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Forum Review

Effect of Gender on Mitochondrial Toxicity of Alzheimer's Aβ Peptide

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ABSTRACT

The aim of this article is to review the role of mitochondria in the pathogenesis of Alzheimer's disease. Additionally, the effect of gender on the incidence of Alzheimer's disease and the pathophysiological mechanisms involved will be discussed. Mitochondria, in the presence of Alzheimer's amyloid-β peptide, increase the formation of reactive oxygen species which act both as damaging agents and also as signaling molecules. These radicals, in fact, unleash a mechanism involving the liberation of cytochrome c that leads to neuronal apoptosis. Notably, young females appear protected against the mitochondrial toxicity of amyloid-β, likely due to the upregulation of antioxidant enzymes which occur in females. Estrogens are responsible for this effect. Overall, the findings support the notion that amyloid-β causes intracellular toxicity via the increased production of oxidant species. Reactive oxygen species generated by mitochondria act as a signal to start the mitochondrial apoptotic pathway. There is a possibility of prevention, and indirect evidence shows that estrogenic compounds (either endogenous estradiol or phytoestrogens such as genistein) may increase the expression of antioxidant enzymes, leading to a lowering of oxidative stress and thus protection against intracellular toxicity of amyloid-β peptide. These ideas open up the possibility of using phytoestrogens to prevent the onset of Alzheimer's disease. More studies are required to determine whether estrogens and/or phytoestrogens fulfill these expectations. *Antioxid. Redox Signal.* 9, 1677–1690.

INTRODUCTION: EFFECT OF GENDER ON ALZHEIMER'S DISEASE INCIDENCE AND PREVALENCE

World Alzheimer's Day, Sept 21, 2006, marked the centenary of the discovery and naming of the clinicopathological entity that we now recognize to be the major cause of dementia syndrome, and one of the most burdensome conditions of later life.

Alzheimer's disease (AD) is a progressive neurodegenerative disease of the central nervous system associated with unrelenting deterioration of cognition and memory, resulting in dementia (88). AD is the most common age-related neurodegenerative disorder and the most frequent form of dementia affecting >25 million people worldwide (113). Early symptoms include memory loss and amnesia, which starts as minor forgetfulness and become more and more pronounced with the progression of the disease. Eventually, cognitive impairment causes aphasia, loss of coordinated movement, and agnosia. AD may also result in behavioral changes, such as outbursts of violence or excessive apathy in people who have no previous history of such behavior. In the later stages, deterioration of musculature and mobility, leading to inability to feed oneself and

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incontinence, will be seen if death from some external cause (*e.g.*, heart attack or pneumonia) does not intervene. Typical duration of the disease is ~7–10 years, although there are cases where reaching the final stage occurs within 4–5 years, or in some reported cases up to 21 years. The pathological processes underlying the disease consist mainly of neuronal loss or atrophy, principally in the temporoparietal cortex, but also in the frontal cortex, with a brain weight loss of around 20%. Neuropathologically, the disease is characterized by the accumulation of extracellular plaques, essentially formed by amyloid-β (Aβ) peptide, as well as by intracellular neurofibrillary tangles (NFT) which consist of hyperphosphorylated tau proteins and loss of neurons, neuronal processes, and synapses in the cerebral cortex and certain subcortical regions (62). Synapse loss is an early phenotypic sign in the pathology of AD (60, 100, 103). One hypothesis for the cause of neurotoxicity in AD is the generation of Aβ that is a 39–43 amino acid peptide derived by cleavage of the amyloid-β protein precursor (AβPP) by the enzymes β and γ secretase (98). This is the amyloidogenic pathway. Several studies indicate that Aβ is important in the etiology of AD, inducing neurotoxicity and neuronal death by an apoptotic pathway. Microinjection of Aβ peptide in cultured human primary neurons produces toxicity (119). Much evidence implies apoptosis in the neuronal death in the AD. In postmortem analysis of AD brain, there is DNA fragmentation in neurons and glia in hippocampus and cortex (98), increased expression of Bcl-2 family members (23), and increased caspase activities. Cleavage of caspase substrates has also been detected in AD brain (115). The cerebellum is not affected. Many genes relevant to the disease have been recognized. Some of these are involved in early-onset forms of the disease and have a direct causal effect: the AβPP gene on chromosome 21 and presenilin genes 1 and 2 located on chromosomes 14 and 1. Presenilin 1 and 2 are the principal components of the γ-secretase enzyme. Mutations in these genes produce excessive Aβ production. The apolipoprotein E gene, located on chromosome 19, and other unidentified genes may determine susceptibility in late-onset forms and sporadic cases. Tau protein has also been implicated in the etiopathology of AD and has been shown to inhibit kinesin-dependent transport of peroxisomes, neurofilaments, and Golgi-derived vesicles into neurites. It also inhibits the transport of AβPP. Disturbance in trafficking of proteins and organelles may impair cellular functions and result in neurodegeneration. The Framingham study determined the incidence of dementia and probable AD in a general population, showing that AD is clearly an age-related disease. In fact, age is the greatest risk factor for developing AD and we are now facing an increase in the number of cases of AD as populations are getting older. The incidence of dementia increases with age, doubling in successive 5-year age groups, such that the incidence of dementia increased from 7 per 1000 at ages 65–69 to 118 per 1,000 at ages 85–89. The importance of gender in the incidence of AD has been repeatedly studied. A high proportion of women are affected by this disease, especially at a very advanced age, which may be primarily associated with the fact that women live longer. Differences in educational status between genders, especially in developing countries, may also play a role. Some studies suggest that incidence rates are increased in women. Early papers demonstrated that gender is an independent risk factor for AD and it was postulated that gender itself might play a role in the pathogenesis of this disease (115). For this reason, the influence of estrogens on the brain (and the decrease of it during menopause) is of special interest. After menopause, circulating levels of estrogens obviously decline, influencing several brain processes predicted to influence AD risk (89). The control of estrogens on oxidative stress, inflammation, and the cerebral vasculature might also be expected to increase AD risk. Estrogen receptors are found in brain areas important to verbal memory, working memory, and retrieval such as the hippocampus and the frontal lobes. Experimental studies in rodents show that estrogen administration enhances the cholinergic function that is impaired in AD (55).

