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NeuroImage 21 (2004) 364–371

Estrogen- and tamoxifen-associated effects on brain structure and function

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Received 25 June 2003; revised 19 August 2003; accepted 25 August 2003

We evaluated the effects of estrogen and tamoxifen, a selective estrogen receptor modulator, on positron emission tomography (PET) measures of brain glucose metabolism and magnetic resonance imaging (MRI) measures of hippocampal atrophy. Three groups of postmenopausal women were studied, women taking estrogen (ERT+), women with breast cancer taking tamoxifen (TAM), and women not taking estrogen or tamoxifen (ERT−). All subjects received a PET scan, an MRI scan, and cognitive testing. The TAM group showed widespread areas of hypometabolism in the inferior and dorsal lateral frontal lobes relative to the other two groups. The ERT− group showed lower metabolism in the inferior frontal cortex and temporal cortex with respect to the ERT+ group. The TAM group also showed significantly lower semantic memory scores than the other two groups. Finally, the TAM group had smaller right hippocampal volumes than the ERT+ group, an effect that was of borderline significance. Both right and left hippocampal volumes were significantly smaller than the ERT+ group when a single outlier was removed. The ERT− group had hippocampal volumes that were intermediate to the other two groups. These findings provide physiological and anatomical evidence for neuroprotective effects of estrogen.

Keywords: Estrogen; Tamoxifen; Hippocampal atrophy

Introduction

Considerable evidence supports the biological credibility of estrogen as a neuroprotective agent with potential effects on the pathogenesis of Alzheimer's disease (AD). Several epidemiological studies showed that estrogen use in postmenopausal women was associated with a lower incidence and prevalence of AD (Paganini-Hill and Henderson, 1996; Tang et al., 1996; Zandi et al., 2002). In addition, a number of studies have examined the effects of estrogen on cognitive function, both in normal postmenopausal women and in women with AD. Several studies have shown that while estrogen appears to be ineffective in treating AD (Mulnard et al., 2000), it may reduce the risk of cognitive loss and the development of AD in postmenopausal women (Amaducci et al., 1986; Baldereschi et al., 1998; Heyman et al., 1984; Kawas et al., 1997; Paganini-Hill and Henderson, 1996; Tang et al., 1996). However, it was recently reported that estrogen plus progestin therapy increased the risk of dementia in postmenopausal women 65 years or older and did not prevent mild cognitive impairment (Shumaker et al., 2003). Conflicting findings may be due to differences in the types of hormone therapy given, specifically the addition of progestin. More work is needed to further evaluate the effects of unopposed estrogen.

Neuroimaging studies have shown that both structural and functional brain changes occur before the onset of AD and may be used to identify those at risk for the disease. Hippocampal atrophy, as measured by magnetic resonance imaging (MRI), is a good marker of both AD and presymptomatic AD (de Leon et al., 1993; Jack et al., 1992, 1999). Older subjects who are at risk for AD by virtue of poor memory or genetics show hippocampal atrophy in studies using both MRI and computed tomography (CT) (de Leon et al., 1993; Soininen et al., 1995; Tohgi et al., 1997). Similarly, functional neuroimaging studies have led to the identification of functional markers of AD. Reduced blood flow and glucose utilization in the temporoparietal neocortex, as shown with both positron emission tomography (PET) (Frackowiak et al., 1981; Friedland et al., 1983) and single photon emission computed tomography (SPECT) (Claus et al., 1994; Eberling et al., 1993; Small et al., 2000) have been reported consistently in subjects with AD and subjects at genetic risk for AD. Both hippocampal atrophy and temporoparietal hypometabolism are used as biological markers of AD and presymptomatic AD. These same markers may help to identify postmenopausal women who are at risk for AD due to low estrogen levels.

There have been only a few neuroimaging studies evaluating the effects of estrogen on neuroimaging markers of AD in postmenopausal women without dementia. We recently reported greater hippocampal volumes in women taking estrogen than women not taking estrogen (Eberling et al., 2003). To date, this is the only study to report an association between estrogen use and hippocampal volume in postmenopausal women. Two SPECT studies found increased cerebral blood flow in women taking estrogen (Greene, 2000; Ohkura et al., 1995), while one PET study found no effect of estrogen on cerebral blood flow, although very few subjects were studied (Moses et al., 2000). One of the SPECT studies (Greene, 2000) showed reduced temporoparietal blood flow at baseline with

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Available online on ScienceDirect (www.sciencedirect.com.)

