ELSEVIER

Neurobiology of Aging 30 (2009) 71–80

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Menopausal transition: A possible risk factor for brain pathologic events

S.M. Bonomo [a,e], A.E. Rigamonti [a,e], M. Giunta [a,e], D. Galimberti [b,e], A. Guaita [c], M.G. Gagliano [d], E.E. Müller [a,e], S.G. Cella [a,e,*]

[a] *Department of Medical Pharmacology, University of Milan, Fondazione IRCCS Ospedale Maggiore Policlinico, Milan, Italy*
[b] *Department of Neurological Sciences, University of Milan, Fondazione IRCCS Ospedale Maggiore Policlinico, Milan, Italy*
[c] *Geriatric Institute "Camillo Golgi", Abbiategrasso, Italy*
[d] *Department of Transfusion Medicine, "San Carlo Borromeo" Hospital, Milan, Italy*
[e] *Center of Excellence for Neurodegenerative Diseases (CEND), University of Milan, Italy*

Received 26 January 2007; received in revised form 15 May 2007; accepted 21 May 2007
Available online 2 July 2007

Abstract

Background and objective: Incidence and prevalence of Alzheimer's disease (AD) are higher in postmenopausal women than in age-matched men. Since at menopause the endocrine system and other biological paradigms undergo substantial changes, we thought to be of interest studying whether (and how) the balance between some biological parameters allegedly neuroprotective (e.g. related to estrogen, dehydroepiandrosterone and CD36 functions) and others considered pro-neurotoxic (e.g. related to glucocorticoid and interleukin-6 activities) vary during lifespan in either sex in either normalcy or neurodegenerative disorders.

Subjects and methods: Along with this aim, we evaluated the gene expression levels of estrogen receptors (ERs), glucocorticoid receptors (HGRs), interleukin-6 (IL-6) and CD36, a scavenger receptor of class B allegedly playing a key role in the proinflammatory events associated with AD, in a population of 209 healthy subjects (73M, 106F, 20–91-year old) and 85 AD patients (36M, 49F, 65–89-year old). Results obtained were related to plasma titers of estrogens, cortisol and dehydroepiandrosterone sulfate (DHEAS). Studies were performed in peripheral leukocytes, since these cells (1) are easily obtainable by a simple blood sampling, (2) express many molecules and multiple receptors which are under the same regulatory mechanisms as those operative in the brain and (3) some of them, e.g. monocytes, share many functions with microglial cells.

Results: In healthy men all the study parameters were quite stable during lifespan. In women, instead, at menopausal transition, some changes that may predispose to neurodegeneration occurred. In particular, there was (1) an up-regulation of ERs, and a concomitant increase of IL-6 gene expression, events likely due to the loss of the inhibitory control exerted by estradiol (E_2); (2) an increase of HGRα:HGRβ ratio, indicative of an augmented cortisol activity on HGRα not sufficiently counteracted by the inhibitory HGRβ function; (3) a reduced CD36 expression, directly related to the increased cortisol activity; and (4) an augmented plasma cortisol:DHEAS ratio, widely recognized as an unfavorable prognostic index for the risk of neurodegeneration. In AD patients of both sexes, the expression of the study parameters was similar to that found in sex- and age-matched healthy subjects, thus indicating their unrelatedness to the disease, and rather a better correlation with biological events.

Conclusions: Menopausal transition is a critical phase of women's life where the occurrence of an unfavorable biological *milieu* would predispose to an increased risk of neurodegeneration. Collectively, the higher prevalence of AD in the female population would depend, at least in part, on the presence of favoring biological risk factors, whose contribution to the development of the disease occurs only in the presence of possible age-dependent triggers, such as beta-amyloid deposition.

Keywords: Menopausal transition; Hormone receptors; CD36; Leukocytes; Alzheimers disease; Estrogen receptors; Glucocorticoid receptors; IL-6

* Corresponding author at: Department of Medical Pharmacology, University of Milan, via Vanvitelli 32, 20129 Milan, Italy. Tel.: +39 02 50317015; fax: +39 02 50317011.
E-mail address: silvano.cella@unimi.it (S.G. Cella).

0197-4580/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2007.05.017

1. Introduction

As the age distribution of the population shifts toward an increase, the dementing disorders, especially Alzheimer's disease (AD), are emerging as a major worldwide health problem. To ameliorate the comprehension of the pathogenetic events underlying neurodegeneration, many prevalence studies on dementia and AD have been conducted in various population subgroups. In particular, the effects of gender have been investigated. Although conflicting data have been reported (Brayne et al., 1995; Nilsson, 1984), most of the studies support a higher prevalence and incidence of AD in women, even after adjusting for their differential survival (Bachman et al., 1992, 1993; Gao et al., 1998). This has obviously focused the attention on the role of female hormones, e.g. estrogens, whose production dramatically decreases at menopause.

The role of estrogens in AD has been investigated in a variety of *in vivo* and *in vitro* models. In these studies, estrogens have been shown to be potent neuroprotective agents. In fact, they (a) augment the cerebral blood flow in the hippocampus and temporal lobe, two brain areas involved in the early pathological changes of AD (Maki and Resnick, 2000, 2001); (b) exert neurotrophic actions on different neuronal populations (Gibbs and Aggarwal, 1998; Granholm et al., 2002, 2003; Leranth et al., 2000; McEwen, 2002); (c) decrease cholesterol levels and modulate the expression of the gene encoding apolipoprotein E (ApoE) (Brinton et al., 2000; Lambert et al., 2004); (d) prevent the formation of beta-amyloid (βA) fibrils and protect the cells against their cytotoxic effects (Granholm et al., 2003; Thomas and Rhodin, 2000); (e) inhibit the chronic inflammatory reaction that has a pathogenetic role in AD (Thomas and Rhodin, 2000); (f) induce the synthesis of thioredoxin, a multifunctional protein endowed with antioxidant and neuroprotective actions (Chiueh et al., 2003). Inferential support to the protective role of estrogens in AD rests on the observation that cognitive function is improved by hormone therapy (HT) in postmenopausal women (Jacobs et al., 1998; Phillips and Sherwin, 1992).

Despite this large body of evidence, other studies have denied the alleged protective role of estrogens, leaving the problem unsettled (den Heijer et al., 2003; Espeland et al., 2004; Shumaker et al., 2003, 2003). Moreover, the Women's Health Initiative Memory Study (WHIMS), a wide randomized placebo-controlled clinical trial for HT in postmenopausal women, has recently shown that in women with an average age of 63 years at entry, HT increases the risk of probable dementia (Shumaker et al., 2003, 2004), and hypothesized that the negative effect may be related to the HT-induced increased risk of stroke, standing the strong relationship existing between microinfarcts in the brain and susceptibility to AD (Shumaker et al., 2003, 2004). For a thorough discussion, see Turgeon et al. (2006).