Based on the above, it is reasonable to think that estrogen might play an important protective role against the deterioration in these cognitive functions that occur with normal aging. Several observational studies reported beneficial effects of hormonal therapy (HT) on cognition but these results are not confirmed by clinical trials (26). The Women's Health Initiative Memory Study (WHIMS) is a substudy of the Women's Health Initiative (WHI) in which 7,479 women, mean age of 65 years, enrolled in WHI were randomized to treatment with either unopposed estrogen (Premarin) if they were hysterectomized, or a combination of Premarin and a progestational (Prempro) agent if they retained a uterus. The principal end point was all cause dementia. The Prempro arm was stopped in 2002 because of an increase in heart attack and breast cancer and the Premarin arm was stopped in 2004 because of excessive strokes and the absence of cardiovascular benefit. Analysis of each arm separately and both arms combined revealed an increase in the cumulative hazard for probable dementia (90–92). On the basis of current evidence, hormone replacement therapy (HRT) is not indicated for the prevention of AD.

There is strong experimental evidence that oxidative stress is involved in the pathogenesis of AD, although it is not yet clear whether the resulting alterations act as a causative agent of neuronal degeneration (111). The free radical theory of aging, which was proposed many years ago by Denham Harman, posits that reactive oxygen species (ROS) whose rate of formation increase with age, results in damage to major components of cells: nucleus, mitochondrial DNA, membranes, and cytoplasmic proteins. The imbalance between the generation of free radicals and ROS may be involved in the pathogenesis of most of the neurodegenerative disorders, including AD (5, 110). Pioneering work from George Perry and Mark Smith showed that all risk factors for this disease are related to oxidative stress (96). The fact that age is a key risk factor in AD provides support for the free radical hypothesis, because the effects of the attacks by free radicals can accumulate over the years. There are many observations that support this, the oxidation of mitochondrial DNA and nuclear DNA has been observed in the parietal cortex of AD patients (65). Protein oxidation has also been observed in elderly individuals with and without AD, but is more marked in AD patients in the regions presenting the most severe histopathologic alterations (39, 43). Several studies have shown increased lipid peroxidation in the temporal lobe of AD patients (76). A healthy brain is protected from oxidative stress by antioxidant defenses, including ascorbate, vitamin E, and glutathione. There is experimental evidence showing that impairment in cellular total antioxidant capacity plays a key role in AD. The activities of antioxidant enzymes are reduced in the

frontal and temporal cortex of AD patients (59). Significant biological changes related to oxidative stress have been found not only in brain, but also in peripheral tissues of AD patients. Many studies deal with the search for soluble peripheral biomarkers of oxidative stress in biological fluids such as cerebrospinal fluid, peripheral blood, or urine, and in peripheral tissues such as fibroblasts or blood cells. The isoprostane 8,12-isoiPF2alpha-VI, which is a marker for *in vivo* lipid peroxidation, is elevated in the urine, blood, and cerebrospinal fluid of patients with AD (78, 82). Levels of 8-OHdG, a marker of oxidative damage to DNA, are elevated in lymphocyte DNA from AD patients (64). In the same study, it was shown that these patients have lower plasma levels of antioxidants than healthy persons of the same age.

A number of studies have been directed towards determining the possible contribution of mitochondria to the pathophysiology of AD because mitochondria are one of the major sources of oxidative stress. Defects in the electron transport chain within the mitochondria are major factors that contribute to the production of free radicals. Many studies have shown a low level of oxidative phosphorylation in AD. This results in energy deficits as well as in the potentially toxic production of free radicals. Many observations have confirmed alterations in mitochondrial function in the course of aging and neurodegenerative diseases such as AD (44, 59, 79). Defects of mitochondrial function can result in excessive production of ROS, opening of the permeability transition pore (PTP), and release of small proteins that trigger the initiation of apoptosis, such as cytochrome c which activates the caspase cascade (82). Direct evidence for mitochondrial dysfunction in AD comes from reports of cytochrome c oxidase (COX) deficiency in AD brain (71, 94). Current evidence shows that Aβ causes mitochondrial dysfunction, resulting in oxidative stress and caspase activation (42, 74), and subsequently in the neuronal apoptosis seen in AD. In isolated mitochondria, Aβ causes a loss of mitochondrial membrane potential, and inhibits the activities of mitochondrial electron transport chain complexes, such as cytochrome c oxidase and pyruvate dehydrogenase (1).

Our laboratory, in recent years, has been involved in determining the effect of gender on life span in several species, including humans. It is well known that females live longer than males in species such as the rat, mouse, and human (108). We have recently studied the role of Aβ on mitochondrial function, particularly on the rate of peroxide production by mitochondria. Work by Moreira *et al*. (68) showed that Aβ exacerbates age-associated mitochondrial dysfunction. Thus we concerned ourselves with studying the possibility that gender is a risk factor for the toxicity of Aβ on mitochondria (109). In this review, we summarize evidence relating gender, mitochondrial toxicity and mitochondrial dysfunction in AD with the background view that AD increases the normal decay of mitochondrial function with age.

MITOCHONDRIAL TOXICITY OF ALZHEIMER'S Aβ PEPTIDE

The original hypothesis for Aβ toxicity was that extracellular deposits of the toxic peptide were responsible for the cellular damage observed in the disease. This view, which had been prevalent for many years, has been changed, and researchers are today increasingly recognizing the toxic role of Aβ inside the cell (13). Gouras and co-workers found Aβ 1–42 inside human neurons (37). Moreover, intracellular aggregates of Aβ are observed before the appearance of senile plaques and this correlates with cognitive impairment. AβPP is an integral membrane protein with a large extracellular glycosylated N-terminus and a shorter cytoplasmic C-terminus. AβPP can be processed by β-secretase (BACE) and γ-secretase enzymes. When AβPP is cleaved by BACE, a peptide composed of 99 residues is formed that is internalized and further processed by γ-secretase to produce Aβ 1–40 or 1–42 peptides in endocytic compartments. The 99 amino acid fragment can translocate to the nucleus where it may regulate gene expression, including the induction of apoptotic genes (53). A new cleavage of the 99 amino acid peptide by caspases produces a neurotoxic peptide composed of 31 amino acids (54). Functional complexes with γ-secretase activity, which is essential to cleave AβPP and create Aβ, have been found inside mitochondria (40). Aβ is located at the cell surface or on the luminal side of the endoplasmic reticulum, mitochondria, and Golgi membranes, with part of the peptide embedded in the membrane. Of note, Anandatheerthavarada *et al.* have shown that AβPP carries a dual leader sequence, permitting targeting to the endoplasmic reticulum (ER) or to mitochondria (2). In a recent study, these authors have extended their experiments to postmortem AD human brain samples (23) and showed that levels of mitochondrial AβPP were higher in more affected brain areas and in subjects with more advanced disease. It seems that AβPP interacts with two translocases of the outer and inner mitochondrial membranes (TOM40 and TIM23), suggesting that AβPP blocks this machinery.

