In Vivo Effects of Estrogen on Human Brain

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ABSTRACT: Age-related brain disorders such as Alzheimer's disease (AD) are becoming increasingly prevalent. Estrogen replacement therapy (ERT) has shown potential both as a preventive measure and treatment for such disorders. Good evidence from basic science demonstrates that estrogen has multiple protective effects on neurons and neurotransmitter systems, and the effects of ERT can be demonstrated on the human brain using techniques such as functional neuroimaging. However, the evidence for estrogen's having a clinical role in the treatment and prevention of neuropsychiatric disorders is not well established. In this article we review research into the effects of estrogen on the human brain and we consider the role for ERT as a therapeutic tool.

KEYWORDS: estrogen; cognition; depression; aging; Alzheimer's disease; serotonin; acetylcholine; dopamine; noradrenaline

INTRODUCTION

As the proportion of elderly people worldwide increases, age-related disorders such as Alzheimer's disease (AD) as well as normal age-related cognitive decline will become increasingly prevalent. Any measure that can reduce the incidence and morbidity of such problems will have enormous quality-of-life and financial implications for the individual, their carers, and society in general. One intervention that has shown promise is estrogen replacement therapy (ERT). ERT has measurable protective effects on higher cognitive function in postmenopausal women and is thought to lower the risk for an individual in whom AD develops. Fluctuations in endogenous estrogen are hypothesized to play a role in the etiology of several other neuropsychiatric disorders and estrogen may have some peripheral value in their treatment. Although the biological basis of these effects remains unknown, considerable insight has now been gained into the mechanisms of action of estrogen on the brain. Below we review studies that have contributed to our understanding of the effects and mechanisms of the action of estrogen, and we examine the evidence for ERT as a therapeutic tool.

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NEUROPSYCHOLOGICAL STUDIES

The Effect of Estrogen on Age-Related Memory Impairment

In healthy individuals, there is a global decline in higher cognitive abilities from late middle age.[1] The most frequent finding in postmenopausal women on ERT is that verbal memory decline is prevented.[2,3] This is also true when women undergo surgical menopause[4] and of "add-back" estrogen given after gonadotrophin releasing hormone agonist–induced menopause.[5] Maki *et al.* demonstrated that both immediate and delayed verbal recall were enhanced in women taking ERT,[2] in a sample well controlled for education, thereby controlling for the "healthy user bias" (the tendency for hormone replacement therapy [HRT] users to be healthier than nonusers), a potential confounding factor.[6] Not all studies have confirmed a positive effect on verbal memory and the effects on cognition were labelled "small and inconsistent" by one thorough meta-analysis.[7] The variability in findings may be related to issues including differing preparations of HRT, healthy user bias, differing plasma levels of estradiol, and treatment duration.[7] There have been less-well-replicated findings that ERT prevents decline in other cognitive domains, such as visual memory.[8,9] In addition, benefits have been shown on tests of working memory.[10,11] Thus, estrogen replacement therapy may prevent age-related decline in verbal memory (and possibly other aspects of higher cognitive function) in postmenopausal women. Verbal memory is also one of the aspects of neuropsychological functioning severely affected by AD. However, the extent of this protective effect is controversial—larger prospective studies that take into account the shortcomings of earlier studies are needed to determine the true importance of ERT in the prevention of normal cognitive decline.

The Effect of Estrogen on Alzheimer's Disease

Prevention of Alzheimer's Disease

The prevalence of AD in women increases after the menopause and is higher in women than in men, even after adjusting for the greater longevity of women.[12] One possible explanation for this sex difference is the decline in estrogen levels seen after menopause. Early case–control studies of the relationship between use of HRT and the risk of developing AD suggested that taking HRT conveyed no protection on the risk of developing AD.[13,14] Subsequent epidemiological studies found not only a decrease in the relative risk of AD in those taking ERT, but also that the risk was lower both with higher doses of estrogen and longer duration of treatment[15,16] (for review, see Henderson[17]). In two prospective analyses,[18,19] relative risk was reduced to 0.40 and 0.46, respectively, and this was confirmed in two meta-analyses,[7,20] which found relative risks of 0.56 and 0.71, respectively. Thus, ERT appears to lower the risk of developing Alzheimer's disease in postmenopausal women. However, there remain differences between the studies (e.g., differing preparations of ERT and progesterone use). Larger randomized placebo-controlled trials are needed to determine whether estrogen conveys sufficient benefit to warrant its use prophylactically in at-risk or even low-risk individuals.