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doi:10.1016/j.neuroimage.2003.08.037

increased blood flow to these regions following estrogen treatment. However, this study was not placebo controlled and should therefore be interpreted cautiously. In a different study, women not taking estrogen showed a pattern of glucose utilization that was similar to women with AD with levels of glucose metabolism intermediate to women taking estrogen and women with AD (Eberling et al., 2000). A recent longitudinal PET study found increased glucose metabolism in the lateral temporal cortex over time in women taking estrogen but no change in women not taking estrogen and men (Rasgon et al., 2001). These PET findings are consistent with a neuroprotective effect of estrogen use in non-demented postmenopausal women. Another longitudinal PET study evaluated the effects of estrogen on brain activation (Maki and Resnick, 2000). Women taking estrogen showed greater activation in several brain regions including regions that are critical for memory and that are typically involved in the early stages of AD.

Tamoxifen is a selective estrogen receptor modulator (SERM) that has estrogen antagonistic effects in some tissues and is used for the treatment and prevention of breast cancer. Initially, tamoxifen was used primarily to treat estrogen receptor-positive breast cancers because of its antiestrogenic effects. The use of tamoxifen has expanded, however, to include all forms of breast cancer, and more recently as a preventative agent for women at high risk for breast cancer. The use of tamoxifen in high-risk women is particularly controversial due to the potential risks associated with tamoxifen side effects, and the unknown risks of tamoxifen use in healthy women (Davidson, 1992; DeGregorio, 1992). While few studies have been performed, it has been reported that tamoxifen elicits symptoms common to menopause, postpartum depression, and premenstrual syndrome, including memory and cognitive dysfunction (Arpels, 1996). Perhaps the unifying factor is the drop in estrogen activity associated with all of the aforementioned conditions (Arpels, 1996), although the effects of tamoxifen in the brain have not been well investigated.

Studying postmenopausal women receiving tamoxifen may provide a model for investigating the effects of estrogen on cognition and brain function and could have implications for the clinical care of postmenopausal women and women at-risk for breast cancer. Because tamoxifen has estrogen antagonistic effects in at least some brain regions, we expected that the decrease in estrogen activity associated with menopause would be potentiated by tamoxifen making any changes in brain metabolism and cognitive function more easily detectable. We used PET, MRI, and cognitive testing to evaluate the effects of estrogen and tamoxifen on cerebral glucose metabolism, hippocampal volume, and cognitive function in three groups of postmenopausal women: women taking unopposed estrogen, women taking tamoxifen, and women not taking estrogen or tamoxifen. We used PET and the tracer [^{18}F]-fluorodeoxyglucose (FDG) to evaluate the effects of estrogen and tamoxifen on cerebral glucose metabolism with the hypothesis that tamoxifen use would be associated with reductions in glucose metabolism in at least some brain regions. MRI was used to evaluate hippocampal volume with the hypothesis that estrogen use would be associated with greater hippocampal volumes and tamoxifen use would be associated with reduced hippocampal volumes.

Materials and methods

Subjects were 40 postmenopausal women volunteers who were recruited by advertisement. Ten of the women were undergoing tamoxifen treatment for breast cancer (TAM). Five had also undergone radiation treatment but none had been treated with chemotherapy. The remaining 30 women included 15 women who were currently taking unopposed estrogens (ERT+) and 15 women who were not taking estrogen (ERT−). Of the women not taking estrogen, 12 had never taken estrogen and 3 had not taken estrogen for 7 years or more. Thirteen of the women in the ERT+ group were taking Premarin, one was taking estradiol, and one had an Estraderm patch. Nineteen of the women had undergone hysterectomy, 13 in the ERT+ group, 2 in the ERT− group, and 4 in the TAM group. Aside from breast cancer, all of the women were in good health, had no history of alcohol or drug abuse, and had not been treated for depression or any other neurological or psychiatric condition. Informed consent was obtained from the subjects after the nature of the experimental procedures was explained. Each subject underwent an MRI scan, PET scan, and cognitive testing over a period of 3 months or less. Cognitive testing included the Mini Mental Status Exam (MMSE), the Center for Epidemiological Studies—Depression Scale (CES-D), and tests of semantic memory, attention span, and pattern recognition (Mungas et al., 2000). Briefly, semantic memory was tested by an object-naming task. This task assesses the ability to retrieve verbal information from semantic memory store. Subjects are shown color pictures and are asked to name specific objects. The pattern recognition task assesses the ability to discriminate black and white designs. A stimulus design is presented with six target alternatives, one that is identical to the stimulus, and the subject must indicate which target is the same as the stimulus. The verbal attention span scale measures fixed attention span. Subjects repeat a string of digits read at a rate of one per second. Some items contain non-random sequences of digits, included to facilitate chunking of information and produce subtle gradients of item difficulty. All procedures were done in accordance with the ethical standards of the Institutional Review Board and with the Helsinki Declaration of 1975, as revised in 1983.

