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Effects of Long-Term Hormone Therapy on Cholinergic Synaptic Concentrations in Healthy Postmenopausal Women*

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ABSTRACT

Experimental evidence suggests that gonadal steroids regulate brain neurochemical systems associated with cognitive function, such as the cholinergic system. This study examines the effect of long-term postmenopausal hormone therapy on the brain concentrations of cholinergic synaptic terminals in women using single photon emission computed tomography and the radiotracer [^{123}I]iodobenzovesamicol ([^{123}I]IBVM). [^{123}I]IBVM labels the vesicular acetylcholine transporter (VAChT) located in the presynaptic terminals of these neurons. Sixteen healthy women treated with hormone therapy since the menopause and 12 women not treated with hormones were studied. There were no significant differences in regional IBVM binding indexes between the 2 groups. The length of hormone replacement therapy correlated positively with VAChT binding indexes in multiple cortical areas ($P < 0.05$): frontal cortex (Spearman rank correlation: $\rho = 0.79$), parietal cortex ($\rho = 0.62$), temporal cortex ($\rho = 0.80$), anterior cingulate ($\rho = 0.71$), and posterior cingulate ($\rho = 0.63$), but not in the basal ganglia ($\rho = 0.35$; $P = 0.2$). An earlier onset of menopause in hormone-treated women was associated with higher VAChT indexes in the anterior cingulate ($\rho = -0.56$; $P = 0.02$) and posterior cingulate ($\rho = -0.63$; $P = 0.01$). The opposite was found in the posterior cingulate of women not treated with hormones ($\rho = 0.58$; $P = 0.04$). Women treated with estrogen alone also showed higher VAChT indexes than women treated with estrogen and progestin in the posterior cingulate cortex (by Mann-Whitney U test: $z = 2.42$; $P = 0.015$). Although an overall effect of postmenopausal hormone therapy was not found, associations between an index of cortical cholinergic terminal concentrations and the length of hormonal replacement suggest that hormone therapy may influence the survival or plasticity of these cells in postmenopausal women. The data also suggest possible differential effects of estrogen and estrogen with progestin treatments in brain areas critical for cognitive processing. (*J Clin Endocrinol Metab* **86:** 679–684, 2001)

FOR MANY POSTMENOPAUSAL women estrogen replacement therapy (ERT) has improved their quality of life by ameliorating hot flashes and genital atrophy, and preventing heart disease and osteoporotic fractures. Recently, several lines of evidence have suggested that estrogen may also be important in the protection of the central cholinergic system, which is intimately involved in memory and cognition. Normal aging is associated with reductions in cholinergic functional markers, such as choline acetyltransferase (ChAT), but a relative preservation of cholinergic cells and terminals (1). Alzheimer's disease (AD) is associated with severe reductions in ChAT and diffuse degeneration of cholinergic terminals, which is more prominent in the temporal lobes (2, 3). Epidemiological data indicate that postmenopausal hormone therapy may reduce the risk or delay the onset of Alzheimer's disease, yet the neurochemical substrates involved in this decreased risk are unknown (4–10).

Studies in experimental animals have suggested that estrogen may influence brain function by 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 (11). Estrogen is known to provide trophic support to cholinergic cells and to regulate various markers of cholinergic function, including ChAT and acetylcholine release (12–14). In this regard, estrogen and intranuclear estrogen-binding sites colocalize and regulate the expression of neurotropins such as nerve growth factor, its receptor trkA, and brain-derived neurotropic factor (11, 15). In turn, these are involved in regulating neuronal survival, regeneration, and plasticity (16). The relationship between menopausal status or postmenopausal hormone therapy and the integrity of markers of cholinergic cells and their projections has not been explored directly in humans.