With these disparate findings in mind, different authors have hypothesized the existence of a "critical temporal window", likely coincident with the menopausal transition, within which the estrogens manifest their positive effects and over which, instead, they become detrimental (Kesslak, 2002; Smith and Levin-Allerhand, 2003; Zandi et al., 2002). Along this line, it is noteworthy that in postmenopausal women the reduction of the risk of dementia is related to the previous and not to the current use of estrogens (Zandi et al., 2002).

Among elderly, and particularly in AD patients, a disrupted hypothalamo-pituitary-adrenal function may also play a role in neurodegeneration (Murialdo et al., 2001). Higher glucocorticoid levels, in fact, may alter the function of hippocampal neurons and glial cells, rendering these elements more vulnerable to metabolic insults, such as hypoglycaemia and hypoxia. They also cause synaptic disruption and are involved in neuronal cell death (Müller, 2001; Sapolsky et al., 1991).

In the last decade, search for biological and hormonal markers of dementia expressed in easily accessible tissues has been intensified. This led to identify several molecules, whose diagnostic potential is now under investigation. Among them, particularly promising would be CD36, a multifunction protein belonging to the family of the class B scavenger receptors. CD36 is expressed on microglia of normal and AD brains and would play an important role in the proinflammatory events associated with AD (Christie et al., 1996; Coraci et al., 2002; El Khoury et al., 1996; Husemann et al., 2001; Maxeiner et al., 1998). Recently, we have shown that CD36 is also expressed by peripheral leukocytes and that its expression by these cells is lower in AD patients than in age-matched controls (Giunta et al., 2006).

Peripheral leukocytes express virtually all hormones and hormone receptors, which are under the same regulatory mechanisms that control their expression in the brain (Hori et al., 1991; Kim and de Vellis, 2005). Importantly, the prevailing view about origin of microglia is that it derives from peripheral leukocytes, particularly, monocytes which, during embryonic development, enter the brain from the bloodstream and then differentiate into brain resident microglia, displaying several cell surface antigens described in monocytes (Kim and de Vellis, 2005). Hence, these cells, easily obtainable *via* a simple blood sampling, may be profitably used as tools to investigate the changes occurring in brain areas reportedly inaccessible in humans.

These premises, dictated the study of the leukocyte expression of some biological parameters in a large group of normal non-dementing subjects and AD patients of either sex, the aim being that of evaluating how the balance between neuroprotective/neurotoxic influences varies across life. Our attention focused on the expression of estrogen and glucocorticoid receptors and the production of interleukin-6 (IL-6), a proinflammatory molecule likely involved in the pathogenesis of AD (Papassotiropoulos et al., 2001). Results were compared to the leukocyte expression of CD36 and related to the circulating levels of estrogens, cortisol, and dehydroepiandrosterone sulfate (DHEAS).

2. Subjects and methods

2.1. Subjects

This study was reviewed and approved by an institutional independent Ethic Committee.

Control subjects were recruited among the members of a private volunteer blood donors association ($n = 97$; 51M and 46F, 20–50-year old), or among outpatients of the Service of Oral Anticoagulant Therapy of an important city hospital in Milan ($n = 132$; 72M and 60F, 51–91-year old). All of them were bearing a cardiac valvular prosthesis, but had no other systemic diseases. None of the enrolled women had ever been treated with estrogens alone or combined to progestins, either for contraceptive purposes or HT.

AD patients ($n = 85$; 36M and 49F, mean age 77.1 ± 2.5 years) were recruited from the Department of Neurological Sciences of the University of Milan, Fondazione IRCCS Ospedale Maggiore Policlinico, Milan and the Camillo Golgi Geriatric Institute in Abbiategrasso, Milan. The clinical diagnosis of probable AD was based on the National Institute of Neurological and Communicative Diseases, and Stroke–Alzheimer's Disease and Related Disorders Association criteria (McKhann et al., 1984). Routine laboratory measurements, including magnetic resonance imaging (MRI), were performed to exclude other causes of cognitive impairment. No subject with familial AD was included in the study.

A population of 314 subjects was studied. Informed consent was obtained from all of them or their caregivers when necessary. For both control subjects and AD patients, exclusion criteria were the presence of acute infectious or inflammatory diseases, endocrine and/or metabolic diseases, chronic hepatic diseases, chronic obstructive pulmonary diseases, alcoholism, cancer, intake of hormones, steroid or NSAIDs, all conditions which might spuriously modulate the expression of the selected parameters.

Fasting blood samples were drawn at 08.00–8.30 a.m. from patients with AD and non-dementing subjects.

Demographic characteristics of the subjects included in the study are depicted in Table 1.

Table 1
Demographic characteristics of the study subjects

	N	Age (years)	Sex
AD patients	85	77.10 ± 2.50	36M, 49F
Controls			
21–30	28	23.48 ± 0.76	14M, 14F
31–40	38	35.39 ± 0.58	20M, 18F
41–50	31	45.42 ± 0.52	17M, 14F
51–60	35	55.36 ± 0.57	20M, 15F
61–70	32	65.31 ± 0.48	19M, 13F
71–80	28	77.39 ± 0.52	13M, 15F
>81	37	84.72 ± 0.43	20M, 17F

2.2. Leukocyte expression of biological markers

2.2.1. Isolation of peripheral blood leukocytes

Blood samples were collected into tubes containing heparin and immediately processed for the isolation of peripheral leukocytes. Mononuclear cells were isolated by Vacutainer® CPT™ (Becton Dickinson, New Jersey, USA), a sterile cell preparation tube with sodium citrate. Cells recovered from the layer just under the plasma layer were washed twice with PBS pH 7.4 and, after a last centrifugation, the pellet was suspended again by using an adapted amount of a specific buffer, which stabilizes cellular RNA (Ambion United Kingdom, distributed in Italy by Celbio, Milan). This cellular suspension was then stored at −80 °C until RNA extraction.