PET data acquisition

PET data were acquired using the Siemens-CTI ECAT EXACT (model 921) 47-section scanner in 3D acquisition mode imaging the radiotracer, [^{18}F]-FDG. All subjects were studied in a quiet room with eyes and ears unoccluded 40 min following the injection of approximately 5 mCi of FDG. Initially a transmission image was obtained in 20 min of imaging using a rotating ^{68}Ge source consisting of three rods of about 2 mCi/rod. Subsequently, emission data were acquired in 3D mode for 20 min.

The PET data were analyzed using both statistical parametric mapping with SPM99 (Wellcome Department of Cognitive Neurology, Functional Imaging Laboratory, London) and a region of interest (ROI) approach using software developed in our laboratory (Klein et al., 1997). Images were coregistered and aligned into a standard stereotaxic coordinate system, smoothed at 16 mm full-width at half-maximum, and normalized to mean global activity. The ROI approach was used to further evaluate those brain regions that showed significant differences using the SPM analysis. ROIs were drawn on individual subjects' MRI data using a standard approach previously employed in our laboratory and described in several publications (Klein et al., 1997; Kwan et al., 1999). Because PET and MRI data sets were coregistered, radioactivity counts could be extracted from the PET data. These radioactivity

counts were corrected for the partial volume effects of cerebral atrophy using a two-compartment (brain vs. non-brain) method (Klein et al., 1997). ROIs were normalized to whole brain radioactivity and used in data analyses as count ratios.

MRI methods

All MR images were collected on a GE 1.5 T Signa Horizon LX NV/i System. Four sequences were obtained: a sagittal fast spin echo T2-weighted pulse sequence, an axial oblique spin echo T2-weighted sequence, a T1-weighted, coronal 3D fast spoiled gradient recalled echo (FSPGR), and a fluid-attenuated inversion recovery (FLAIR).

Hippocampal volumes were drawn by a single rater blind to subject classification using an in-house program to create a volume out of the areas traced on contiguous slices (Klein et al., 1997) on the FSPGR data. Volumes for a subset of subjects were drawn twice by two raters to determine inter- and intra-rater reliability using intraclass correlations.

First, the MR data set was resliced, using the sagittal view to align the coronal data set perpendicular to the long axis of the left hippocampal formation. The boundaries of the hippocampus were manually traced on contiguous 1.6-mm coronal slices in the anterior to posterior direction. While the boundaries of the hippocampus were traced on coronal slices, the corresponding sagittal and axial planes for any point on the coronal image could also viewed.

Within the term "hippocampus" we include the dentate gyrus, the hippocampus proper (CA3, CA2, and CA1), the subiculum, and the pre- and parasubiculum. The entorhinal cortex is not included in our measurements. The boundaries of the hippocampus in each subject were identified using anatomical landmarks, as previously described (Wu et al., 2002). To correct for differences in head size, hippocampal volumes were divided by intracranial volume for each subject. Intracranial volume was determined by manually outlining the margin of the inner table of the skull on contiguous 10-mm axial slices.

Statistics

Analysis of variance (ANOVA) was used to evaluate group differences on demographic and neuropsychological variables, along with Fisher's PLSD post hoc tests when the overall ANOVA was significant. Planned comparisons were performed to evaluate differences on neuroimaging variables. For the SPM analysis, comparisons were made using a t statistic with the voxel height threshold (T) set to 0.01 and the extent threshold set to 100 voxels. We did not correct for multiple comparisons at the voxel level because we used a high extent threshold and therefore only large clusters of significantly different voxels were considered. Associations between continuous variables were evaluated by regression analysis.

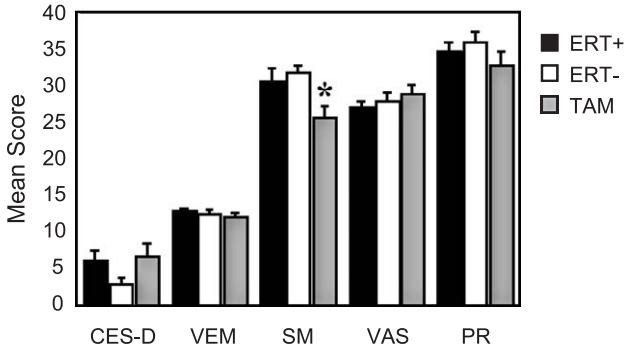

Fig. 1. Neuropsychological test results. Mean scores for each group on the Center for Epidemiological Studies—Depression Scale (CES-D), verbal episodic memory (VEM), semantic memory (SM), verbal attention span (VAS), and pattern recognition (PR). ERT+, women taking estrogen; ERT−, women not taking estrogen; TAM, women taking tamoxifen. Error bars represent standard errors of the mean. * TAM < ERT+, ERT−, $P < 0.05$.