The first experiments showing death of neurons in culture induced by Aβ date back to the 1990s (116). There is ample evidence that intracellular toxicity of Aβ peptide leads to apoptotic cell death. Apoptosis includes changes in the cytoplasm, endoplasmic reticulum, mitochondria, and nucleus. In fact, the role of mitochondria has emerged as key in the development of the disease. Furthermore, work from the group of Oliveira has shown that intact mitochondria are required for the toxicity of Aβ. These researchers showed that cells lacking mitochondrial DNA did not show toxicity of Aβ. The general conclusion that can be drawn from the work of Oliveira's group is that Aβ interferes directly with mitochondria, particularly by inhibiting or decreasing the efficiency of the mitochondrial respiratory chain.

The relationship between respiratory chain and Aβ toxicity was proposed years ago. Parker and co-workers showed defects in electron transport chain in the brain of AD patients (80). The authors proposed that all electron transport chain complexes show a depression of activity but this depression was most marked in COX activity. Indeed COX has been purified from brain of AD patients and displayed anomalous kinetic behavior compared with that of control brain. Thus, the COX from AD patients in brain may be structurally abnormal contributing to the bioenergetic defect seen in AD (81). COX activity has been related with the sAPP secretion in fibroblast from AD patients. Inhibiting COX activity increased the released of AβPP (30).

This deficiency in the electron transport chain and an impairment of mitochondrial energy metabolism has been well

documented in brain and in peripheral cells from AD patients (4, 6, 33). Altered levels of oxidative phosphorylation enzymes have been found to be directly responsible for a decrease in energy production in the brains of AD patients and the rate of cerebral metabolism in general is reduced in AD (4). Bosetti *et al.* have shown a decrease in the COX and F1F0-ATPase activities in platelets from patients with AD (10). This defect has been related with somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication (18) and with differential expression of oxidative phosphorylation genes in AD patients (58).

An immediate conclusion from these studies is that the radical production by mitochondria will be increased in the presence of Aβ. Indeed, there is a general misconception that increased flow of electrons through the respiratory chain will lead to an increased production of free radicals. This is derived from the idea that 2% of all oxygen utilized by cells is converted to free radicals. This conclusion cannot be drawn from the pioneer work of the group of Britton Chance and his colleagues. What these authors stated is that 2% of all oxygen used in mitochondria when they are in state 4 is converted to ROS. However, when mitochondria turn to state 3 (*i.e.*, when there is availability of ADP and thus an active oxidative phosphorylation), the proportion of free radicals derived from mitochondria decreases to $<10\%$ that of state 4. Therefore, an increased flow of electrons through the respiratory chain normally leads to a lower production of radicals in mitochondria. This is usually the case in physical exercise. However, the case with Aβ is the contrary: lower respiratory chain will lead to an increased radical production by mitochondria. This kind of experiment was undertaken in our laboratory. Our experiments show that the rate of oxidant production by mitochondria is increased approximately fourfold in the presence of Aβ peptide. The reverse, nontoxic peptide (Aβ 42–1) does not cause an increase in radical production by mitochondria (Fig. 1). Several groups have found that Aβ interacts with mitochondria, impairing mitochondrial function and increasing free radical generation (14, 22, 56, 58, 109).

Another molecular explanation, complementary with the ABAD hypothesis, is that Aβ binds to heme, particularly heme present in the respiratory chain and in particular in cytochrome c. This leads to a lower activity of COX and therefore to a decreased flow of electrons through the respiratory chain. We postulated that the toxic effect of Aβ in the presence of mitochondria could be due to its interaction with heme. We thus pretreated Aβ with heme and then incubated this complex with mitochondria. We found that under these conditions Aβ did not cause an increase in the production of oxidants by mitochondria. Therefore, we propose that Aβ peptide causes a direct mitochondrial toxicity via the following mechanism: Aβ binds to heme present in the cytochrome c which is in the internal mitochondrial membrane. This causes a deficiency in cytochrome c which in turn leads to a lower rate of respiration by mitochondria. This in turn causes an increased production of oxidants and is important to explain the cellular oxidative stress which is observed in AD (109).

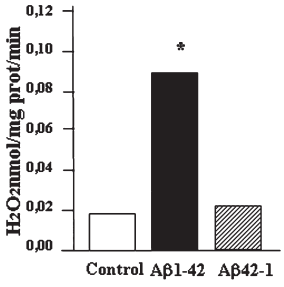

FIG. 1. Mitochondrial Aβ toxicity. Hydrogen peroxide generation in brain mitochondria from 6-month-old male Wistar rats incubated with 10 m*M* Aβ 1–42 peptide. The number of experiments was 12. The statistical difference is indicated as follows: $*p < 0.05$ *versus* control.

If this is really the case, and mitochondria (and other organelles like lysosomes) are impaired in AD, axonal transport and its function may play an important role in the pathogenesis of the disease. Trimmer and Borland (103a) have created a cybrid cell line that model AD pathology by fusing platelets containing mitochondria from age-matched platelets from AD and control volunteers with mitochondrial DNA-free SH-SY5Y human neuroblastoma cells. The authors observed that the mean velocity of mitochondrial movement, and the percentage of moving mitochondria, was significantly reduced in AD cybrids. These results suggest that the axonal transport machinery may be compromised in cybrid cell lines that contain mitochondrial DNA derived from AD patients. Reduced mitochondrial and lysosomal movement in susceptible neurons may compromise synaptic function and participate in the neurodegeneration that occurs in AD.