In human subjects, there are data suggesting that estrogen therapy may be beneficial in the prevention of AD, but not necessarily in the treatment of established cognitive impairment. Postmenopausal ERT in healthy women is associated with improvement or preservation of various cognitive functions (17, 18). Studies initially found that estrogen may reduce cognitive decline in memory-impaired postmenopausal

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women (19) and improve the effectiveness of tacrine, a cholinesterase enzyme inhibitor used in the treatment of AD (20). However, a recent placebo-controlled multicenter trial in mild to moderate AD did not support beneficial effects of ERT in the treatment of postmenopausal women with AD (21). Thus, the study of estrogen as prevention against cognitive impairment is of particular importance. This study was therefore designed to examine whether long-term postmenopausal hormone therapy has neuroprotective effects on the cholinergic system in healthy women, using the vesicular acetylcholine transporter (VAChT) as an index of cholinergic synaptic terminal density (3, 22–24).

We examined whether healthy postmenopausal women treated with long-term ERT or estrogen plus progestin replacement therapy (HRT) would show higher regional brain concentrations of cholinergic synaptic terminals than comparable women never given hormone therapy. To further determine the relationship between hormone replacement and cholinergic synaptic terminal measures, their associations with the length of replacement and the age at menopause were also examined as possibly contributing to the variance in the data. For that purpose, we used single photon emission tomography (SPECT) with the radiotracer [^{123}I]iodobenzovesamicol ([^{123}I]IBVM), a marker of VAChT. This protein is exclusively located in cholinergic neurons, mediating the transport of acetylcholine from cytoplasm into synaptic vesicles (24). Its distribution is thought to reflect and provide a measure of cholinergic synaptic concentrations (22). VAChT concentrations, as measured with [^{123}I]IBVM and SPECT, decline slightly with advancing age and are reduced in neurodegenerative disorders that affect cholinergic cell survival (23). In the present report [^{123}I]IBVM binding indexes were obtained in neocortical areas thought to be involved in various forms of cognitive processing and in the pathophysiology of AD.

Subjects and Methods

Subjects

Two groups of healthy postmenopausal women were recruited by advertisement. They were selected so that they were 60 yr old or older and had either received hormone therapy without interruption after the menopause or no replacement (no-HRT). The subjects receiving hormone therapy all started it within 2 yr of menopause. All subjects on replacement were using the same dose and preparation of estrogen (conjugated equine estrogens, Premarin, Wyeth-Ayerst, Philadelphia, PA; 0.625 mg/day) with or without cyclic or continuous medroxyprogesterone acetate at various doses ranging from 2.5–10 mg. Menopause was defined as the absence of menstrual periods for 1 yr or the time of hysterectomy with bilateral salpingo-oophorectomy. In subjects with a prior hysterectomy without bilateral salpingo-oophorectomy, menopause was defined as the onset of hot flashes. Eight subjects had been treated with continuous ERT, and eight with estrogen and progestin. The hormonally treated and no-HRT samples were group age matched. The timing of the scanning varied and was not standardized to a specific time in the progestin cycle for the eight combined estrogen and progestin users.

After an initial phone screening, subjects were personally interviewed for medical, neurological, and psychiatric histories and review of systems. The Mini-Mental State Examination (25) and Geriatric Depression Scale (26) were also administered at that time. Subjects were free of significant general medical, neurological, or psychiatric illness; head injury with loss of consciousness; and drug or alcohol abuse or dependence and were right handed, nonsmokers, and taking no medications with actions on the central nervous system. Mini-Mental State Examination scores were higher than 26 for all subjects. After a full description of the study, written informed consent was obtained. All procedures were approved by the University of Michigan's institutional review board and radiation safety committee.

Hormone assays

Blood samples for the determination of estradiol and testosterone were obtained immediately before scanning. After collection, all samples were refrigerated, centrifuged, and stored at −30 C until analysis. Plasma estradiol and testosterone were determined using an enhanced chemiluminescence immunoassay instrument (reagents from Amersham Pharmacia Biotech UK Ortho Clinical Diagnostics, Inc., Rochester, NY). For the estradiol assay, the intra- and interassay coefficients of variation were less than 5.0% and 8.92%, respectively. The sensitivity of the estradiol assay was 15 pg/mL. The cross-reactivity at 50% of zero standard binding was less than 0.5% for estriol, less than 3.1% for estrone, and 0% for other steroids. For the testosterone assay, the intra- and interassay coefficients of variation were less than 5.0% and 7.90%, respectively. The cross-reactivity at 50% of zero standard binding was less than 0.5% for androstenedione, less than 0.6% for 5α-dihydrotestosterone, less than 0.3% for 5β-dihydroxytestosterone, less than 6.2% for 11β-dihydrotestosterone, less than 10% for 11-ketotestosterone, less than 3.6% for nortestosterone, and 0% for all other steroids.