Results

As shown in Table 1, the groups did not differ on age, education, onset of menopause, duration of menopause, MMSE, or body mass index (BMI). The TAM group showed significantly ($P < 0.05$) poorer performance than the other two groups on the semantic memory test. The groups did not differ significantly ($P > 0.05$) on any other cognitive test or on the CES-D. These data are shown in Fig. 1.

The results of the SPM analysis are shown in Fig. 2. Both the TAM and ERT− groups showed significant reductions ($P < 0.01$) in glucose metabolism with respect to the ERT+ group. The ERT− group showed differences in the left inferior frontal cortex, and right superior temporal gyrus. The TAM group showed more widespread bilateral differences predominately in the superior and inferior frontal cortex but also extending into the parietal cortex. These reductions also tended to be greater in the left hemisphere. A similar pattern was seen when comparing the ERT− and TAM groups.

Because the frontal lobes showed the greatest group differences using the SPM analysis, we compared the hand-drawn ROIs for both dorsal lateral and orbital frontal cortex. As shown in Fig. 3, the TAM group showed the lowest mean metabolic ratio values across the frontal regions, with the ERT− group intermediate to the other two groups. These differences were not statistically significant in the dorsal lateral frontal cortex. In the right and left orbital frontal cortex, the TAM group showed lower metabolic ratios than the

Table 1
Subject characteristics

	Duration ERT/TAM (years)	Age	Education (years)	Menopause onset (age)	Menopause duration (years)	BMI	MMSE
ERT+	16.7 (8.7)	67.3 (7.1)	15.7 (2.2)	44 (6.3)	22 (10.2)	27.2 (2.9)	29.5 (0.7)
ERT−		66.5 (6.4)	15.9 (2.0)	48 (5.5)	18 (10.6)	27.0 (4.5)	29.6 (0.6)
TAM	2.3 (1.5)	64.7 (6.7)	15.7 (2.4)	48 (4.4)	17 (8.5)	28.1 (3.7)	29.2 (1.3)

ERT+, women taking estrogen; ERT−, women not taking estrogen; TAM, women taking tamoxifen. Values are means and (standard deviations).

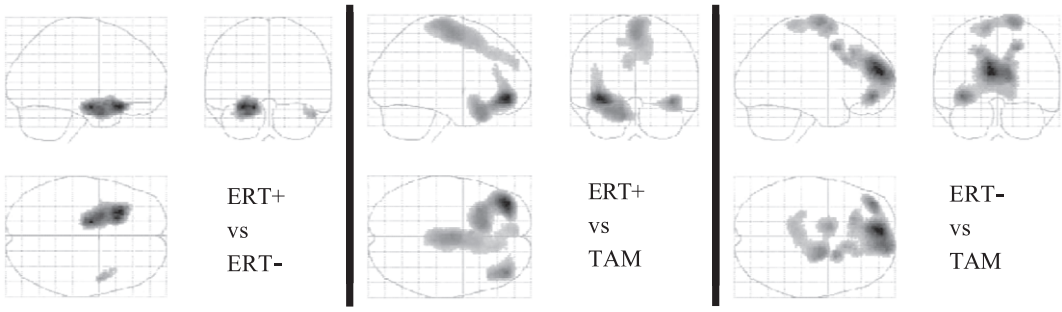

Fig. 2. SPM maps of PET findings. SPM maps showing comparisons between women taking estrogen (ERT+) and women not taking estrogen (ERT−), women taking estrogen and women taking tamoxifen (TAM), and women not taking estrogen and women taking tamoxifen. The shaded areas indicate areas of significant ($P < 0.01$) differences. The right side of the map represents the right side of the brain. ERT+ > ERT−, ERT+ > TAM, ERT− > TAM. Significant clusters for the ERT+ vs. ERT− comparison include Talairach coordinates [−22, −6, −20] left inferior frontal gyrus, [44, 8, −26] right superior temporal gyrus. Significant cluster for the ERT+ vs. TAM comparison include [−8, 58, 28] left superior frontal gyrus, [−38, 46, 10] left middle frontal gyrus, [38, 44, 10] right middle frontal gyrus, [4, −10, 62] right medial frontal gyrus. Significant clusters for the ERT− vs. TAM comparison include [−12, −2, 66] left superior frontal gyrus, [−8, 56, 20] left medial frontal gyrus, [−22, −42, 66] left postcentral gyrus, [20, −16, 72] right superior frontal gyrus, [8, 56, 20] right medial frontal gyrus.