Wang and co-workers have quantified oxidized bases in nuclear and mitochondrial DNA of frontal, parietal, and temporal lobes and cerebellum from brain of AD patients, shortly after death and from age-matched control subjects. They found that the levels of oxidized bases in AD brain specimens were significantly higher in frontal, parietal, and temporal lobes compared to control subjects, and that mitochondrial DNA had approximately 10-fold higher levels of oxidized bases than nuclear DNA. These data are consistent with higher levels of oxidative stress in mitochondria. DNA from temporal lobe showed the most oxidative damage, whereas cerebellum was only slightly affected in AD brains. These results confirm that oxidative damage to mitochondrial DNA may contribute to the neurodegeneration of AD (112).

After the demonstration of the role of oxidative stress in AD, many researchers have tried to reduce it with antioxidants. In our results, reduced glutathione (GSH) prevented high free radical levels produced after incubation with Aβ (109). Veereshwarayya and co-workers reported that Hsp70 protects cultured neurons from cell death caused by intraneuronal Aβ. They have recently shown that Hsp60, Hsp70, and Hsp90 both alone and in combination provide protection against intracellular Aβ stress through the maintenance of mitochondrial oxidative phosphorylation and functionality of tricarboxylic acid cycle enzymes (106). A decrease in mitochondrial superoxide dismutase (SOD) activity accelerates the onset of behavioral changes in human AβPP transgenic mice. Whereas reductions in mitochondrial SOD would be expected to trigger or exacerbate neuronal and vascular pathology in AD, increasing SOD activity might be of therapeutic benefit (27). In a parallel study, Anan-

tharaman and co-workers showed that MnSOD levels do not change in the APPNLH/NLH X PS-1P264L/P264L double knockin mouse model of AD, but MnSOD activity and mitochondrial respiration decreased in knockin mice, suggesting compromised mitochondrial function (3). This chronic deficiency in the electron transport chain could explain the increase in ROS generation leading to neuronal dysfunction.

On the other hand, the peroxisomal content in hippocampal neurons has been related to oxidative stress in AD. An increase in the number of peroxisomes, with a concomitant increase in catalase activity decreased the production of ROS, cytoplasmic calcium uptake, and mitochondrial potential in hippocampal neurons exposed to Aβ (86).

But the definitive link between mitochondria and AD was established when Wallace and co-workers found a correlation between the progression of the disease and mtDNA tRNA MTTQ*ADPD433C mutation, in some cases of AD (17). Moreover, additional mtDNA mutations have been described: MTND1*ADPD3397G, in 12S rRNA gene and a heteroplasmic 16S rRNA mutation, MTTRNR2*ADPD3196A. Levels of 8-hydroxyguanosine, an oxidative mutational modification in DNA, have been found to be threefold higher in mtDNA from AD patients in comparison to control brains (15). Recently, Kazuno and co-workers have shown that the mtDNA polymorphisms 10398A, associated with an increased risk of AD, may play a role in the pathophysiology of this complex disease by affecting mitochondrial matrix pH and intracellular calcium dynamics (47). Nevertheless, Elson and co-workers have recently published the analyses of the complete mtDNA coding region sequences from 270 AD patients and normal controls to determine if inherited mtDNA mutations contribute to the etiology of AD. The authors concluded that inherited mtDNA mutations do not constitute a major etiological factor in sporadic AD (25). Only a small proportion of AD patients carry a pathogenic mtDNA mutation and a small proportion of cognitively normal aged individuals carry a mtDNA mutation that reduces the risk of AD. In contrast, van der Walt and co-workers have examined the association of mtDNA variation with AD risk in 989 cases and 328 controls, testing the effect of individual haplogroups and single nucleotide polymorphisms (SNPs). Haplogroups were defined by ancestral polymorphisms that are continent-specific. Nine primary mt haplogroups have been identified in European populations (H, I, J, K, T, U, V, W, X). Differences relative to gender in haplogroup U have been found. Males classified as haplogroup U showed an increase in risk of AD relative to the most common haplogroup H, while females prevented a significant decrease in risk with haplogroup U (105). Coskun *et al*. have investigated the sequence of the mtDNA control region from AD brains for possible disease-causing mutations. Sixty-five percent of the AD brains harbored the T414G mutation, whereas this mutation was absent from all controls. Moreover, cloning and sequencing of the mtDNA control region from patient and control brains revealed that all AD brains had a 63% increase in heteroplasmic mtDNA control region mutations and that AD brains from patients 80 years and older had a 130% increase in heteroplasmic control region mutations. In addition, these mutations preferentially altered known mtDNA regulatory elements (18).

To conclude this section, we want to emphasize the new idea from George Perry's and Mark Smith's group that oxidative markers seen in AD may be products of an adaptative situation. Aβ deposition and hyperphosphorylated tau function could be a compensatory response and downstream adaptation to ensure that neuronal cells do not succumb to oxidative damage (67). Extracellular accumulation of A in the form of plaques are proposed not as a cause of the neuronal lesions, which occur in AD, but rather as a protective mechanism against cellular damage in AD (69). Previous results from the same group, demonstrated an increase in mtDNA and an overexpression of mRNA, in postmortem brain specimens from AD patients compared with age-matched healthy controls. The excess of mtDNA is due to a mtDNA replication specific for neurons that undergo oxidative damage. Reddy and co-workers suggest that the increase in mtDNA must be due to an accumulation of products from degraded mitochondria. The connection between decreased COX and increased mRNA has been interpreted as a compensatory mechanism in AD (83). Strazielle *et al*. also suggested that, the increased cytochrome oxidase activity in transgenic mice might be the result of functional compensation on the part of surviving neurons (97).

MITOCHONDRIA FROM FEMALES PRODUCE FEWER OXIDANTS THAN THOSE FROM MALES

AD, as stated above, is highly gender dependent. Thus, it is relevant to comment on the importance of gender on the radical production by mitochondria. This has been a major concern in our laboratory for the last 10 years. We have been interested in why females live longer than males. A major challenge of gerontology is to find reliable markers of biological, as opposed to chronological, aging. In many cases, the search for reliable parameters of biological aging has been sterile. The level of 16S rRNA is an interesting marker of aging. Work by the group of Marco in Madrid (12) showed an important decrease in mitochondrial transcripts of 16S rRNA with aging. Moreover, they reported that these changes correlate with the shape of the life span curve. Work by the group of Davies showed that 16S rRNA degradation is associated with oxidative stress (21). Our group demonstrated that mitochondria from female animals have a higher expression of 16S rRNA than those from males. The 16S rRNA expression was over 700% higher in females than in males. Glutathione (GSH) is another biomarker of aging with a similar concentration compared to glucose (107). Mitochondrial GSH levels are doubled in females. The intracellular level of the oxidized form of glutathione (GSSG) is considered a biological marker of aging (41), and we have found that mitochondrial GSSG is related to the damage associated with aging (87). Thus, we conclude that biological markers of aging indicate that females behave as if they were younger than males of the same chronological age.