Scanning procedures and image processing

Images were obtained in a Prism 3000 SPECT tomograph (Picker International, Cleveland, OH). Subjects were positioned in the scanner gantry, and an iv line was placed in an antecubital vein. Blood samples for the determination of estrogen and testosterone levels were obtained through the same line immediately before radiotracer administration.

IBVM was prepared by oxidative radioiodination of the respective (−)-5-tributyltin precursor, with specific activity greater than 1.1×10^9 MBq/mmol (30,000 Ci/mmol) (27), and administered iv as a bolus at a dose of 370 MBq (10 mCi). Oral Lugol's solution (one drop, three times daily) was administered for 1 day before and for 3 days after the study to minimize iodine uptake by the thyroid gland. An oral laxative (Dulcolax, Ciba-Geigy, Summit, NJ) was administered the evening after the [^{123}I]IBVM injection to reduce radiation exposure to the bowel (22). Under these conditions, the effective dose equivalent for [^{123}I]IBVM was 14.2 mSv (1.42 rem).

The scanning protocol consisted of three imaging periods: 1) 0–60 min posttracer administration, providing an index of tracer transport and general brain anatomy; 2) 3–4 h, for striatum and cortex anatomy; and 3) 22–23 h. This latter measure was used to determine the VAChT binding index, as previously described and validated (22, 23). After correction for attenuation using a first order Chang algorithm, images were reconstructed to a full width-half maximum resolution of 13.5 mm in the center of the axial and transverse planes.

Head motion within each scanning period was corrected for by using scalp radioactive fiducial markers. Frames obtained on the first and second days were coregistered using a computer algorithm based on image similarity. Image sets were then aligned to the intercommisural line and transformed anatomically to a standard stereotactic coordinate system, as described in previous publications employing this radiotracer (3, 22, 23).

Regional tracer activities were extracted using stereotactically based predefined volumes of interest (VOIs) applied to surface projection maps for subsequent analyses. These predefined VOIs and their corresponding Brodmann cortical areas (BA) were: frontal cortex: BA, 6, 8, 9, 10, 11, 44, 45, 46, and 47; temporal cortex: BA, 21, 22, 37, and 38; parietal cortex: BA, 5, 7, 39, and 40; anterior cingulate cortex: BA, 24 and 32; and posterior cingulate cortex: BA, 23 and 31. Cerebellar activity was also extracted using a predefined VOI. VOI activities were averaged (area weighted) within each VOI and measured separately for both hemispheres (23). Left- and right-sided measures were further averaged to provide a mean value for each anatomical region and reduce the number of multiple comparisons. One additional area rich in cholinergic terminals, but typically not found altered in AD, the striatum (caudate nucleus and putamen) (23), was selected as a control region to test for the regional specificity of findings.

A VAChT binding site density index was obtained by dividing the

TABLE 1. Demographics and subject characteristics

	Hormone-treated group			No HRT group (n = 13)
	All HRT subjects (n = 16)	Estrogen only (n = 8)	Estrogen + progestin (n = 8)	
Age (yr)	65 ± 4	64 ± 3	65 ± 4	67 ± 6
Education (yr)	15 ± 2	15 ± 2	15 ± 3	16 ± 3
Age at menopause (yr)	48 ± 5	45 ± 6	50 ± 2	51 ± 3
Bilateral SO	4	4	0	0
Unilateral SO	2	2	0	1
Yr postmenopause	17 ± 6	19 ± 5	15 ± 5	17 ± 6
Alcoholic beverages/week	4 ± 6	1 ± 2	7 ± 7	3 ± 3
MMSE scores	28.0 ± 1.7	27.4 ± 1.6	28.6 ± 1.6	27.5 ± 1.8
Plasma estradiol (pg/mL)	89 ± 39[a]	97 ± 50	82 ± 26	20 ± 13
Plasma testosterone (ng/mL)	0.28 ± 0.12	0.25 ± 0.15	0.31 ± 0.09	0.27 ± 0.07