2.2.2. Total RNA isolation and RT-PCR assay

Total RNA was extracted from leukocytes according to the single-step acid guanidinium thiocyanate–phenol–chloroform extraction method. Single strand cDNA was synthesized following standard procedures of reverse transcription. PCR amplification was performed with a synthetic CD36-specific primer (CD36 primer sense = 5′-GAGAACTGTTATGGGGCTAT-3′; CD36 primer antisense = 5′-TTCAACTGGAGAGGCAAAGG-3′; product length = 389 bp), HGRα-specific primer (HGRα primer sense = 5′-GCCAAGTCTTGGCCCTCTAT-3′; HGRα primer antisense = 5′-CCTAAGGACGGGCTGAAGAGC-3′; product length = 450 bp), HGRβ-specific primer (HGRβ primer sense = 5′-CCTAAGGACGGTCTGAAGAGC-3′; HGRβ primer antisense = 5′-CCACGTATCCTAAAAGGGCAC-3′; product length = 377 bp), ERα-specific primer (ERα primer sense = 5′-GATGGTCAGTGCCTTGTTGATGC-3′; ERα primer antisense = 5′-GCAGATTCATCATGCGGAACCGAG-3′; product length = 380 bp), ERβ-specific primer (ERβ primer sense = 5′-GTCCATCGCCAGTTATCACATC-3′; ERβ primer antisense = 5′-GCCTTACATCCTTCACACGA-3′; product length = 250 bp), IL-6-specific primer (IL-6 primer sense = 5′-AAAGAGGCACTGGCAGAA-3′; IL-6 primer antisense = 5′-AGCTCTGGCTTGTTCCTCAC-3′; product length = 183 bp) (Gibco Life Technologies, Milan, Italy). The expression of these specific genes was normalized by amplifying glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (primer sense = 5′-GCCATCAACGACCCCTTCATTG-3′; primer antisense = 5′-TGCCAGTGAGCTTCCCGTTC-3′; product length = 500–600 bp) gene from the same RNA sample. For a full description of the RT-PCR method, see Giunta et al. (2006).

2.3. Hormone radioimmunoassays

Blood samples were collected in tubes containing heparin and immediately chilled. Plasma was frozen until assayed for estradiol, estrone, cortisol and DHEAS by double-antibody RIA with commercial kits (DSL, Texas, USA). The sensitivities of the assays were the following: estradiol, 1.5 pg/ml; estrone, 1.2 pg/ml; cortisol, 0.5 nmol/l; DHEAS, 5 μg/dl. To

avoid possible inter-assay variation, all samples of a given experiment were assayed in a single RIA. No sample fell below the limits of detection of each kit.

2.4. Statistical analysis

Non-dementing subjects were divided according to age (decades) and gender, whereas AD patients were not separated in age groups, since among them no age-related differences were found. Data are expressed as mean ± S.E.M. Comparisons among groups were performed by parametric one-way analysis of variance (ANOVA), followed by the post hoc Tukey's test. Correlation was verified by linear regression analysis (Pearson's product moment correlation). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Estrogen receptors

In non-dementing men, the rate of leukocyte expression of both α- and β-estrogen receptors (ERα and ERβ) was quite constant, with only a slight decline starting from the beginning of the sixth decade of life (i.e. 51–60 years) (Fig. 1A). In women, instead, ERs expression augmented more than 2.5-fold in the same decade (when in most of women the menopausal transition occurs), after which it returned to pre-menopausal values (Fig. 1B). In AD patients, the expression of ERs was superimposable on that of gender- and age-matched non-dementing subjects (Fig. 1A and B).

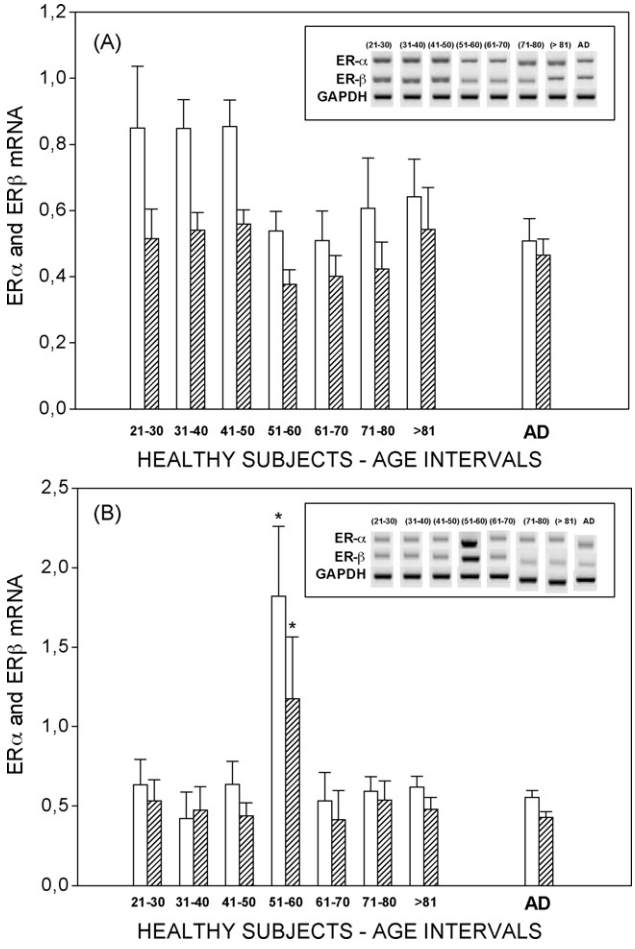

Fig. 1. Leukocyte expression of ERα (white bars) and ERβ (striped bars) in male (panel A) and female (panel B) healthy subjects and AD patients. Healthy subjects were divided according to age (decades) and gender, whereas AD patients were not separated in age groups. Representative blots for ERα and ERβ are shown. ERα: α-estrogen receptor; ERβ: β-estrogen receptor; AD: Alzheimer's disease; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

3.2. Glucocorticoid receptors

In non-dementing men, the expression of both α- and β-glucocorticoid receptors (HGRα and HGRβ) was lower than in aged-matched women and remained stable with increasing age. In women, the expression of HGRα declined from the peak in the 51–60-year-old group for all samples between 60 and 80 years, while in very old females (>81-year old) it was similar to that of younger people. The expression of HGRβ showed a similar trend, but it declined earlier and more intensely, starting from 40 years of age. In AD patients of both sexes HGR expression was significantly lower than that of younger non-dementing individuals (data not shown). In control women, the ratio HGRα:HGRβ exhibited a biphasic trend with a maximum in 51–60-year old subjects and lower values in younger and older subjects. In men of all age groups, instead, this ratio was quite stable. In AD patients of both sexes, HGRα:HGRβ ratio was not significantly different from that of age-matched non-dementing subjects (Fig. 2).

3.3. CD36

In non-dementing men, leukocyte expression of CD36 was not age-related. In women, instead, it was significantly reduced in the age interval 51–80 years, but not in the very old subjects (>81-year old). In AD patients of both sexes the expression of CD36 was significantly lower than that of non-dementing subjects (Fig. 3A).

3.4. IL-6

In non-dementing men, the leukocyte expression of the gene encoding IL-6 did not vary significantly among age groups, although there was a trend toward higher values in the older. In women, instead, a peak of expression was evident between 51 and 60 years of age. In both male and female AD patients, IL-6 expression was similar to that observed in younger control subjects (Fig. 3B).