Treatment of Established Alzheimer's Disease

Initial studies looking at the effectiveness of ERT as a treatment for AD suggested it may be beneficial.[21] Recently, several well-controlled placebo studies have addressed this question in detail.[22–24] Mulnard *et al.* found no benefit on several cognitive outcomes after 12 months on conjugated equine estrogens[22]; indeed placebo was superior to CEE on several outcomes. A temporary improvement on the Mini Mental State Examination at 2 months was seen, however, and in support of this Asthana *et al.* found a benefit of estrogen replacement up to eight weeks with transdermal estradiol.[25] Thus, despite a possible transitory improvement in functioning, recent randomized placebo-controlled trials suggest that ERT taken for 3 months or longer conveys no benefit on established AD. It appears that there may be a "window of opportunity" that is already past when the condition becomes symptomatic. Nevertheless, the only study to use transdermal estradiol was the only positive one, and therefore the type of ERT may be important—an extended study period may yet be warranted. Moreover, in a study examining the benefits of tacrine in AD, women who were established on HRT before starting tacrine had a better outcome than those taking only tacrine.[26] Therefore combining ERT with other treatments for AD may prove beneficial and future studies should address this possibility.

The Effect of Estrogen on Depression

Epidemiological evidence suggests that a vulnerable subgroup of women are more prone to suffer depression at times of reproductive change (e.g. in the postpartum period there is a peak of incidence[27]). This observation has prompted studies examining the effect of estrogen therapy on depression. The small number of studies and methodological difficulties present in many have meant that at present we cannot be sure of the role of estrogen in depression: it may be useful as a prophylaxis against postpartum depression (PPD)[28] and as a treatment for PPD,[29] as well as having a possible adjunctive role in treatment-resistant depression in women.[30] Reports that estrogen may be useful as an antidepressant in the perimenopause[31,32] are difficult to substantiate as estrogen may only be treating menopausal symptoms with the incidental effect of reducing secondary depressive symptoms. ERT is, however, effective in reducing mild depressive symptoms after the menopause.[33] Thus, the extent to which changes in endogenous estrogen are important in the genesis of depression remains unclear. Moreover, estrogen cannot on current evidence be recommended as an antidepressant, except perhaps in circumstances of non-response to standard therapy in women.

The Effect of Estrogen on Schizophrenia

It has been suggested that the later age of onset of schizophrenia in females than males reflects a loss of the antidopaminergic effect of estrogen as levels decline in older age. This hypothesis is partially supported by the observation that psychotic symptoms emerge in some females when estrogen levels are low, for example, during the perimenopausal[34,35] and postpartum periods,[36] and during low estrogen phases of the menstrual cycle.[37] Furthermore, adjunct estrogen treatment has been reported to improve response to antipsychotic medication.[38] Again, however, there is no clear etiologic link between endogenous estrogen and schizophrenia or a ther-

apeutic indication for exogenous estrogen in schizophrenia. Moreover, the relationship between estrogen and dopamine is complex and given acutely may enhance the dopaminergic system (see below).

MECHANISMS OF ACTION OF ESTROGEN ON THE CNS

Animal and in Vitro *Work*

Animal and *in vitro* work has greatly informed our understanding of the basis of observed effects of estrogen on normal aging and neuropsychiatric disorders. For example, ovariectomy in rats leads to a significant decrease in dendritic spine density in the CA1 area of the hippocampus (an area vital for memory function), although estrogen prevents this decline and estradiol levels are significantly related to synaptic spine density.[39] Estrogen is also associated with an increase in *N*-methyl-D-aspartate receptor binding and an increase in the sensitivity of CA1 pyramidal cells to NMDA receptor–mediated synaptic input in rat hippocampal neurons.[40] NMDA is thought to be involved in long-term potentiation, which is a theoretical mechanism for memory formation. Therefore estrogen has biological action on systems and brain areas that are implicated in memory and AD. Estrogen also modulates several processes that are known to form part of the pathology of AD. For example, ovariectomized guinea pigs display increased levels of β amyloid and estradiol treatment significantly reverses this change.[41] Moreover, cultured human microglia show enhanced β amyloid uptake when pre-treated with estrogen.[42] Ovariectomized rats display increased activity of the acetylcholine synthesizing enzyme choline acetyl transferase in the basal forebrain when administered acute estrogen.[43] In addition, estrogen has a number of neuroprotective effects including reducing damage by glutamate.[44] Thus, estrogen displays biological activity in animal and *in vitro* models that is consistent with a protective effect against AD: formation of neural plaques, upregulating the cholinergic system, and decreasing glutamate damage.