Data are expressed as the mean ± SD. SO, Salpingo-oophorectomy; HRT, hormone replacement therapy; MMSE, Mini-Mental State Examination. Plasma levels of estradiol and testosterone were obtained immediately before scanning.

[a] Significantly different from no HRT group, by Mann-Whitney *U* test; z = 4.43; $P < 0.0001$.

activity within each VOI by that in the cerebellum. This measure has been shown to be proportional to and to introduce less statistical uncertainty than that of more complex and cumbersome three-compartment tracer kinetic analyses (23).

Statistical analysis

Data are expressed as the mean ± SD. Possible differences in regional VAChT binding indexes between groups were examined with independent nonparametric Mann-Whitney *U* tests with transformation to z-scores, at a statistically significant level of $P < 0.05$. No correction for multiple comparisons was applied to the data. Correlations between regional binding levels and measures of estrogen exposure and menopausal age were tested by Spearman rank correlations with correction for ties, at a significance level of $P < 0.05$. The significance of these correlations was also interpreted based on their consistency across regions and measures of estrogen usage.

Results

Effects of hormone therapy on cholinergic synaptic density in postmenopausal women

Subject groups were not different in age, educational level, age at menopause, years postmenopause (by Mann-Whitney *U* test, $P > 0.05$; Table 1). A larger proportion of women treated with long-term postmenopausal hormone therapy had received hysterectomies and bilateral salpingo-oophorectomies (four women *vs.* none in the no-HRT group), which may account for the slightly lower (albeit not significantly different) age at menopause in this group (Table 1). Plasma estradiol levels were higher in the women treated with hormones, as would be expected (z = 4.43; $P < 0.0001$), although a high degree of variability was noted in the plasma estrogen levels for both groups of women (Table 1).

No significant differences in regional VAChT binding measures were detected between women treated with long-term postmenopausal hormone therapy and those who did not receive hormone therapy after the menopause (Table 2). As an equal number of women taking hormones had been treated with or without progestin, a secondary analysis was performed to test whether the mode of replacement affected VAChT binding indexes. Women treated with estrogens alone showed significantly higher VAChT concentrations than women treated with estrogen and progestin (Table 3) in the posterior cingulate (z = 2.42; $P = 0.015$), with nonstatistically significant trends in the same direction for the temporal cortex (z = 1.79; $P = 0.07$). These differences between hormone-treated subgroups could not be explained solely by demographics or subject characteristics, including age, age at menopause or length of replacement, which were not significantly different between them (by Mann-Whitney *U* test, $P > 0.05$).

TABLE 2. Regional [^{123}I]BVM binding indexes to VAChT sites in postmenopausal women treated with or without long-term hormone replacement therapy

	Hormone-treated group (n = 16)	No-HRT group (n = 13)
Frontal cortex	0.90 ± 0.22	1.02 ± 0.18
Parietal cortex	0.73 ± 0.16	0.78 ± 0.16
Temporal cortex	0.93 ± 0.24	1.03 ± 0.24
Anterior cingulate	1.30 ± 0.34	1.41 ± 0.26
Posterior cingulate	0.86 ± 0.20	0.94 ± 0.25

Data are expressed as the mean ± SD. HRT, Hormone replacement therapy. No significant differences were detected between groups (by Mann-Whitney *U* test, $P > 0.05$).