3.5. Circulating hormone levels

As expected, in control men plasma estradiol levels were not age-related, while in women both estradiol and estrone

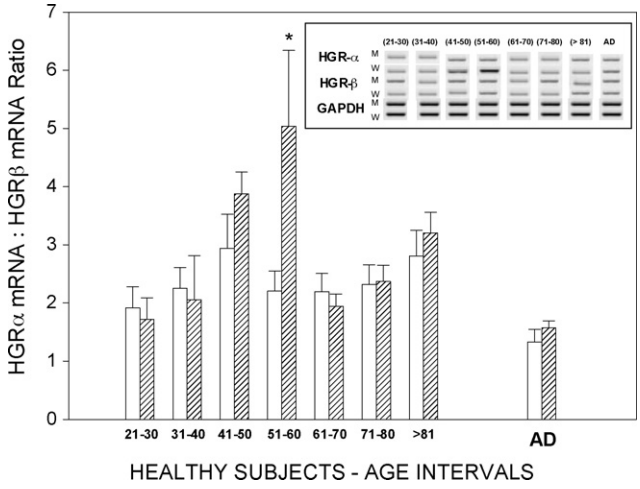

Fig. 2. Ratio of leukocyte expressions of HGRα:HGRβ in male (white bars) and female (striped bars) healthy subjects and AD patients. Representative blots for HGRα and HGRβ are shown. HGRα:HGRβ: ratio between α- and β-glucocorticoid receptors. See legend of Fig. 1 for further details.

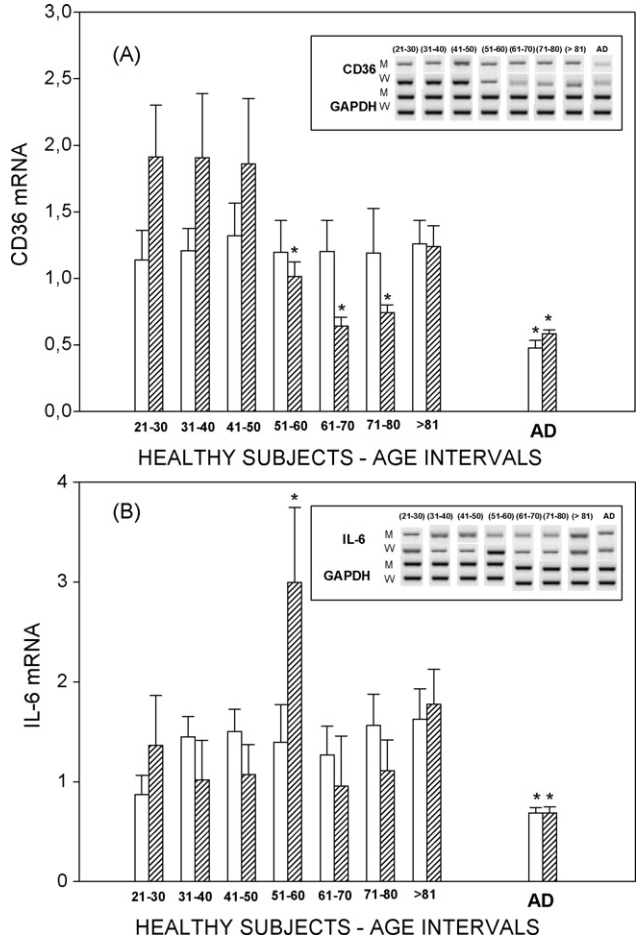

Fig. 3. Leukocyte expression of CD36 (panel A) and IL-6 (panel B) in male (white bars) and female (striped bars) healthy subjects and AD patients. Representative blots for CD36 and IL-6 are shown. IL-6: interleukin-6. See legend of Fig. 1 for further details.

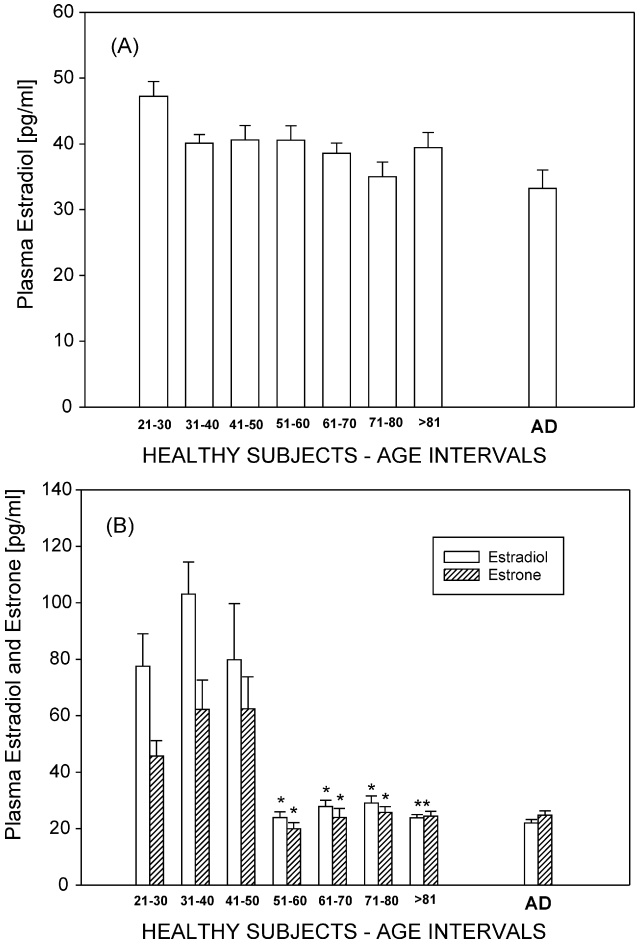

Fig. 4. Plasma concentrations of estradiol (white bars) and estrone (striped bars) in male (panel A) and female (panel B) healthy subjects and AD patients. See legend of Fig. 1 for further details.

dramatically declined after menopause (Fig. 4A and B). In AD patients circulating estrogen concentrations were superimposable on those of age-matched non-dementing subjects (Fig. 4A and B).

Morning cortisol concentrations did not vary with increasing age in normal subjects and AD patients of both sexes (data not shown).

Plasma levels of DHEAS progressively declined in normal individuals and AD patients of both sexes. The decline occurred earlier and was more intense in women than in men (data not shown). In all subjects, the ratio between the concentrations of cortisol and DHEAS constantly augmented with increasing age (Fig. 5).

3.6. Correlations

None of the biological parameters investigated was related to age, except for the plasma levels of estrogens in women and DHEAS in either sex. A direct correlation was found in both genders between ERs and IL-6 gene expression and between HGRs and CD36 expression (Table 2).