Human Studies

A number of studies have provided evidence that ERT modulates neurochemical systems in postmenopausal women. We reported that women treated with ERT show significantly greater growth hormone (GH) response to neuroendocrine challenge with pyridostigmine.[45] In addition, there was a correlation between length of ERT use and GH response. These data provide support to the notion that ERT increases the responsivity of the cholinergic system and that the length of ERT use is related to the integrity of this system. Thus, ERT appears to protect a neurochemical system that is profoundly implicated in AD and memory function, providing a theoretical link between the prophylactic effect of ERT against AD, as well as its protection of verbal memory. We have also reported that central 5-hydroxytryptamine (5-HT) tone (as measured by the prolactin response to *d*-fenfluramine) is similar to that in young women in those taking ERT, but is significantly lower in ERT never-users compared to young women.[46] Therefore the "tone" of the serotonergic system may also be maintained following the menopause in ERT users. Serotonin is also implicated in memory function, is also severely depleted in AD, and is important in mood disor-

ders. Hence, the finding of a protective effect on the serotonergic system is important as it may further help to explain the findings in AD and memory as well as in depression. Dopamine research has reported a significant correlation between plasma estradiol concentration and GH response to apomorphine (a dopamine agonist) in women after estradiol implants.[47] Also, preliminary data from our group suggest that ERT enhances the GH response to apomorphine challenge (Craig *et al.*, unpublished data). Thus the same protective effect of estrogen may also be present in the dopaminergic system, a neurotransmitter system important in schizophrenia.

NEUROIMAGING STUDIES

Structural Studies

There have been two studies that have examined anatomical differences between women on and off ERT. The first of these did not find any differences between the groups in total grey matter, white matter, and lobar brain volumes using a semiautomated technique.[48] However, using voxel-based morphometry, a more sensitive technique that allows the examination of regional differences in grey and white matter volumes, we have found regions where ERT users had a significantly greater volume of grey matter (Robertson *et al.*, unpublished data). These regions were bilateral orbitofrontal cortex, inferior temporal cortices, medial and bilateral cerebellum, right inferior frontal cortex, and left paracentral and right precentral cortices. Thus, ERT may protect grey matter integrity in regions important to higher cognitive functioning and vulnerable to the pathology of AD.

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a technique that allows examination of spectra of energy emitted from regions of interest in the brain. From peaks in this spectrum, we can infer local concentrations of metabolites, such as choline (Cho) (precursor to phosphatidyl choline and hence a measure of membrane turnover) and *N*-acetyl aspartate, a measure of neuronal density. We have used MRS to examine differences in metabolites between ERT users, never-users, and young women.[49] The Cho signal in women who had never taken ERT was significantly higher than that in both young women and ERT users in the hippocampus and parietal lobe, but there was no significant difference between ERT users and young women. In addition, there was a significant association between increased membrane turnover and reduced memory. Therefore, this suggests that the increased membrane catabolism seen with normal aging is reduced by ERT. Furthermore, this membrane catabolism is correlated with visual short-term memory impairment.

Receptor Studies

Advances in neuroimaging and radioligand synthesis now allow us to directly visualize receptor systems in the living human brain. Relatively few studies have capitalized on this technique in the study of the effect ERT on receptor systems. Smith *et al.* studied the effect of estrogen replacement on the brain concentrations of cholinergic synaptic terminals.[50] They found that although there was no overall effect

of HRT, there were relationships between cortical terminal concentrations and length of HRT use. However, some subjects were also using progesterone and so it is difficult to distinguish between the effects of estrogen and progesterone. Thus, there are some data to suggest that ERT may have an effect on cholinergic synaptic terminals, but further studies are required to address this question in women who use estrogen-only HRT. Only one published study has examined the effect of ERT on serotonin (5-HT) receptor density. 5-HT2A receptor density was increased in several brain regions (including orbitofrontal cortex and hippocampus) in women who took 8–14 weeks' ERT, followed by the addition of progesterone for 2–6 weeks.[51] This effect cannot be attributed entirely to the estrogen—there was no significant difference between baseline and the first scan in the estrogen-only phase. However, a trend of increased binding potential was present at the culmination of the estrogen-only stage. A more recent study has found an increase in 5HT2A receptor binding among postmenopausal women in several frontal regions after short-term estrogen administration.[52] Therefore, at present, there is some evidence to suggest that ERT affects the 5HT2A receptor density. No studies have directly looked at the effect of ERT on dopamine receptors, although positron emission tomography (PET) studies of age-related differences in the dopaminergic system of human adults report reducing dopamine D2 receptor concentration with increasing age—especially in frontal and basal ganglia regions[53]—and that this decline is more pronounced in women than in men.[54] Women exhibit a linear decline in caudate D2 density, whereas men exhibit a decline best represented by a second-degree polynomial regression with a much steeper fall between 20 and 40 years of age. Thus, although there have been no studies directly examining the effect of ERT on dopamine receptors, there is evidence that women may show a more pronounced decline in dopamine receptor density with age. Therefore, estrogen may play a role in the maintenance of dopamine receptor systems, and studies examining the effect of ERT on dopamine receptor densities would fill this gap in our knowledge.

