study showing that ApoE3 and ApoE4 isoforms have a protective effect against the toxicity of amyloid aggregates (Puttfarcken et al., 1997). Accordingly, there is a gradient in the ApoE mass, with ApoE2> ApoE3>>ApoE4 (Yamada et al., 1995). These data suggest that ApoE may protect against the progression of AD.

Also relevant are the known differences in the lipid interaction between the different isoforms and the binding for their receptors. Both ApoE2 and ApoE3 have preference for HDL, the lipoprotein naturally synthesized in the brain, whereas ApoE4 has a preference for VLDL. Nevertheless, both ApoE3 and ApoE4 bind with the same affinities to LRP and to LDL-R, whereas ApoE2 binds both receptors to a much lesser extent, particularly in the case of LDL-R (Weisgraber, 1994; Mahley, 1996). Therefore, the lower affinity of ApoE2 for its receptors could result in a greater availability of the protein in AD patients and its preferential association with HDL-like particles.

Interestingly, the protective role of estrogen in postmenopausal women has been shown to be independent of the ApoE phenotype (Tang et al., 1996). This supports the notion that part of the protective effect of estrogen could be related to the regeneration of injured brain induced by the neurotrophic action of ApoE itself, which is independent of the isoform expressed (Boyles et al., 1989; Poirier et al., 1991; 1993; Masliah et al., Puttfarcken et al., 1997). The cause of brain injury is not entirely clear. It is therefore feasible that estrogen treatment could override the possible reduction in endogenous estrogen normally produced by astrocytes (Zwain et al., 1997), and in this way induce the synthesis of ApoE in astrocytes and glial cells. This would result in a positive response in the regeneration of neurons, especially at the cholinergic level in the basal forebrain and hippocampus, regions particularly affected in AD. In addition, estrogen could also simultaneously modulate the expression of ApoE receptors either directly (as occurs with LDL-R in liver) or indirectly, enhancing the NGF-mediated pathway that is known to induce ApoE-R2 in PC12 cells (Kim et al., 1996).

Estrogen Regulation of the Amyloid Precursor Protein (APP)-Processing Mechanism

Amyloid-precursor protein (APP) is a transmembrane glycoprotein that acts as a precursor protein of the amyloid-β-peptide (Aβ), a 40–42-amino-acid peptide that forms the principal constituent of senile plaques and cerebrovascular deposits in AD (Soto et al., 1994). The physiological role of APP in the brain is not well-understood. The secreted form of APP (APPs) can function as an autocrine factor to stimulate cell proliferation (Saitoh et al., 1989; Alvarez et al., 1995) and can promote cell-substratum adhesion, possibly through an interaction with extracellular-matrix components such as laminin (Breen et al., 1991; Chen and Yankner, 1991; Kibbey et al., 1993; Milward et al., 1992; Schubert et al., 1991; Bronfman et al., 1996).

The hypothesis that APP or Aβ may play a role in the pathogenesis of AD is supported by at least five lines of evidence:

1. The association of AD with inherited APP mutations (Selkoe, 1996).
2. The association of AD with APP overexpression in Down's syndrome (Selkoe, 1996).
3. The neurotoxicity of Aβ fibrils (Yankner, 1996).
4. The fact that other AD-related genes: presenilin I (chromosome 14) and presenilin II (chromosome 1) increase the levels of released Aβ1-42 (Selkoe, 1996).
5. Individuals expressing the *apo*E4 allele (chromosome 19) present plaques with increasing β-amyloid deposition in their brains (Selkoe, 1996).

In addition to its direct neurotoxic effect, Aβ can potentiate the toxic effects of a variety of neuronal insults including excitatory amino acids (Koh et al., 1990; Mattson et al., 1992), glucose deprivation (Copain et al., 1991), and oxidative stress (Lockhart et al., 1994). There is

evidence suggesting that the structure of the fibril, rather than the sequence of its constituent peptide, is responsible for its toxicity. A possible model postulates that the interaction of amyloid fibrils with cell-surface receptors that recognize β-sheet structure as their ligand, results in an aberrant activation of signal-transduction pathways (Yankner, 1996).

APP is cleaved by at least two distinct enzymatic activities (Haass and Selkoe, 1993; Selkoe, 1994), one of which, designated α-secretase, yields a large soluble amino-terminal fragment of the initial APP: sAPP (soluble APP). The other activity, designated β-secretase, produces a truncate sAPP-like molecule (Seubert et al., 1993; Shoji et al., 1992) and a cell-associated potentially amyloidogenic carboxyl-terminal fragment believed to be further processed to generate soluble Aβ. There is evidence to suggest that estrogen promotes the formation of soluble nonamyloidogenic APP by regulating the metabolism of this protein; in fact, Jaffe et al. (Jaffe et al., 1994) used a ZR-75-1 human breast-carcinoma-cell line to test this hypothesis. These cells, which contain very high estrogen-receptor levels, were treated with 17-β-estradiol for 9 d; levels of sAPP were found to be higher in the medium of treated cells than in the medium of control cells.