Moreover, we observed that mitochondria from females produce approximately half the amount of peroxides than those from males (9). This leads to a mitochondrial oxidative stress which can be observed by measuring the glutathione redox ratio in mitochondria and, more importantly the amount of DNA oxidation in these organelles (9). We have observed that glutathione levels in mitochondria from males are significantly

lower than those from females and that the rate of oxidation of mitochondrial DNA in females is fourfold lower than that in males. Mitochondrial DNA is a key component of the mitochondrion, and its degree of oxidation is directly related to aging. The level of 8-oxodeoxyguanosine, an excellent indicator of oxidative damage to DNA, is four times as great in mitochondria from males than in those from females (9). This is the most pronounced oxidative change we have observed in mtDNA in any physiological situation, and it indicates that the higher chronic and continuous H_2O_2 production in males results in marked oxidative damage to mtDNA and in mutagenic lesions to DNA. Moreover, we observed that mitochondria from postmitotic tissues are much more damaged than those from tissues with active mitotic activity. Interestingly, in the brain, mitotic and postmitotic cells coincide: synaptic mitochondria are neuronal (*i.e.*, postmitotic and nonsynaptic mitochondria are mainly glial, that is, mitotic cells). Figure 2 shows that the rate of peroxide production from mitochondria from postmitotic cells such as neurons is approximately double that of mitotic tissues such as hepatocytes or glia. Figure 2 also shows that in all cases mitochondria from females produce approximately half the amount of peroxides than those from males. In our search for an explanation for this phenomenon, we came to the conclusion that it is due to the protective effects of estrogens (for a review, see Ref. 108). Estrogens can play important neurotrophic and neuroprotective roles during the lifetime of women. In fact, estrogen-induced synaptic plasticity is clearly seen during puberty and the ovarian cycles. Estrogen appears to be important for the regulation and maintenance of network integrity of several brain areas related to cognition (29). In addition to changes in the cortex accompanying cognitive tasks, estrogen regulates the anatomy and connectivity of the hippocampus and associated structures. In postmenopause, neurotransmitters, neuropeptides, and neurosteroids undergo important changes as a consequence of the failure of gonadal hormone production at a time when many CNS activities deteriorate, particularly those associated with hippocampal functions such as memory, attention, cognition, and autonomic control (31). This neuroendocrinological aging process represents a unique opportunity to investigate the actions of gonadal hormones and their specific receptors in the nervous system. It is important to point out that estrogens do not act as antioxidants *per se* (*i.e.*, due to the phenolic ring in their chemical structure) but rather because they catalytically activate genes by interaction with estrogen receptors and subsequent activation of the MAP kinase–NFKB pathway which leads to the upregulation of antioxidant genes, particularly those of superoxide dismutase and glutathione peroxidase (7, 8, 57).

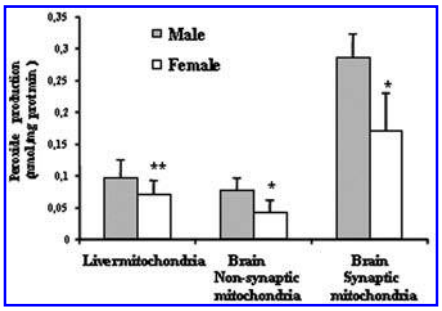

FIG. 2. Oxidant production by synaptic (brain) and nonsynaptic (brain and liver) mitochondria. Hydrogen peroxide generation in brain mitochondria from male and female Wistar rats. The number of experiments was 3–5 for synaptic mitochondria and 7–9 for nonsynaptic (brain or liver) mitochondria. The statistical difference is indicated as follows: $*p < 0.05$, $**p < 0.01$ *versus* male rats.

Thus, estrogens upregulate the expression of mitochondrial superoxide dismutase and of glutathione peroxidase. The amount of ROS that are formed by mitochondria is the consequence of the interaction between production (mainly at the mitochondrial respiratory chain) and detoxication (by peroxidase and superoxide dismutase). Since these last two enzymes are upregulated by estrogens, the total amount of oxidants released by mitochondria must be lower in mitochondria from females than in those from males. This results in a chronic oxidative stress which is higher in males than in females. A major aim of our laboratory is to define nutritional or other kinds of physiological measurements (including changes in lifestyle) that may result in lowering the rate of oxidant formation in males to levels similar to those found in females. This may result in an increase in life span of ~15% in the male population (*i.e.*, to equal the high longevity of females).

MITOCHONDRIA FROM YOUNG, BUT NOT OLD, FEMALES ARE PROTECTED AGAINST Aβ PEPTIDE TOXICITY

Cognitive decline is well recognized during aging. It is often accelerated in women after menopause. There are significant gender differences in aging brain with significantly greater changes in brain structure, function, and metabolism. One of the most exciting areas of research in women's health over the past 10 years involves growing appreciation that estrogens play important neurotrophic and neuroprotective roles during adulthood. This brings new meaning to the potential impact of the prolonged postmenopausal hypoestrogenic state on learning and memory and the increased vulnerability of aging women to brain injury and neurodegenerative diseases. The increase in female life expectancy during the past century has meant that women now live one-third of their lives beyond cessation of their ovarian function. This evolution in demography has increased the need for the development of new therapeutic strategies to promote successful aging, defined as low probability of disease, high cognitive and physical capacity, and active engagement in life. Because changes in the aging nervous system are delicate, it may be possible to reverse them and to improve cognitive performance by pharmacological treatment (32).