ERT+ group but these differences were only of borderline significance ($P = 0.06$). The ERT− group showed significantly ($P < 0.05$) lower metabolic ratios than the ERT+ group in the left orbital frontal cortex. Because both the ERT− and TAM groups showed reductions in orbital frontal cortex using the SPM analysis, we combined these groups into a single "no estrogen" group. Both right ($P = 0.03$) and left ($P = 0.02$) orbital frontal ratios were significantly lower than the ERT+ group.

Hippocampal and intracranial volumes were both drawn with high intra-rater and inter-rater reliability (rho = 0.96–0.97). Normalized hippocampal volumes are shown in Fig. 4. The TAM group showed smaller right hippocampal volumes than the ERT+ group that were borderline significant ($P = 0.05$) with the ERT− group intermediate. The left hippocampal volumes were also smallest for the TAM group but this difference was not statistically significant ($P > 0.05$). However, one of the subjects in the TAM group had hippocampal volumes that were the largest for all three groups and more than two standard deviations larger than the hippocampal volumes for the other subjects in the TAM group.

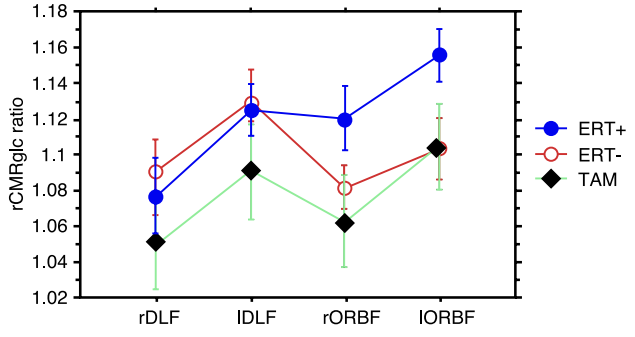

Fig. 3. ROI analysis. Regional cerebral glucose metabolism (rCMRglc) ratios for women taking estrogen (ERT+), women not taking estrogen (ERT−), and women taking tamoxifen (TAM). Regions are right and left dorsal lateral frontal cortex (DLF) and right and left orbital frontal cortex (ORBF). The TAM group showed lower metabolic ratios for rORBF and lORBF than the ERT+ group that were of borderline significance ($P = 0.06$). When the ERT− and TAM groups were combined into a single group, rORBF and lORBF ratios were significantly lower than the ERT+ group ($P < 0.05$).

When this outlier (Moore and McCabe, 1989) was removed, the TAM group showed significantly smaller hippocampal volumes than the ERT+ group in both right and left hemispheres ($P < 0.05$). The ERT− group did not differ significantly from either of the other groups.

Given the hippocampal volume findings, we decided to evaluate glucose metabolism in the hippocampus to determine if the structural changes were associated with physiological changes. Although the SPM analysis did not reveal any group differences

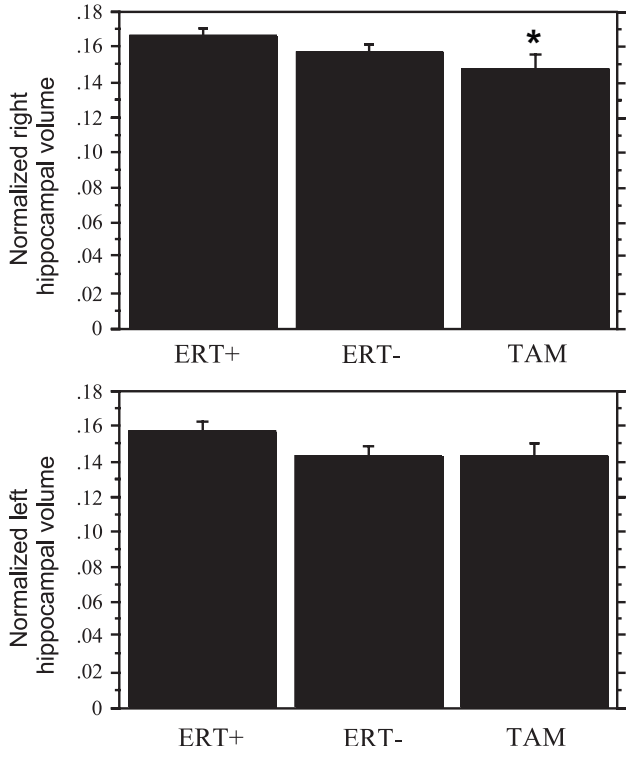

Fig. 4. Normalized hippocampal volumes. Mean normalized hippocampal volumes for the women taking estrogen for each group. Error bars indicate standard deviations. ERT+, women taking estrogen; ERT−, women not taking estrogen; TAM, women taking tamoxifen. * TAM < ERT+, $P = 0.05$.

in the hippocampus, we further explored hippocampal glucose metabolism using the hand-drawn hippocampal regions that were used for the volumetric analysis and normalizing to whole brain metabolism. A simple group comparison of normalized hippocampal glucose metabolism did not show any significant group differences. In addition, a regression analysis did not reveal any significant relationship between hippocampal volume and normalized hippocampal glucose metabolism.