Table 2
Correlation among the biological parameters investigated in women (panel A) and men (panel B)

	Age	ERα	ERβ	HGRα	HGRβ	CD36	IL-6	E_2	Cortisol	DHEAS
(A) Women										
Age	–	No	No	No	No	No	No	$R^2 = 0.56$, $P < 0.05$	No	$R^2 = 0.52$, $P < 0.05$
ERα	No	–	$R^2 = 0.39$, $P < 0.05$	No	No	No	$R^2 = 0.36$, $P < 0.05$	No	No	No
ERβ	No	$R^2 = 0.39$, $P < 0.05$	–	No	No	No	$R^2 = 0.50$, $P < 0.05$	No	No	No
HGRα	No	No	No	–	$R^2 = 0.40$, $P < 0.05$	$R^2 = 0.84$, $P < 0.01$	No	No	No	No
HGRα	No	No	No	$R^2 = 0.40$, $P < 0.05$	–	$R^2 = 0.52$, $P < 0.05$	No	No	No	No
CD36	No	No	No	$R^2 = 0.84$, $P < 0.01$	$R^2 = 0.52$, $P < 0.05$	–	No	No	No	No
IL-6	No	$R^2 = 0.36$, $P < 0.05$	$R^2 = 0.50$, $P < 0.05$	No	No	No	–	No	No	No
E_2	$R^2 = 0.57$, $P < 0.05$	No	No	No	No	No	No	–	No	No
Cortisol	No	No	No	No	No	No	No	No	–	No
DHEAS	$R^2 = 0.52$, $P < 0.05$	No	No	No	No	No	No	No	No	–
(B) Men										
Age	–	No	No	No	No	No	No	No	No	$R^2 = 0.48$, $P < 0.05$
ERα	No	–	$R^2 = 0.54$, $P < 0.05$	No	No	No	$R^2 = 0.54$, $P < 0.05$	No	No	No
ERβ	No	$R^2 = 0.54$, $P < 0.05$	–	No	No	No	$R^2 = 0.59$, $P < 0.05$	No	No	No
HGRα	No	No	No	–	$R^2 = 0.36$, $P < 0.05$	$R^2 = 0.76$, $P < 0.01$	No	No	No	No
HGRβ	No	No	No	$R^2 = 0.36$, $P < 0.05$	–	$R^2 = 0.49$, $P < 0.05$	No	No	No	No
CD36	No	No	No	$R^2 = 0.76$, $P < 0.01$	$R^2 = 0.49$, $P < 0.05$	–	No	No	No	No
IL-6	No	$R^2 = 0.54$, $P < 0.05$	$R^2 = 0.59$, $P < 0.05$	No	No	No	–	No	No	No
E_2	No	No	No	No	No	No	No	–	No	No
Cortisol	No	No	No	No	No	No	No	No	–	No
DHEAS	$R^2 = 0.48$, $P < 0.05$	No	No	No	No	No	No	No	No	–

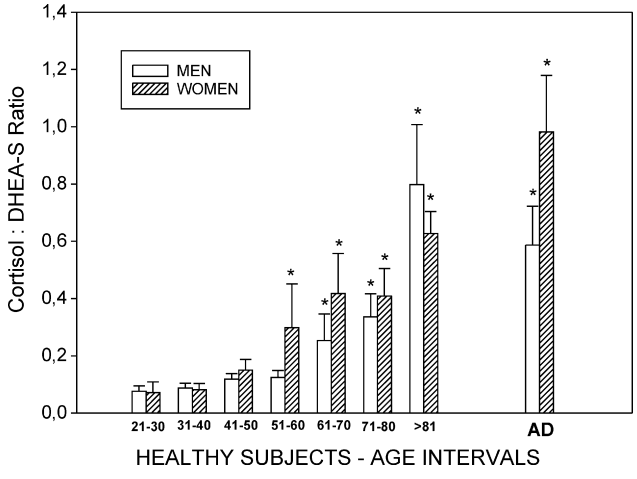

Fig. 5. Cortisol and DHEA plasma level ratio in male (white bars) and female (striped bars) healthy subjects and AD patients. DHEA: dehydroepiandrosterone. See legend of Fig. 1 for further details.

4. Discussion

In this study, none of the biological parameters investigated was related to age, except for the plasma levels of estrogens in women and DHEAS in either sex. In addition, most of the potentially neurotoxic alterations found in the perimenopausal period were absent in the very old healthy women. This, inferentially, would confirm the view that the higher prevalence of AD in the older population (Gao et al., 1998) is not a direct effect of age *per se*. More likely, it depends, instead, from the presence of favoring risk factors whose contribution to the development of the disease occurs only in the presence of possible age-dependent triggers. This view also is supported by the recognition that among very old individuals the prevalence of AD seems to level off or even decline (Ritchie and Kildea, 1995).

The higher prevalence of AD reportedly present in postmenopausal women (Bachman et al., 1992, 1993) led us to

consider estrogen deprivation as a putative favoring risk factor in the female population. Consistent with this view, in our study menopausal transition, which resulted in a sudden failure of the hypothalamic-pituitary-gonadal axis, up-regulated the leukocyte expression of the ERs, likely due to the loss of the estrogen ligand. Similar phenomena have already been described, e.g. estrogen down-regulated ERs in a rat pituitary cell line (Schreihofer et al., 2000) and, conversely, estrogen deficiency up-regulated ERs in the brain of hypogonadal mice (Chakraborty et al., 2005). Surprisingly, in the older age groups the leukocyte expression of both ERα and ERβ was similar to those of younger subjects, despite the persistent reduction of plasma estrogen levels. Though information on the regulation of ERs at menopause is scarce, it can be argued that several factors, which for the sake of brevity we cannot allude to in details, may account for this phenomenon (Ben-Hur et al., 1995; Metha et al., 1987; Meza-Munoz et al., 2006; Nelson and Bulun, 2001; Santner et al., 1993; Simpson, 2003).

In men the expression of both ERα and ERβ was rather uniform and also circulating levels of estradiol were rather stable. This is likely due to the preserved pool of testosterone in men, which undergoes aromatisation to estrogens also in advanced age (Vermeulen et al., 2002). That leukocyte ERs expression in AD patients was similar to that found in age- and sex-matched control subjects would deny a direct relationship between this parameter and the disease.

The widespread presence of ERs in multiple cell types of the immune system and their participation to the inflammatory response is remarkable. ERα and, in some cases, ERβ are present in front line immune and cytokine-producing cells, such as macrophages and microglia, and activated ERs have been shown *in vitro* to affect release of proinflammatory cytokines from these cells and to interfere with the action of cytokines (Mor et al., 1999; Pfeilschifter et al., 2002; Salem, 2004).

In our study, leukocyte IL-6 expression peaked in 51–60-year-old women, whereas in men it remained constant over time. In this context, an interesting feature is the direct correlation found in either sex between ERs and IL-6 gene expression. These findings would confirm that estrogens are important to maintain under inhibitory control IL-6 production and so to prevent tissue damage. Hence, during menopausal transition, the abrupt fall of estrogens may predispose to an excessive CNS inflammatory response induced by triggers, such as βA deposition.