There are many mechanisms that could account for the effect of estrogen on sAPP accumulation. This could potentially occur through the activation of APP gene transcription via a member of the steroid/thyroid/retinoid superfamily of nuclear receptors (Landers and Spelsberg, 1992; McEwen et al., 1986), although this seems unlikely considering that cellular APP holoprotein levels were not affected by the 17-β-estradiol treatment (Jaffe et al., 1994). A more plausible explanation is that estrogen stimulates either the amount or the activity of the α-secretase enzyme in ZR-75-1 cells. The latter could in turn be caused by an estrogen-induced increase in the level of or activity of protein kinase C (pkC), seeing as estrogen has been shown to regulate pkC in both normal and neoplastic tissue (Drouva et al., 1990; Maizels et al., 1992). Finally, the effect of estrogen on pkC might be mediated via polypeptide growth factors (Dickson et al., 1986), many of which are known to activate pkC.

This regulatory pathway seems possible, since as reported above, an estrogen-NGF-receptor-related system exists in many areas of the basal forebrain. Furthermore, the fact that cholinergic neurons in the hypothalamus, preoptic area, and basal nuclei of Meynert can be regulated either by neurotrophins and estrogen raises the possibility of a therapeutic approach based on estrogen's capacity to regulate the processing of APP.

Estrogen as an Antioxidant Agent

Evidence of increased oxidative stress in the AD brain has come from studies showing increased lipid peroxidation (Subbarao et al., 1990), increased carbonyl modification of proteins (Smith et al., 1991) and increased oxidation of mitochondrial DNA (Mecocci et al., 1994) in cases of AD. Oxidative stress and the associated free-radical-mediated oxidative damage have been linked to diseases such as atherosclerosis (Parthasarathy et al., 1992) and an increasing number of neuropathological conditions (Coyle and Puttfarcken, 1993).

In the case of oxidative damage to neurons, this has been causally linked to neurological diseases and neurodegenerative disorders such as amyotrophic lateral sclerosis and Parkinson's disease (Coyle and Puttfarcken, 1993; Brown, 1995).

Some evidence suggests that hydrogen peroxide (H_2O_2) mediates the toxicity of Aβ, since this peptide causes the intracellular accumulation of H_2O_2 (Behl et al., 1994). In addition to its direct toxic effect, Aβ increases the susceptibility of neurons to excitotoxins in rodent and human neurons (Yankner, 1996).

To study neuronal-cell degeneration produced by oxidative stress, Behl et al. used the immortalized mouse hippocampal cell line HT22 which is sensitive to Aβ. They investi-

gated induced cell death caused by the neurotoxins Aβ-peptide, H_2O_2 and glutamate, as well as the neuroprotective potency of the lipophilic free-radical scavenger vitamin E and especially of steroid hormones, both of which are known to protect neurons from Aβ toxicity (Behl et al., 1992; 1995). The protection afforded by a concentration of 10^{-5} *M* 17-β-estradiol was as effective as that afforded by 2.3×10^{-4} *M* vitamin E (92 ± 3% survival vs 90 ± 3% survival, respectively). This is in accordance with previously reported data showing that estrogen is a more potent inhibitor of iron-catalyzed lipid peroxidation in brain tissue than vitamin E, as determined in a comparative study of ischemia in male and female mongolian gerbils (Behl et al., 1992; Hall et al., 1991).

The high concentration of 17-β-estradiol necessary for cellular protection in the aforementioned study strongly suggested a hormone-receptor-independent effect, probably mediated by the antioxidant activity of the hormone. HT22 cells did not express functional estrogen receptors as shown after transfection of a reporter plasmid containing estrogen-responsive elements (Behl et al., 1995; Newton et al., 1994) followed by stimulation with 17-β-estradiol. Moreover, in another study, it has been shown that 17-α-estradiol (17-β-estradiol nonestrogenic stereoisomer), which does not bind to estrogen receptors, is also neuroprotective (Behl et al., 1997). Therefore, the neuroprotective antioxidant activity of this hormone appeared to be nonreceptor mediated (for Estrogen's structure, *see* Fig. 1). This antioxidant potential could be explained by the structural analogy of 17-β-estradiol to 21-aminosteroids, the latter being inhibitors of lipid peroxidation at similar concentrations (Braughter and Pregenzer, 1989). In addition, estradiol was also shown to react with peroxyl radicals (Braughler and Pregenzer, 1989; Mukai et al., 1990) and to inhibit cell membrane phospholipid oxidation (Sugioka et al., 1987).

Since it was previously demonstrated that the oxidation of LDL was inhibited by 17-β-estradiol, it has been suggested that this substance might protect against atherosclerosis by acting as an in vivo antioxidant (Rifici and Khachadurian, 1992).