A regression analysis was performed to determine if semantic memory performance, the only neuropsychological test that differed between groups, was associated with either hippocampal volumes or glucose metabolism. None of these relationships were statistically significant ($P = 0.1–0.9$).

Discussion

The effect of estrogen on the brain has been the focus of much recent attention especially with regard to its potential neuroprotective properties. While a number of studies support a protective effect, the underlying mechanisms remain poorly understood and it is not known if there are differential effects of unopposed estrogen and estrogen plus progestin. The effects of unopposed estrogen are of particular interest given the recent report of an increased risk of dementia in women taking estrogen plus progestin (Shumaker et al., 2003). Here, we used tamoxifen as a means of exploring dose–response effects of estrogen on brain structure and function with the assumption that tamoxifen has estrogen antagonistic effects in at least some brain regions. We report that women taking tamoxifen had lower frontal lobe glucose metabolism and smaller hippocampal volumes than women taking unopposed estrogen, with women not taking estrogen intermediate to the other two groups. While the findings in the hippocampus were only of borderline significance, the removal of a single outlier from the TAM group increased the significance level for both right and left hippocampus. These findings support an antagonistic effect of tamoxifen in both the frontal lobes and hippocampus, although the effects in the hippocampus should be regarded as fairly weak.

Estrogen agonistic and antagonistic effects

Tamoxifen is a mixed estrogen agonist/antagonist, with estrogen antagonistic effects in some tissues (e.g. breast) and estrogen agonistic effects in other tissues (e.g. uterine tissue) (Barkhem et al., 1998). The reasons for these mixed agonist/antagonist effects are not clear. There are at least two estrogen receptors in the brain, ERα and ERβ (Shughrue and Merchenthaler, 2000), and tamoxifen binds to each with similar affinity (Kuiper et al., 1997). While these two receptors appear to have different roles in mediating certain biological events (Dubal et al., 2001), the differences between ERα and ERβ are not well understood. While the current findings support an antagonistic effect in the brain, others report agonistic effects, especially in the hippocampus (see below). The mechanism underlying the mixed antagonistic and agonistic effects of tamoxifen, as well as other estrogen antagonists such as CI-628 and raloxifene, appear to be related to different pathways of estrogen action (McEwen, 2002) that involve both genomic and non-genomic mechanisms. Mixed agonist/antagonist genomic effects of tamoxifen may be due to differences in the distribution of ERα and ERβ and involve differences in DNA response elements and important coregulator proteins (Diel, 2002; Horwitz et al., 1996). ERα and ERβ activate genes similarly in cases involving the estrogen response element (ERE). However, in cases in which transcription is regulated via the AP-1 response element, E2 activates transcription with ERα but not ERβ (Paech et al., 1997). Tamoxifen appears to be mainly an antagonist in pathways that involve the ERE as the site of genomic action, but more of an agonist in cases involving the AP-1 enhancer element and ERβ and to a lesser extent ERα (Paech et al., 1997). Thus, ERα and the AP-1 response element appear to be involved in cases in which both estrogen and tamoxifen show agonistic effects. Tamoxifen can be expected to have both agonistic and antagonistic effects on estrogen activity in the brain depending on the pathway of estrogen action (McEwen, 2002). Also, in addition to genomic actions, estrogen and estrogen antagonists can act via non-genomic effects, and may involve both membrane ERs and membrane-associated ERs (Kelly and Levin, 2001; Razandi et al., 1999). Thus, there are several mechanisms whereby tamoxifen may produce either agonistic or antagonist effects. Unfortunately, the effects of tamoxifen on brain tissue have not been well characterized, especially with regard to regional effects.