The aim of our study was to determine the rate of oxidant production by mitochondria in the presence of Aβ. We were puzzled by the fact that Aβ increases the rate of oxidant production in mitochondria from males but not from females. This was indeed very surprising in view of the higher incidence of AD in women than in men. The solution came when we measured the rate of oxidant production from aged animals and found that mitochondria from aged females were not protected

against toxic effects of Aβ as those from young females. Thus, estrogens protect against the increased rate of oxidant production by mitochondria caused by Aβ. It must be pointed out that in the rat, unlike humans, estrogen levels do not fall at old age. However, a decreased sensitivity to estrogens is observed. In any case, estrogen action decreases with age in old rats as it does in older women and in that case Aβ causes an increased oxidant production by mitochondria. Figure 3 shows the rate of oxidant production in the presence of Aβ in young male rats, young female rats, and old female rats.

Several experimental data show that estrogen treatment can protect against a wide range of neurotoxic insults, including free radical generators, excitotoxicity, Aβ-induced toxicity, and ischemia (16). The neuroprotective effects of estrogen have a range from the chemical to the biochemical to the genomic mechanisms. It is not yet clear whether there is one unifying neuroprotective cascade induced by estrogen or whether estrogen induces multiple mechanisms that are selectively neuroprotective against different neurotoxins or whether it is a combination of both. The hippocampus is a brain region that is involved in episodic, declarative, contextual, and spatial learning and memory, as well as in serving as a component in the control of autonomic and vegetative functions (46). The hippocampus is vulnerable to damage by stroke and susceptible to damage during aging and repeated stress (24). Estrogen effects on memory have been reported in animal models and in studies in humans. The memories affected are ones in which the hippocampus plays a role along with the basal forebrain cholinergic system and other neurochemical systems. Rather than one estrogen-regulated process, many types of estrogen actions on different neurochemical and neuroanatomical substrates are likely to cause the actions of estrogens on cognition and other aspects of behavior such as mood, pain perception, and nociception (63). One of the best-known processes regulated by ovarian hormones is the cyclic formation and breakdown of excitatory synapses in the hippocampus (114). Estrogen treatment increases dendritic spine density on CA1 pyramidal neurons in the hippocampus within 24–72 h of acute administration (36). When progesterone is administered after estradiol priming, spine density increases during the first 6–8 h, followed by a rapid return to low baseline levels. Memory performance in rats is strictly temporally related to the hormonal status: 10 μg injection of estradiol is able to improve memory retention until 72 h (85).

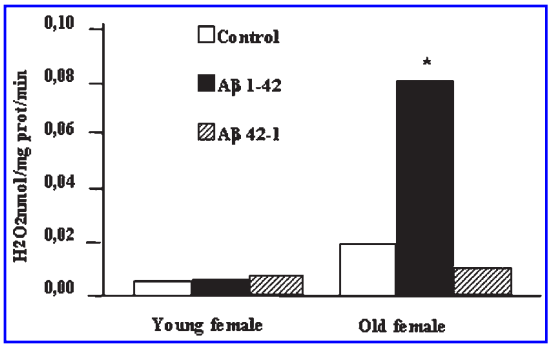

FIG. 3. Gender and age differences in mitochondrial Aβ toxicity. Hydrogen peroxide generation increases in brain mitochondria incubated with 10 m*M* Aβ 1–42 peptide in old female rats but not in young female rats. The number of experiments was 12. The statistical difference is indicated as follows: $^*p < 0.05$ *versus* control.

Furthermore, estrogens are also involved in another mechanism related to the CNS protection: the immune system exerts a series of significant activities in the CNS. It maintains homeostasis, the silent cleaning and refurbishing of the brain. The brain is constantly assaulted by free radical, metabolic waste, trauma, infectious disease, and so on. While the immune response is fundamental to the maintenance of CNS homeostasis, if excessive or not regulated, it can damage brain cells and disrupt neural processes (93). Estrogens may modulate cellular and humoral immune responses. The regulation of checkpoint proteins, such as the Fas/Fas-ligand as well as the CD40–CD40 ligand systems, is built up by estrogens. Inside the CNS there are different cells, including astroglia and microglia, and estrogens regulate microglial cells at different levels, in particular modulating microglial expression of cytokines and growth factors. In the absence of estrogens, it may be expected that less restricted immune responses speed up clinical brain disorders (66). Glial cells are involved in age-related brain modifications, and specific neuroanatomical changes may be observed. For instance, modifications of extrasynaptic 'volume' transmission can be seen in aged brains because of the loss of extracellular matrix and of narrower intercellular clefts. Progressive loss of the orientation and number of glial processes (anisotropy) and replacement of neurons by hypertrophy and proliferation of glial processes are also seen with aging (gliosis). Deposition of macromolecules (*e.g.*, amyloid-β) can be seen with aging. These are a typical feature of pathological conditions such as AD. Structural and functional modifications contribute to the reduction in diffusion of neuroactive substances in the extracellular space, therefore, leading to the progressive decline of synaptic and extrasynaptic transmission and of synaptic plasticity, ultimately helping to explain reduced brain performance (99).

In addition to the abundant experimental data demonstrating the positive effect on cholinergic activity, estrogen also influences two key proteins implicated in AD pathology: tau and Aβ. Estrogen induces tau formation, a process that coincides with the enhanced growth of axons and dendrites. Estrogen also acts to reduce the formation of Aβ, blunting its neurotoxic effects. The large AβPP, encoded by a normal chromosome 21 gene, can be proteolytically processed at alternative sites. At physiological concentrations, estradiol leads to degradation products that are unable to accumulate as Aβ (38).

Estrogens have been shown to affect the nervous system in many different ways: via binding to estrogen receptors but also via many other pathways. The results of small randomized trials and larger observational studies suggest a beneficial effect of estrogen therapy on cognitive function in symptomatic postmenopausal women. AD is two to three times more common in women than in men. Based on currently available data, routine therapeutic use of estrogens in women with AD is not justified but it may have a role in the prophylaxis of AD. The existing evidence supports the use of HRT therapy in women with menopausal symptoms for a few years following menopause (11).

MITOCHONDRIA GENERATE SIGNALS THAT ARE IMPORTANT IN ALZHEIMER'S DISEASE PATHOPHYSIOLOGY

Mitochondria play a central role in both cell life and death. The role of mitochondria was classically centered on the generation of energy via the respiratory chain–oxidative phosphorylation. Mitochondria were also the site of the Krebs cycle enzymes and therefore their role in energy metabolism was, and is indeed prevalent. Mitochondria are not only essential for energy production via the Krebs cycle–the respiratory chain–oxidative phosphorylation, they are also responsible for several other important cellular functions, including heme production, synthesis of some steroids, and ureogenesis and apoptosis. Furthermore, mitochondria regulate intracellular Ca^{2+} homeostasis and are the principal generators of intracellular ROS. Mitochondria also play a key role in controlling pathways that lead to apoptosis. These mitochondrial roles are critical in the brain, given its high energy demand that is driven by the need to maintain ion gradients across the plasma membrane and it is critical for the generation of action potentials.