PET Dynamic Studies

The effect of estrogen replacement can also be demonstrated on cerebral blood flow (CBF), both at rest and during the performance of cognitive tasks. At rest, the results of two studies examining CBF in women on and off ERT are conflicting.[48,55] In the former (cross-sectional) study, no differences were found, but in the latter (longitudinal) study, there were increases in the CBF over 2 years. The discrepancy between the two studies is likely to represent in part methodological differences, as other studies (e.g., Ref. 56) in different groups have shown an increase in CBF in those taking ERT. Regional cerebral glucose metabolism ratios are significantly higher in ERT users than in women with AD, whereas ERT never-users have metabolic ratios that are intermediate to those of the ERT user and AD groups, but not significantly different to women with AD.[57] In studies of PET brain activation during cognitive tasks, ERT affects regional cerebral blood flow. For example, when ERT users were compared to non-users cross-sectionally on a delayed verbal and visual recognition task, they displayed significant differences in relative brain activation patterns.[48] In the verbal task the non-users showed greater activation in the right inferior frontal cortex, whereas ERT users showed greater deactivation in the right parahippocampal gyrus, right precuneus, and right dorsal frontal gyrus. In the figural

task, ERT users showed greater relative activation in right inferior parietal lobe and deactivation in right parahippocampal gyrus. In a longitudinal study reported in 2000, Maki and Resnick found that ERT users showed increased cerebral blood flow relative to non-users in the hippocampus, parahippocampal gyrus, and middle temporal gyrus over a two-year period.[55] These brain regions are vital to memory and display decreased blood flow in individuals susceptible to AD. A different approach was taken by Berman *et al.,* who studied the effect of a pharmacologically induced menopause (using Lupron) on rCBF during an executive function task.[58] During treatment with Lupron, there was an attenuated pattern of activity that was restored by adding back both estrogen and progesterone. Thus, ERT can modulate blood flow to brain regions that are important to higher cognitive function and that are implicated in the genesis of AD. In addition, ERT is associated with increased rCBF during cognitive tasks.

Functional Magnetic Resonance Imaging

Functional magnetic resonance imaginf (fMRI) is another tool that can be used to compare differences in brain activity between women who take ERT and never-users. For several reasons, this is likely to become the tool of choice for this kind of study: it gives higher resolution than PET, it does not use radioactivity, and it does not involve cannulation. In a placebo-controlled crossover study using verbal and nonverbal working memory tasks, treatment with CEE increased activation during storage of verbal material in the inferior parietal lobule and when storing nonverbal material decreased activation in the same region.[59] CEE also increased activation in the right superior frontal gyrus during retrieval tasks, with greater left hemisphere activation during encoding. This represents a "sharpening" of the hemisphere encoding/retrieval asymmetry effect. Thus, the only fMRI study to date to examine the effect of ERT on brain activation patterns confirms PET findings that ERT is associated with increased blood flow in brain regions associated with higher cognitive function during cognitive paradigms. This is important, as greater rCBF is an indicator that ERT modulates neuronal activity in these regions.

CONCLUSION

Estrogen has been demonstrated to have an extraordinary complexity and range of actions on the central nervous system, including actions on cellular function, neurotransmitter activity, and macroscopic effects on brain structure and function. From the foregoing evidence, we can conclude that: (1) estrogen has numerous neuroprotective effects on the brain at the cellular, neurochemical, and metabolic levels; (2) ERT may protect aspects of higher cognitive functioning from age-related decline; (3) ERT use may be associated with a lower relative risk for AD; (4) ERT modulates regional cerebral blood flow in regions that are involved in higher cognitive function and implicated in AD; and that (5) the links between (1) and (2)-(4) remain unclear. However, despite these conclusions, the role of estrogen in the treatment of neuropsychiatric disorder such as depression and schizophrenia is not clear. At present, estrogen cannot be recommended as a treatment for AD or as a first-line treatment for any other disorders, such as depression or schizophrenia. However, if ERT proves to

have a role in the prevention of AD, the implications for the quality of life of sufferers and financial cost to health care systems would be enormous.

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