In AD patients, contrarily, IL-6 expression was lower than in age-matched non-dementing subjects. It is tempting to speculate that this occurred for the progressive loss of cytokine-producing cells induced by cortisol (see below) and/or by other factors, such as the reduced expression of CD36, which is essential for the release of many proinflammatory agents, including cytokines and reactive oxygen species (Coraci et al., 2002).

The neuropathological hallmarks of AD are very prominent in the hippocampus, a brain area pivotal to the regulation of the hypothalamic-pituitary-adrenal (HPA) system. An age-related dysregulation of the HPA axis is well recognized in animals, in which steroid detrimental effects on cognition may occur *via* the hippocampus, a major site of corticosteroid action, and an important structure involved in learning and memory (Miller and O'Callaghan, 2005; Müller, 2001).

HGRs are member of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Among the many variants of HGRs, the HGRα isoform is recognized as the classical HGR and the primary mediator of glucocorticoid actions (Yudt and Cidlowski, 2002). The HGRβ isoform – generated through alternative splicing and transcriptionally inactive – does not bind agonists or antagonists and has a dominant negative effect on HGRα-mediated transactivation. HGRβ is physiologically important, since it attenuates the HGRα response, thus dampening an excessive increase of the glucocorticoid actions (Bamberger et al., 1995). Hence, it is impossible to correctly appraise the activity of glucocorticoids disregarding interactions between the two receptor isoforms. Accordingly, in our study calculating the ratio HGRα:HGRβ expression, as a dynamic index of global glucocorticoid activity, it emerged that in women the ratio increased during the menopausal transition, likely, to signify that this critical phase of the female life also is driven by an hyper-activity of cortisol and an exacerbation of its pro-neurotoxic effects. Such changes would not be dependent on changes in the production of adrenal steroids, at least based only on the morning plasma cortisol levels, which were constant trough life in either sex. More likely, the alterations present in women at menopausal transition were due to a prevalent reduction of HGRβ-positive leukocytes, as described in cultured HGR-positive hippocampal neurons, whose absolute number decreased following exposure to elevated cortisol concentrations (Packan and Sapolsky, 1990).

In the present study, we confirmed previous data of ours indicating that in male AD patients the expression of CD36 is lower than in age-matched healthy subjects (Giunta et al., 2006). The most interesting data, however, was the observation that in women, starting from the menopausal transition, the expression of CD36 fell and became similar to that present in AD patients. We do not have an easy explanation which accounts for this phenomenon, but, recalling that a direct correlation occurred in either sex between CD36 and HGRs expression, it is conceivable that an excessive cortisol activity caused a loss of CD36-positive cells. Were this also occurring in the brain, the most likely consequence would be the progressive inability of microglial elements to remove the βA protein, thus favoring its accumulation.

Dehydroepiandrosterone (DHEA) is an androgenic precursor endowed with positive effects on many brain functions (Baulieu, 1997; Vallee et al., 2001; Yen et al., 1995). In blood, most DHEA is found as sulfate (DHEAS), which represents a buffer and reservoir of free DHEA and whose measurement is preferable to that of DHEA, its levels being more stable. An elevated cortisol:DHEA ratio is generally recognized as an unfavorable prognostic index for the risk of neurodegenera-

tion, since it indicates that the neurotoxic actions of glucocorticoids are not well balanced by the neuroprotective effects of DHEA (Herbert, 1998). Our data indicate that this ratio increased with advancing age both in men and women, but the augmentation occurred earlier in the female population, being yet present in the decade of menopausal transition (i.e. 51–60 years), whereas in men it took place about 10 years later.

Collectively, in a large group of control non-dementing subjects and in AD patients of either gender, evaluation of leucocyte expression of some biological parameters evidenced, in general, their unrelatedness to ageing, but rather a better correlation with the hormonal events. This was particularly evident in women, where the estrogen deprivation occurring in the transitional period (51–60 years) towards a more advanced menopause, induced clearcut, specific changes in some hormonal/biological paradigms (e.g. peak HGRα:HGRβ ratio; peak IL-6 expression). Concerning the leucocyte expression of CD36, a paradigm of neurodegeneration, AD women, as previously observed in men, presented with lower values than in non-dementing subjects within a wide interval of their lifespan (51–80 years); here, the correlation found in AD patients of either sex between CD36 and HGRs expression, would imply a pathogenetic role for the HPA function in AD. Disentangle of different biological markers in target peripheral tissues (as circulating leucocytes) of either animals or humans, and evaluation of their interactions, may allow better insight into the physiopathology of neurodegenerative disorders.

This study suffers of some limitations, the principal one being its cross-sectional design. However, due the wide range of age intervals considered (20–91 years), too many years would have been necessary to apply for a prospectic design, thus making the project unfeasible. A second flaw rests on the evaluation of the HPA activity, based on a single cortisol morning sample, rather than a more appropriate circadian evaluation. Finally, the whole leucocyte population, rather than the more selected monocytes, was evaluated.

Regardless these and other limitations, data here-in reported indicate that to globally appraise an endocrine function it is mandatory considering not only circulating hormone titers, but also the tissue expression of the receptors and, possibly, post-receptor events. Hence, easily obtainable tissues (such as peripheral leukocytes), expressing multiple hormone receptors, as target of several hormones, should be considered useful tools.

Studies are now in progress to investigate whether during menopausal transition an HT can prevent the appearance of an unfavorable endocrine/biological *milieu*, thus contributing to inplement neuroprotective influences and to reestablish a proper hormonal balancing.

Disclosure statement

All authors of the present manuscript have no actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within 3 years of beginning the work submitted that could inappropriately influence (bias) this work.

Acknowledgements

We thank Prof. Elio Scarpini for advice and helpful discussion. The studies were supported in part by Cariplo Foundation, Milan, Italy. Thanks are given to Mrs. Maria Lupo for careful secreterial work.

References

Bachman, D.L., Wolf, P.A., Linn, R., Knoefel, J.E., Cobb, J., Belanger, A., D'Agostino, R.B., White, L.R., 1992. Prevalence of dementia and probable senile dementia of the Alzheimer type in the Framingham Study. Neurology 42 (1), 115–119.

Bachman, D.L., Wolf, P.A., Linn, R.T., Knoefel, J.E., Cobb, J.L., Belanger, A.J., White, L.R., D'Agostino, R.B., 1993. Incidence of dementia and probable Alzheimer's disease in a general population: the Framingham Study. Neurology 43 (3 Pt 1), 515–519.

Bamberger, C.M., Bamberger, A.M., de Castro, M., Chrousos, G.P., 1995. Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. J. Clin. Invest. 95, 2435–2441.