Estrogen-associated effects in the frontal lobes

While previous neuroimaging studies have found the greatest effects of estrogen on temporal and parietal blood flow and metabolism (Eberling et al., 2000; Greene, 2000; Rasgon et al., 2001), we found the greatest differences in the frontal lobes in the ERT− and TAM groups. Because very few studies have been done to date, it is difficult to put the current findings into context. However, activation studies have shown increased activations in a variety of regions, including the frontal lobes, in association with estrogen use and memory performance (Resnick et al., 1998; Shaywitz et al., 1999). While it is not clear why the frontal lobes were disproportionately affected, it is worth noting that estrogen loss is associated with reduced activity of several neurotransmitter systems that could affect frontal lobe function, although the effect of tamoxifen on most of these systems has not been well studied. For example, rodent studies have shown an increase in 5-HT_{2A} receptor density following estrogen treatment that is blocked by tamoxifen (Sumner et al., 1999), and human PET studies have shown that 5-HT_{2A} receptor density is increased in the dorsal lateral and orbital frontal cortex following estrogen treatment (Moses et al., 2000). Tamoxifen use has been associated with depression and depression is associated with both serotonin dysfunction and reductions in blood flow and metabolism in the prefrontal lobe (Baxter et al., 1989; Kennedy et al., 2001). PET studies have shown increased frontal lobe metabolism and 5-HT_{2A} receptor density following treatment with selective serotonin reuptake inhibitors (Kennedy et al., 2001; Zanardi et al., 2001). Recently, it was reported that tamoxifen blocked the stimulatory effects of estrogen on 5-HT_{2A} receptors and the serotonin reuptake transporter when both E_2 benzoate and tamoxifen were given to ovariectomized rats but had no effect when given alone (Sumner et al., 1999). These findings are consistent with a genomic action involving the ERE or a non-genomic pathway in which tamoxifen acts as an antagonist. Additional studies are needed to determine if the estrogen- and tamoxifen-mediated effects on the serotonin system seen in rodents are likely to generalize to humans. While we excluded subjects with a history of or current depression, subclinical suppression of serotonin

function is a possible mechanism for the frontal lobe hypometabolism we observed. More work is needed to further investigate the effects of tamoxifen on serotonin and other neurotransmitter systems.

Estrogen- and tamoxifen-associated effects on the hippocampus

A number of rodent studies that specifically looked at the effects of estrogen on the hippocampus have shown evidence of neuroprotection and neurotrophism. For example, estrogen increases the density of dendritic spines and synapses in the CA1 region (Gould et al., 1990; Woolley and McEwen, 1992) and regulates the cyclic breakdown of excitatory synapses on dendritic spines (Weiland et al., 1997). In addition, ovariectomy results in a loss of dendritic spine and synapse density in hippocampal pyramidal cells that is prevented or reversed by E2 treatment (Green and Simpkins, 2000), and cell proliferation in the hippocampus is highest when estrogen levels are highest (Tanapat et al., 1999). In an experimental model of apoptotic death in hippocampal granule cells, ovariectomy intensified granule cell apoptosis, which was ameliorated by E2 (Liu et al., 2001).

While there is good evidence that estrogen has protective effects in the hippocampus, the effects of tamoxifen are less clear with evidence for both estrogen agonistic and estrogen antagonistic effects. Tamoxifen has been shown to block the effects of estrogen on apoptotic cell death (Harms et al., 2001), mossy fiber sprouting (Teter et al., 1999), and the toxic effects of amyloid β peptides (Aβ) (Zhang et al., 2001), although it has also been reported that both E2 and tamoxifen showed protective effects against Aβ (Gursoy et al., 2002). There have been several reports that tamoxifen, like estrogen, has neuroprotective effects. Tamoxifen was shown to increase synaptic density in the CA1 region of the hippocampus in ovariectomized rats (Silva et al., 2000), although these findings are in contrast to an earlier in vitro study that found that tamoxifen completely blocked the estrogen-mediated increase in dendritic spine density in hippocampal CA1 cells (Murphy and Segal, 1996). Similarly, another estrogen antagonist, CI-628, blocks estrogen-mediated synapse formation in the hippocampus (McEwen et al., 1999).

Both E2 and tamoxifen have been reported to prevent the decrease in glutamate specific binding to NMDA receptors in CA1 and dentate gyrus in rats following ovariectomy (Cyr et al., 2001). Agonistic effects have also been reported on cholinergic neurotransmission. Tamoxifen and estrogen were equally effective at restoring choline acetyltransferase (ChAT) activity in the hippocampus of ovariectomized rats (Wu et al., 1999). One neuroimaging study used proton magnetic resonance spectroscopy to evaluate concentrations of myo-inositol (MI), a compound implicated in glial metabolism, in frontal white matter, basal ganglia, and hippocampus (Ernst et al., 2002). MI concentrations were lower in women taking estrogen or tamoxifen than in women who were taking neither. This effect is interpreted as neuroprotective. However, there were several shortcomings of the study, including the possibility that the lifetime exposure to endogenous and exogenous estrogens was likely greater in the two treatment groups (Ganz et al., 2002). Thus, these findings need to be cautiously interpreted. Conflicting findings may be due to differences in experimental procedures, including differences in dosages. In fact, the protective effects of tamoxifen against both Aβ and glutamate-induced cell death reportedly show inverted U-shaped dose-response curves (Gursoy et al., 2002). It is worth noting that much of the work done to date is in rodents and does not necessarily apply to humans. In addition, while our MRI findings suggest an antagonistic effect, we cannot say whether this is a direct or indirect effect on the hippocampus or whether the effect is mediated by nuclear or non-nuclear ERs.