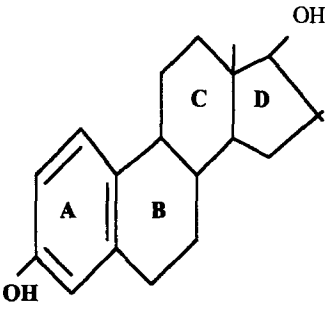

Fig. 1. Molecular structure of estradiol (E2): 17-α-estradiol and 17-β-estradiol have the same structure, differing in the orientation of the 3-OH group on the A ring, which is necessary for the neuroprotective activity (Behl et al., 1997). Both stereoisomers have antioxidant activity, in spite of only 17-β-estradiol binds to ERα and ERβ receptors.

Under the above context, which points to a potential role for estrogen in AD, ERT may serve as a possible treatment for the prevention of postmenopausal neuronal degeneration and dementia (Paganini-Hill and Henderson, 1994; Lobo, 1995).

Potential Benefits of Estrogen Replacement Therapy in AD

ERT has been shown to reduce the risk of cardiovascular disease and osteoporosis in postmenopausal women (Tang et al., 1996; Lobo, 1995). Studies also indicated a reduced risk of stroke and its consequent mortality among estrogen users, as well as a possible role in reducing the risk of AD and increasing a woman's overall quality of life (Bachman et al., 1992; Birge, 1996). Early clinical trials strongly suggested a positive effect for ERT on cognition in cognitively impaired postmenopausal women (Fillit et al., 1986; Honjo et al., 1989).

More recently, postmenopausal women receiving ERT were reported to have a significantly reduced risk of developing AD (Tang et al., 1996; Henderson et al., 1994) which is

known to affect more women than men. Henderson and his group performed an ERT study (Henderson et al., 1994) in which estrogen was given to nondemented elderly women and to women with AD. They found that with regard to age, education, and symptom duration, AD patients receiving oral estrogen replacement did not differ significantly from other AD patients, yet scored significantly better on the cognitive Mini Mental test (mean score of 14.9 vs 6.5, $p < 0.005$). Better cognitive performance by the relatively small number of women with AD who used estrogen compared with the large majority of demented women who did not, has raised the possibility that ERT might also benefit patients who already have this disease (Henderson et al., 1994; Simpkins et al., 1994; Paganini-Hill and Henderson, 1996).

There is retrospective evidence showing that postmenopausal ERT is inversely correlated, both in terms of the dose and duration of the therapy, with the incidence of AD (McEwen et al., 1991; Paganini-Hill and Henderson, 1994). The risk of AD and related dementia decreased with an increased duration of estrogen use, with the lowest risk observed for long-term users taking high doses of the hormone (Tang et al., 1996; Paganini-Hill and Henderson, 1994; 1996). Overall, the above data suggests that estrogen might be highly relevant in AD. In fact, it has been suggested that the relative risk of developing AD is reduced by 30 to 40% among estrogen users (Paganini-Hill and Henderson, 1994), with a fall of approx 50% after 7 or more years of treatment; the severity of the disease is also apparently reduced (Tang et al., 1996; Paganini-Hill and Henderson, 1996).

In long-term studies, Kawas and Morrison examined the records of 524 postmenopausal or perimenopausal women, who were followed up for as many as 16 yr in the Baltimore Longitudinal Study of Aging. They found that women who had received estrogen had a 54% reduction in the risk of developing AD, compared with those who had never taken the hormone (Stephenson, 1996). Although the work showed an association rather than a causal link between ERT and AD, it also suggested that estrogen may have a neuroprotective effect against this devastating illness (for summary, *see* Table 1).

Table 1
Potential Protective and Neurotrophic Effects of Estrogen in the AD Brain

Promotes neurite outgrowth
Promotes synaptogenesis
Increases ChAT levels in cholinergic neurons
Increases expression of neurotrophins
Induces expression of apolipoprotein E in glial cells
Activation of the non-amyloidogenic processing of APP
Increases α-secretase activity → ↑ sAPP
Protects from the cellular damage and death induced by oxidative stress

The exact mechanisms by which estrogen exerts its protective effects are still not fully understood. At this point it is important to mention that the use of ER-α knock-out mice (Lubahn et al., 1993; Korach, 1994) would be very useful in terms of delucidate the molecular targets involved in estrogen's neuroprotective effects, considering that in some tissues as uterus there are effects nonmediated by the two known receptors (Das et al., 1997). Further investigations in this field could lead to a therapeutic approach based on the potential antioxidant and neurotrophic effects of estrogen in the central nervous system, which is strongly affected by neurodegenerative diseases such as AD.

Finally, the data presented in this review hopefully will help to open the possibility that estrogen should be considered for both symptomatic treatment and prevention of AD in pre- and postmenopausal women.

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