Baulieu, E.E., 1997. Neurosteroids: of the nervous system, by the nervous system, for the nervous system. Recent Prog. Horm. Res. 52, 1–32.

Ben-Hur, H., Mor, G., Insler, V., Blickstein, I., Amir-Zaltsman, Y., Sharp, A., Globerson, A., Kohen, F., 1995. Menopause is associated with a significant increase in blood monocyte number and a relative decrease in the expression of estrogen receptors in human peripheral monocytes. Am. J. Reprod. Immunol. 34 (6), 363–369.

Brayne, C., Gill, C., Huppert, F.A., Barkley, C., Gehlhaar, E., Girling, D.M., O'Connor, D.W., Paykel, E.S., 1995. Incidence of clinically diagnosed subtypes of dementia in an elderly population: Cambridge Project for Later Life. Br. J. Psychiatry 167, 255–262.

Brinton, R.D., Chen, S., Montoya, M., Hsieh, D., Minaya, J., 2000. The estrogen replacement therapy of the Women's Health Initiative promotes the cellular mechanisms of memory and neuronal survival in neurons vulnerable to Alzheimer's disease. Maturitas 34 (Suppl. 2), S35–S52.

Chakraborty, T.R., Rajendren, G., Gore, A.C., 2005. Expression of estrogen receptor {alpha} in the anteroventral periventricular nucleus of hypogonadal mice. Exp. Biol. Med. (Maywood) 230 (1), 49–56.

Chiueh, C., Lee, S., Andoh, T., Murphy, D., 2003. Induction of antioxidative and antiapoptotic thioredoxin supports neuroprotective hypothesis of estrogen. Endocrine 21 (1), 27–31.

Christie, R.H., Freeman, M., Hyman, B.T., 1996. Expression of the macrophage scavenger receptor, a multifunctional lipoprotein receptor, in microglia associated with senile plaques in Alzheimer's disease. Am. J. Pathol. 148 (2), 399–403.

Coraci, I.S., Husemann, J., Berman, J.W., Hulette, C., Dufour, J.H., Campanella, G.K., Luster, A.D., Silverstein, S.C., El-Khoury, J.B., 2002. CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to beta-amyloid fibrils. Am. J. Pathol. 160 (1), 101–112.

den Heijer, T., Geerlings, M.I., Hofman, A., de Jong, F.H., Launer, L.J., Pols, H.A., Breteler, M.M., 2003. Higher estrogen levels are not associated with larger hippocampi and better memory performance. Arch. Neurol. 60 (2), 213–220.

El Khoury, J., Hickman, S.E., Thomas, C.A., Cao, L., Silverstein, S.C., Loike, J.D., 1996. Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. Nature 382 (6593), 716–719.

Espeland, M.A., Rapp, S.R., Shumaker, S.A., Brunner, R., Manson, J.E., Sherwin, B.B., Hsia, J., Margolis, K.L., Hogan, P.E., Wallace, R., Dailey, M., Freeman, R., Hays, J., Women's Health Initiative Memory Study, 2004. Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. JAMA 291 (24), 2959–2968.

Gao, S., Hendrie, H.C., Hall, K.S., Hui, S., 1998. The relationships between age, sex, and the incidence of dementia and Alzheimer disease: a meta-analysis. Arch. Gen. Psychiatry 55 (9), 809–815.

Gibbs, R.B., Aggarwal, P., 1998. Estrogen and basal forebrain cholinergic neurons: implications for brain aging and Alzheimer's disease-related cognitive decline. Horm. Behav. 34 (2), 98–111.

Giunta, M., Rigamonti, A.E., Scarpini, E., Galimberti, D., Bonomo, S.M., Venturelli, E., Müller, E.E., Cella, S.G., 2006. The leukocyte expression of CD36 is low in patients with Alzheimer's disease and mild cognitive impairment. Neurobiol. Aging.

Granholm, A.C., Ford, K.A., Hyde, L.A., Bimonte, H.A., Hunter, C.L., Nelson, M., Albeck, D., Sanders, L.A., Mufson, E.J., Crnic, L.S., 2002. Estrogen restores cognition and cholinergic phenotype in an animal model of Down syndrome. Physiol. Behav. 77 (2–3), 371–385.

Granholm, A.C., Sanders, L., Seo, H., Lin, L., Ford, K., Isacson, O., 2003. Estrogen alters amyloid precursor protein as well as dendritic and cholinergic markers in a mouse model of Down syndrome. Hippocampus 13 (8), 905–914.

Herbert, J., 1998. Neurosteroids, brain damage, and mental illness. Exp. Gerontol. 33 (7–8), 713–727.

Hori, T., Nakashima, T., Take, S., Kaizuka, Y., Mori, T., Katafuchi, T., 1991. Immune cytokines and regulation of body temperature, food intake and cellular immunity. Brain Res. Bull. 27 (3–4), 309–313.

Husemann, J., Loike, J.D., Kodama, T., Silverstein, S.C., 2001. Scavenger receptor class B type I (SR-BI) mediates adhesion of neonatal murine microglia to fibrillar beta-amyloid. J. Neuroimmunol. 114 (1–2), 142–150.

Jacobs, D.M., Tang, M.X., Stern, Y., Sano, M., Marder, K., Bell, K.L., Schofield, P., Dooneief, G., Gurland, B., Mayeux, R., 1998. Cognitive function in nondemented older women who took estrogen after menopause. Neurology 50 (2), 368–373.

Kesslak, J.P., 2002. Can estrogen play a significant role in the prevention of Alzheimer's disease? J. Neural Transm. (Suppl. 62), 227–239.

Kim, S.U., de Vellis, J., 2005. Microglia in health and disease. J. Neurosci. Res. 81, 302–313.

Lambert, J.C., Coyle, N., Lendon, C., 2004. The allelic modulation of apolipoprotein E expression by oestrogen: potential relevance for Alzheimer's disease. J. Med. Genet. 41 (2), 104–112.

Leranth, C., Roth, R.H., Elsworth, J.D., Naftolin, F., Horvath, T.L., Redmond Jr., D.E., 2000. Estrogen is essential for maintaining nigrostriatal dopamine neurons in primates: implications for Parkinson's disease and memory. J. Neurosci. 20 (23), 8604–8609.

Maki, P.M., Resnick, S.M., 2001. Effects of estrogen on patterns of brain activity at rest and during cognitive activity: a review of neuroimaging studies. Neuroimage 14 (4), 789–801.

Maki, P.M., Resnick, S.M., 2000. Longitudinal effects of estrogen replacement therapy on PET cerebral blood flow and cognition. Neurobiol. Aging 21 (2), 373–383.

Maxeiner, H., Husemann, J., Thomas, C.A., Loike, J.D., El Khoury, J., Silverstein, S.C., 1998. Complementary roles for scavenger receptor A and CD36 of human monocyte-derived macrophages in adhesion to surfaces coated with oxidized low-density lipoproteins and in secretion of H_2O_2. J. Exp. Med. 188 (12), 2257–2265.