TABLE 3. Regional [^{123}I]BVM binding indexes to VAChT sites in postmenopausal women treated with long-term hormone replacement therapy, with or without progestin

	Estrogen alone (n = 8)	Estrogen + progestin (n = 8)
Frontal cortex	0.97 ± 0.21	0.83 ± 0.21
Parietal cortex	0.80 ± 0.14	0.67 ± 0.16
Temporal cortex	1.01 ± 0.20	0.85 ± 0.26
Anterior cingulate	1.34 ± 0.33	1.26 ± 0.36
Posterior cingulate	0.96 ± 0.14[a]	0.77 ± 0.21

Data are expressed as the mean ± SD.

[a] Significantly different between groups, by Mann-Whitney *U* test: z = 2.42; $P = 0.015$.

Relationship of synaptic density with age at menopause and length of replacement

In women treated with long-term postmenopausal hormone therapy (n = 16), significant positive correlations were obtained between the number of years of replacement and cortical binding indexes, as follows: frontal cortex, $\rho = 0.79$ and $P = 0.0021$ (Fig. 1); parietal cortex, $\rho = 0.62$ and $P = 0.016$; temporal cortex, $\rho = 0.80$ and $P = 0.0019$; anterior cingulate, $\rho = 0.71$ and $P = 0.0063$; and posterior cingulate, $\rho = 0.63$ and $P = 0.014$ (but not in the striatum, $\rho = 0.35$ and $P = 0.2$). No

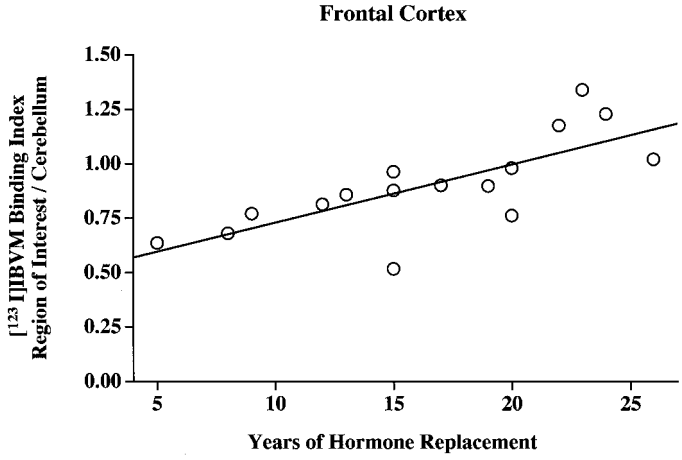

FIG. 1. Relationship between [^{123}I]IBVM binding indexes in the frontal cortex of postmenopausal women and the length of HRT. IBVM binding indexes correlated positively with the years of hormone therapy after the menopause in the frontal cortex (Spearman rank correlations, $\rho = 0.79$; $P = 0.0021$) and other neocortical regions (see text), but not in the striatum.

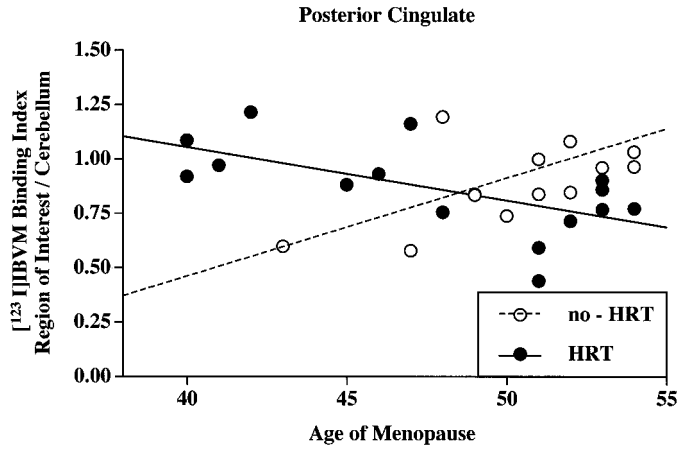

FIG. 2. Relationship between [^{123}I]IBVM binding indexes in the posterior cingulate cortex of postmenopausal women and age at menopause. In hormone-replaced women, IBVM binding indexes correlated negatively with age at menopause in the anterior cingulate ($\rho = -0.56$; $P = 0.02$) and posterior cingulate ($\rho = -0.63$; $P = 0.01$). In the case of women who did not receive hormone replacement after the menopause, positive correlations were observed between age at menopause and IBVM binding indexes in the temporal cortex ($\rho = 0.55$; $P = 0.05$) and posterior cingulate ($\rho = 0.58$; $P = 0.04$).