Tamoxifen-associated effects on cognition

It has been suggested that tamoxifen has detrimental effects on cognitive function due to antiestrogenic effects (Arpels, 1996), although few studies have actually addressed this issue. We report poorer performance on an object-naming task, a test of semantic memory, in the women taking tamoxifen relative to the other two groups. Semantic memory scores were not associated with glucose metabolism or hippocampal volumes. We did not observe group differences on any of the other cognitive tasks nor on the depression scale. A few case studies have reported negative effects of tamoxifen on memory, mood, and psychiatric function (Lundberg et al., 2000; Ron et al., 1992), although self-ratings of premenopausal breast cancer patients enrolled in a randomized trial did not show a significant impact of tamoxifen on symptoms and perception of anxiety and depression (Nystedt et al., 2000). While a few experimental studies have shown little or no effect of tamoxifen on cognitive performance (Schagen et al., 1999; van Dam et al., 1998), one large study found that subjects treated with tamoxifen were more likely to see their physician with memory complaints than nonusers and tended to make more errors on a clock drawing task (Paganini-Hill and Clark, 2000). It should be noted that effects of tamoxifen on cognition and mood are often confounded by concomitant chemotherapy, although none of the subjects in the current study had ever received chemotherapy. While the mechanism for the impaired semantic memory performance that we observed is not known, one possibility is blockade of synapse formation such as has been reported in the hippocampus (see above). It is also worth noting that high-dose E2 treatment has been shown to improve semantic memory performance in AD patients (Asthana et al., 2001).

Limitations

The current findings are limited by the relatively small sample sizes. The PET data were analyzed using two different methods with similar findings, although the results of the ROI analysis were strongest when the TAM and ERT− groups were combined. This is probably due to the small sample sizes and to differences in the locations of the hand-drawn regions relative to the significant clusters of voxels identified by the SPM analysis.

The effects on hippocampal volume were modest and only statistically significant bilaterally when an outlier was removed from the TAM group providing evidence for a weak effect in the hippocampus. These findings are not entirely consistent with our previous report that women taking estrogen had larger hippocampal volumes than women not taking estrogen and men (Eberling et al., 2003). While the women not taking estrogen showed hippocampal volumes that were intermediate to the other two groups, they were not statistically significantly different from either group. Another limitation is that there were differences in estrogen or tamoxifen use with respect to dosage and duration that could affect the results. However, the ERT+ group only included women who were taking unopposed estrogens and most of the women had been

taking estrogen for a long period of time. In addition, while the ERT− group included three women who had previously taken estrogen, none had taken estrogen within the last 7 years. It is also important to consider that unlike the women in the ERT+ and ERT− groups, all of the women in the TAM group had breast cancer. Hormonal differences that may have put these women at higher risk for breast cancer may also have effects on brain structure and function. In addition, at least some of these women underwent surgery that required general anesthesia. This too may have affected brain structure and function. Finally, breast cancer may result in increased levels of stress that may compromise cognitive function and produce symptoms of depression, including elevated cortisol levels. Relevant to this is the finding that recurrent major depression is associated with reduced hippocampal volumes, perhaps due to elevated levels of glucocorticoids (Sheline et al., 1999). As mentioned above, none of the women in this study, including those with breast cancer, were being treated for depression, had a history of depression, or showed signs of depression. Still, at this point in time, we cannot rule out the possibility that any effects we observed in the TAM group were due to factors associated with having breast cancer. Therefore, the effects on both hippocampal volume and glucose metabolism should be regarded as tamoxifen-*associated* effects rather than direct effects.

Summary and conclusions

We report evidence that estrogen is associated with both frontal lobe glucose metabolism and hippocampal size. Women taking estrogen showed higher frontal lobe glucose metabolism and larger hippocampal volumes than women taking tamoxifen and women not taking estrogen. The women not taking estrogen showed values intermediate to the other groups on both measures. These findings support an antagonistic role of tamoxifen in both the frontal lobes and hippocampus, although additional, well-controlled studies are warranted to further explore the association between tamoxifen and these measures.

Acknowledgments

This work was supported by a UC Davis Health System Award.

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