McEwen, B., 2002. Estrogen actions throughout the brain. Recent Prog. Horm. Res. 57, 357–384.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34 (7), 939–944.

Metha, R.R., Valcourt, L., Graves, J., Green, R., Das Gupta, T.K., 1987. Subcellular concentrations of estrone, estradiol, androstenedione and 17 beta-hydroxysteroid dehydrogenase (17-beta-OH-SDH) activity in malignant and non-malignant human breast tissues. Int. J. Cancer 40 (3), 305–308.

Meza-Munoz, D.E., Fajardo, M.E., Perez-Luque, E.L., Malacara, J.M., 2006. Factors associated with estrogen receptors-alpha (ER-alpha) and -beta (ER-beta) and progesterone receptor abundance in obese and non-obese pre- and post-menopausal women. Steroids 71 (6), 498–503.

Miller, D.B., O'Callaghan, J.P., 2005. Aging, stress and the hippocampus. Ageing Res. Rev. 4 (2), 123–140.

Mor, G., Nilsen, J., Horvath, T., Bechmann, I., Brown, S., Garcia-Segura, L.M., Naftolin, F., 1999. Estrogen and microglia: a regulatory system that affects the brain. J. Neurobiol. 40 (4), 484–496.

Müller, E.E., 2001. Steroids, cognitive processes and aging. Recent Prog. Med. 92 (5), 362–372.

Murialdo, G., Barreca, A., Nobili, F., Rollero, A., Timossi, G., Gianelli, M.V., Copello, F., Rodriguez, G., Polleri, A., 2001. Relationships between cortisol, dehydroepiandrosterone sulphate and insulin-like growth factor-I system in dementia. J. Endocrinol. Invest 24 (3), 139–146.

Nelson, L.R., Bulun, S.E., 2001. Estrogen production and action. J. Am. Acad. Dermatol. 45 (3 Suppl.), S116–S124.

Nilsson, L.V., 1984. Incidence of severe dementia in an urban sample followed from 70 to 79 years of age. Acta Psychiat. Scand. 70, 478–486.

Packan, D.R., Sapolsky, R.M., 1990. Glucocorticoid endangerment of the hippocampus: tissue, steroid and receptor specificity. Neuroendocrinology 51 (6), 613–618.

Papassotiropoulos, A., Hock, C., Nitsch, R.M., 2001. Genetics of interleukin 6: implications for Alzheimer's disease. Neurobiol. Aging 22 (6), 863–871.

Pfeilschifter, J., Koditz, R., Pfohl, M., Schatz, H., 2002. Changes in proinflammatory cytokine activity after menopause. Endocr. Rev. 23 (1), 90–119.

Phillips, S.M., Sherwin, B.B., 1992. Effects of estrogen on memory function in surgically menopausal women. Psychoneuroendocrinology 17 (5), 485–495.

Ritchie, K., Kildea, D., 1995. Is senile dementia "age-related" or "ageing-related"?—evidence from meta-analysis of dementia prevalence in the oldest old. Lancet 346 (8980), 931–934.

Salem, M.L., 2004. Estrogen, a double-edged sword: modulation of TH1- and TH2-mediated inflammations by differential regulation of TH1/TH2 cytokine production. Curr. Drug Targets Inflamm. Allergy 3 (1), 97–104.

Santner, S.J., Ohlsson-Wilhelm, B., Santen, R.J., 1993. Estrone sulfate promotes human breast cancer cell replication and nuclear uptake of estradiol in MCF-7 cell cultures. Int. J. Cancer 54 (1), 119–124.

Sapolsky, R.M., Stein-Behrens, B.A., Armanini, M.P., 1991. Long-term adrenalectomy causes loss of dentate gyrus and pyramidal neurons in the adult hippocampus. Exp. Neurol. 114 (2), 246–249.

Schreihofer, D.A., Stoler, M.H., Shupnik, M.A., 2000. Differential expression and regulation of estrogen receptors (ERs) in rat pituitary and cell lines: estrogen decreases ERalpha protein and estrogen responsiveness. Endocrinology 141 (6), 2174–2184.

Shumaker, S.A., Legault, C., Kuller, L., Rapp, S.R., Thal, L., Lane, D.S., Fillit, H., Stefanick, M.L., Hendrix, S.L., Lewis, C.E., Masaki, K., Coker, L.H., Women's Health Initiative Memory Study, 2004. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. JAMA 291 (24), 2947–2958.

Shumaker, S.A., Legault, C., Rapp, S.R., Thal, L., Wallace, R.B., Ockene, J.K., Hendrix, S.L., Jones III, B.N., Assaf, A.R., Jackson, R.D., Kotchen, J.M., Wassertheil-Smoller, S., Wactawski-Wende, J., WHIMS Investigators, 2003. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA 289 (20), 2651–2662.

Simpson, E.R., 2003. Sources of estrogen and their importance. J. Steroid Biochem. Mol. Biol. 86 (3–5), 225–230.

Smith, J.D., Levin-Allerhand, J.A., 2003. Potential use of estrogen-like drugs for the prevention of Alzheimer's disease. J. Mol. Neurosci. 20 (3), 277–281.

Thomas, T., Rhodin, J., 2000. Vascular actions of estrogen and Alzheimer's disease. Ann. NY Acad. Sci. 903, 501–509.

Turgeon, J.L., Carr, M.C., Maki, P.M., Mendelsohn, M.E., Wise, P.M., 2006. Complex actions of sex steroids in adipose tissue, the cardiovascular system, and brain: insights from basic science and clinical studies. Endocr. Rev. 27 (6), 575–605.

Vallee, M., Mayo, W., Le Moal, M., 2001. Role of pregnenolone, dehydroepiandrosterone and their sulfate esters on learning and memory in cognitive aging. Brain Res. Rev. 37 (1–3), 301–312.

Vermeulen, A., Kaufman, J.M., Goemaere, S., van Pottelberg, I., 2002. Estradiol in elderly men. Aging Male 5 (2), 98–102.

Yen, S.S., Morales, A.J., Khorram, O., 1995. Replacement of DHEA in aging men and women. Potential remedial effects. Ann. NY Acad. Sci. 774, 128–142.

Yudt, M.R., Cidlowski, J.A., 2002. The glucocorticoid receptor: coding a diversity of proteins and responses through a single gene. Mol. Endocrinol. 16 (8), 1719–1726.

Zandi, P.P., Carlson, M.C., Plassman, B.L., Welsh-Bohmer, K.A., Mayer, L.S., Steffens, D.C., Breitner, J.C., Cache County Memory Study Investigators, 2002. Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. JAMA 288 (17), 2123–2129.