significant correlations were obtained between regional VAChT binding indexes and chronological age ($P > 0.05$). However, significant negative correlations were observed between regional binding measures for some of the above regions and age at menopause: anterior cingulate, $\rho = -0.56$ and $P = 0.02$; and posterior cingulate, $\rho = -0.63$ and $P = 0.01$ (Fig. 2). Correlational values for other brain regions examined that did not reach statistical significance were: frontal cortex, $\rho = -0.45$ and $P = 0.08$; temporal cortex, $\rho = -0.45$ and $P = 0.08$; parietal cortex, $\rho = -0.19$ and $P = 0.5$; and striatum, $\rho = -0.37$ and $P = 0.17$.

In the case of women who did not receive hormones after the menopause (n = 13), no significant correlations were obtained between years postmenopause or chronological age and regional binding indexes (Spearman rank correlations; $P > 0.05$). However, significant positive correlations were observed between age at menopause and regional VAChT binding for the temporal cortex ($\rho = 0.55$; $P = 0.05$) and posterior cingulate ($\rho = 0.58$; $P = 0.04$; Fig. 2). Nearly significant associations were noted in the parietal cortex ($\rho = 0.54$; $P = 0.06$). No significant correlations were obtained for the frontal cortex ($\rho = 0.47$; $P = 0.1$), anterior cingulate ($\rho = 0.25$; $P = 0.4$), or striatum ($\rho = 0.09$; $P = 0.8$).

Discussion

The present report describes the possible influence of long-term hormone replacement therapy on a measures of cholinergic neuronal terminal concentrations (VAChT binding indexes in neocortical regions) in a sample of healthy postmenopausal women. A positive association between the length of HRT and cholinergic synaptic terminal concentrations in cortical brain regions is described. Also, significant differences in cholinergic terminal measures were noted between women treated with estrogen alone and those treated with estrogen and progestin.

No overall differences in VAChT binding indexes were observed between women treated with long-term postmenopausal hormone therapy and those who did not receive hormones at any point after menopause. However, significant correlations were noted between the length of hormone replacement and the age at menopause, and VAChT binding indexes. In women treated with hormones, VAChT binding indexes were positively correlated with the number of years of hormone replacement in all neocortical areas, with strongest associations in the frontal and temporal lobes and anterior cingulate. These correlations were not observed in the striatum and argue for a preferential effect of postmenopausal hormone therapy on neocortical cholinergic terminal preservation or plasticity. This possibility is supported by experimental data showing trophic effects of estrogen and progesterone on cortical cholinergic neurons in primates (28) and prior studies showing similar effects in hippocampal cells (16).

In the hormone-treated sample, negative correlations were also obtained between the age at menopause and VAChT binding in the anterior and posterior cingulate. The above relationships, although somewhat less robust statistically, appear to be consistent with the hypothesis that postmenopausal hormone therapy is associated with a preservation of cholinergic neuronal terminals: the younger the replacement was initiated or the longer it was maintained, the higher the concentration of VAChT sites.

In the case of women not treated with hormones, the opposite relationships were found, with age at menopause being positively correlated with posterior cingulate and temporal cortex VAChT binding indexes. Again, although statistically not as compelling as the findings in HRT-treated women, this is consistent with a longer exposure to gonadal steroids being associated with trophic support of cholinergic neurons. The later the age at menopause in non-HRT-treated women, the longer the exposure to ovary-produced gonadal steroids and the higher the VAChT concentrations.