However, in recent years, particularly from the turn of this century, the role of mitochondria as generators of signals to understand cell physiology has been highlighted. Reactive oxygen species act as mediators of mitochondrial signaling functions. Defects of mitochondrial function can result in excessive production of ROS, formation of the PTP, and release of small proteins that trigger the initiation of apoptosis, such as cytochrome c and apoptosis-inducing factor (AIF), from the mitochondrial intermembrane space into the cytoplasm. Released cytochrome c binds apoptotic protease-activating-factor-1 (Apaf-1) and activates the caspase cascade (42). Neuronal death can occur by necrosis or apoptosis, which differ morphologically and biochemically. Necrosis is the result of extreme perturbation of the cellular environment, as occurs in ischemic insults or trauma. For this reason, intracellular constituents pour out into the extracellular space. This results in inflammation which normally accompanies necrosis. In contrast, apoptosis, also known as programmed cell death, is dependent of intracellular pathways, resulting in cellular commitment to a defined series of steps resulting in cell suicide. Apoptosis is an important mechanism in normal cell turnover, in growth and development, as well as in maturity. Exposure of acidic phospholipids on the cell membrane during apoptosis is a signal for phagocytic uptake of apoptotic cells. The latter occurs considerably before loss of membrane integrity and unregulated leakage of intracellular contents. It has been generally believed that the predominant mechanism of pathologic cell death in CNS injury is necrotic, and physiological cell death during brain development is regarded as apoptotic. However, recent accumulating evidence strongly suggests that uncontrolled apoptosis might additionally contribute to neuronal death in a variety of neurodegenerative disorders such as AD, Parkinson's, Huntington's diseases, and amyotrophic lateral sclerosis (61). The execution of neuronal apoptosis involves relatively few pathways that converge on activation of the cysteine proteases called caspases. Two principal pathways are well known with respect to their activation: the cell surface death receptor pathway and the mitochondrial pathway. In the death receptor pathway, activation of caspase-8 is the critical event that transmits the death signal. In the mitochondrial pathway, caspase activation is triggered by the formation of an Apaf-1/cytochrome c complex that is fully functional in recruiting and activating procaspase-9. Activated caspase-9 then cleaves and activates downstream caspases, such as caspase-3, -6, and -7. The mitochondrial pathway appears to be regulated by the Bcl-2 family of proteins, and there may be participation of ion channels/nonselective pores, in particular the PTP, that may be activated by pro-apoptotic stimuli.

Mitochondrial dysfunction has been proposed as a potential mechanism in the development and pathogenesis of AD and neuronal apoptosis has been detected in AD brain. In fact, mitochondria from patients with AD are hypofunctional (10, 20) because of a catalytic defect in respiratory complex IV of AD-associated mitochondria (34, 50). Evidence for mitochondrial dysfunction in AD comes from several reports of COX deficiency in AD patients. Histochemical analyses revealed a significant reduction of COX activity in the dentate gyrus and other subfields of the hippocampus. In situ hybridization studies also show decreased mRNA levels of the mtDNA-encoded subunit II, but not the nuclear DNA-encoded subunit IV, of COX. High levels of mtDNA mutations, linked to COX deficiency, are observed more frequently in hippocampal pyramidal neurons of AD patients, compared to age-matched controls (19). Human teratocarcinoma cells expressing mtDNA from AD subjects display reduced COX activity, elevated ROS, and reduced ATP levels, compared with cells expressing mtDNA from age-matched control subjects (16). These observations suggest that alterations in mtDNA may play a key role in mitochondrial dysfunction in AD. Furthermore, current evidence indicates Aβ may cause mitochondrial dysfunction, resulting in oxidative stress and caspase activation. Aβ25–35 inhibits respiratory complex I and decreases cellular ATP content of neuronal cells (15). Aβ25–35 induces translocation of the Second-Mitochondria Derived Activator of Caspase (Smac) from mitochondria to cytosol via AP-1/Bim activation (117). Intracellular Aβ1–42 selectively causes apoptosis in human neurons through p53 and Bax (119). Expression of a mutant form of AβPP (mAPP) PC12 cells and human embryonic kidney cells results in substantial elevation of Aβ levels and is associated with increased levels of nitric oxide (NO) and reduced ATP, finally leading to cell death (48). DAPT (*N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*phenylglycine *t*-butylester), a functional γ-secretase inhibitor, decreases intracellular Aβ production and normalizes NO and ATP levels in the cells expressing mAPP. All these findings lead to the proposal that Aβ directly disrupts mitochondrial function and may contribute to the deficiency of energy metabolism and neuronal apoptosis seen in AD. Our results confirm this hypothesis. Moreover, additional data indicate that Aβ interacts with a binding protein, termed Aβ-binding alcohol dehydrogenase (ABAD), in mitochondria and directly causes mitochondrial dysfunction (101). ABAD is a mitochondrial protein, which was initially identified in a yeast two-hybrid screen against Aβ. The impressive body of evidence collected by these authors argues strongly for a more central role of mitochondria in the etiology of AD

and Aβ. Furthermore, neurons cultured from transgenic mice over-expressing mAPP and ABAD display spontaneous generation of ROS, loss of mitochondrial membrane potential, and decreased ATP, as well as subsequent release of cytochrome c from mitochondria and induction of caspase-3-like activity followed by apoptotic cell death. In summary, mitochondrial dysfunction and release of cytochrome c trigger the onset of neuronal apoptosis in AD. There is accumulating evidence from *in vitro*, *in vivo*, and human studies suggesting that apoptosis is likely to have an essential role in pathogenesis of AD. As such, understanding of this chemical cascade should lead to determining substances able to block this system and antagonize apoptosis. Apoptosis, by definition, is a controlled event; thereby offering the possibility of intervention. Necrosis is a more stochastic process. Many of the propagators in AD are pro-apoptotic and in fact it is not surprising that certain aspects of apoptosis are evident (120).