The data presented are not likely to be influenced by non-

specific effects of HRT or the absence of hormone replacement on regional brain volumes or on regional cerebral blood flow. The utilization of HRT has not been associated with detectable changes in regional brain volumes or ventricular size in comparable samples studied by others (29, 30). Also, the regional brain distribution of [^{123}I]IBVM does not appear to be influenced by even the large reductions in regional cerebral blood flow that is present in neurodegenerative disorders such as AD (23).

Although the lack of group differences in [^{123}I]IBVM binding indexes between estrogen users and nonusers may argue against an overall effect of HRT/ERT on cholinergic cell survival, several factors should be taken into consideration in interpreting this negative result. VAChT binding sites appear well preserved with normal aging, and therefore may not be a sensitive marker of cholinergic function as much as a measure of cholinergic cell survival. This is supported by findings of large reductions in VAChT binding indexes in the neocortex of patients diagnosed with early-onset AD, but much less severe and more localized reductions in late-onset AD and relatively small declines with advancing age (23). It is reasonable to assume that numerous factors, such as health status, genetics, activity level, or substance abuse, influence the preservation of cholinergic cells in older individuals as well as the length of hormone replacement and the age at menopause. Also, it is arguable whether the two samples studied were indeed comparable, because the reasons for selecting postmenopausal hormone therapy were not examined in this study. It would be of interest to examine whether postmenopausal hormone therapy effects would be detected in randomly selected women across broader socioeconomic groups in a larger sample, where the effects of possible confounding variables could be studied.

An unexpected finding of this study was a higher concentration of cholinergic synaptic terminals in the posterior cingulate of women treated with estrogen alone compared with women treated with estrogen and progestin. This finding will need to be confirmed in further studies due to the sample size limitations of the present report. However, its possible importance is underlined by the magnitude of the differences (a mean 25% higher VAChT binding index in women treated with estrogens alone), its regional localization, and its public health implications. In this regard, concerns have been raised about a possible increased risk of breast cancers in postmenopausal women treated with estrogen plus progestins compared with that in women treated with estrogens alone (31).

The posterior cingulate cortex has been recently identified as a brain region involved in very early stages of AD. Profound reductions in the metabolic function of this area were identified in patients who presented with isolated memory impairments and later developed dementia (32). If a differential effect of estrogen and progestins can be confirmed in this brain region, this may have important implications for the prevention of AD and related neurodegenerative disorders and in the selection of hormone replacement regimens in postmenopausal women. Differential effects of estrogen and progesterone on brain-derived neurotropic factor protein and in the regulation of synaptogenesis have been described in some experimental models (16, 33), but not specifically in cholinergic cells (28, 34). Further exploration of the effects of estrogen and progestin in the posterior cingulate region of healthy postmenopausal women appears warranted, considering that the effectiveness of estrogen is questionable once AD is diagnosed (21).

One of the limitations of this study design is the lack of randomization between hormone users and nonusers. Previous imaging studies have provided information on the brain regional effects of short-term estrogen administration (35–37). However, the central effects of long-term estrogen use are more difficult to study because of the length of treatment time required. The lack of randomization to hormone use or nonuse allows selection bias to exist in this study design. In addition, this design relies on patient recall of when medications were initially started and dosages used. Although a tendency for ERT users to be healthier and have higher education levels has been demonstrated (38–40), our subject groups were matched for education level and had no major medical illnesses. In fact, the majority of the women not taking hormones were highly educated (9 of the 13 women in this group had educational levels of 16 yr or more) and decided against replacement after careful consideration of the risks and benefits. This probably does not reflect the views, educational level, or health status of the general population.

The data presented in this report point to important areas of investigation regarding the effects of ERT and HRT on cholinergic neuronal integrity in postmenopausal women. Although an overall effect of postmenopausal hormone therapy was not found, associations between an index of cortical cholinergic terminal concentrations and the length of hormonal replacement suggest that hormone therapy may influence the survival or plasticity of these cells in postmenopausal women. In addition, differences in the concentration of cholinergic terminals in the posterior cingulate between women treated with ERT and those treated with HRT will require replication and further investigation in view of its important health implications.

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