In our experiments, incubation with Aβ resulted in an increased release of cytochrome c from mitochondria. As we note above, it is well known that the release of cytochrome c is a starting event in the physiological pathway leading to apoptosis. In Fig. 4, we show that when mitochondria were incubated with Aβ, a significant release of cytochrome c from the organelles was observed in both young male and old females but it did not occur in young females, showing that mitochondria from young females were resistant to Aβ. This mirrors the effect observed on the release of oxidants from mitochondria. Therefore, we can attribute the release of cytochrome c and its prevention in young females to the effect of signaling via the generation of oxidant species. It is well known that cytochrome c is a starting event in the signaling pathway leading to apoptosis. The fact that Aβ is capable of increasing the release of cytochrome c from mitochondria from males or old females and that this does not occur in young females, may be important in understanding the relationship between ROS and the death of the cells (Fig. 4) (109). Further studies examining the importance of mitochondrial pathophysiology in aging, gender, and AD may provide important insight into neurodegenerative disease pathogenesis and may indeed provide a target for specific therapies.

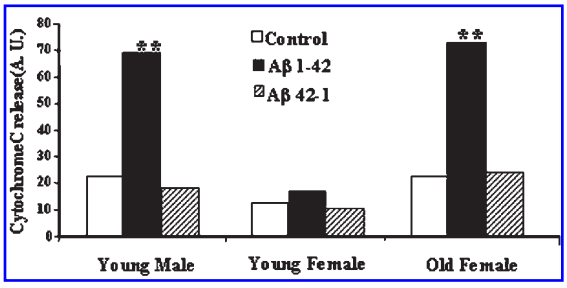

FIG. 4. Release of cytochrome c after incubation of mitochondria with Aβ. Cytochrome c release from rat brain mitochondria was measured by Western blot analysis in the supernatant of mitochondrial suspensions after 6 h incubation with 10 m*M* of the corresponding peptide. The number of experiments was 8. The statistical difference is indicated as follows: $^{**}p < 0.01$ *versus* control.

ESTROGENIC COMPOUNDS ARE ABLE TO DIMINISH Aβ TOXICITY BY PREVENTING ACTIVATION OF THE P38 MAP KINASE

The brain of patients with AD presents a whole series of deficiencies, such as hormones, neurotransmitters, and trophic factors, to name but a few. All of these factors can be a target of pharmacological supplementation in AD patients. Because changes in the aging nervous system are delicate, it may be possible to reverse them and to improve cognitive performance by pharmacological treatments. Administration of estrogens may be particularly interesting. The classical mechanism of hormone action is that estrogens cross the plasma membrane to bind to intracellular estrogen receptors, resulting in translocation of the activated estrogen receptor into the nucleus and eventual regulation of gene transcription. On the other hand, this mechanism of action is insufficient to explain all of estrogen's actions, including the rapidity of some of estrogen's actions in the brain. Alternative mechanisms have been documented, together with the regulation of signal transduction pathways typically associated with growth factor action, including the induction of MAPK and the ability to modulate the NFκB pathways. Estrogens also are important regulators of mitochondrial function. For example, estrogen can elicit the activation of the PI-3K/Akt pathway (45, 95), which can result in the phosphorylation of the proapoptotic protein BAD. When BAD is phosphorylated render, it is rendered inactive and prevents BAX-mediated release of cytochrome c from the mitochondria (14). Furthermore, estrogens have been shown to affect concentrations and localization of antiapoptotic proteins (73), which appear to exert their antiapoptotic effects through maintenance of mitochondrial membrane potential in the face of cellular stresses (51).

The neuroprotective effects of estrogens is another mechanism potentially involved in the maintenance of intracellular calcium homeostasis. Loss of calcium homeostasis correlates with glutamate excitotoxicity and is involved in several brain disorders including AD. Glutamate excitotoxicity results in energy depletion, overactivation of glutamate receptors, excessive calcium influx, and oxidative stress. Hippocampal neurons pretreated with estrogens and then exposed to excitotoxic glutamate respond with an attenuated rise in intracellular calcium and increased cellular survival. Mitochondria are major targets of estrogen action on calcium homeostasis: it has been shown that estrogen treatment in intact neurons potentiates an increase of mitochondrial sequestration of calcium, induced by excitotoxic glutamate through the activation of MAPK. In addition, estrogen effects stabilize mitochondrial membrane potential, prevent ATP depletion, and reduce the generation of oxygen free radical (72).

Estrogen may be of preventive value against the onset of AD. In several prospective studies (77, 102, 118), HRT was associated with reduced risk of AD. However, in a recent study, Mulnard *et al*. (70) reported no benefit of estrogen replacement in women with mild to moderate dementia. Neither estrogen nor progesterone replacement therapy (90, 92) was effective; it increased the adverse effects on global cognitive function in women over 65 years old. Some of this dichotomy could be re-

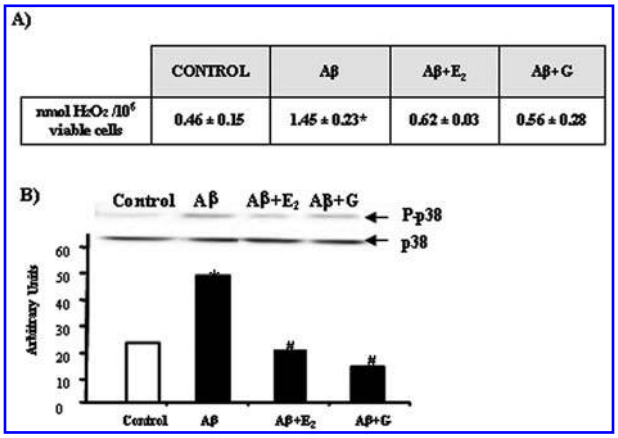

FIG. 5. Estradiol or genistein decreases peroxide levels and prevents p38 MAPK phosphorylation induced by Aβ in neurons in primary culture. (**A**) Peroxide levels were determined in cells treated for 24 h with 5 μM Aβ 40–1 (C), 5 μM Aβ 1–42 (Aβ), 5 μM Aβ 1–42 + estradiol (0.2 nM) (Aβ + E2), or 5 μM Aβ 1–42 + genistein (0.5 μM) (Aβ + G). Neurons were incubated with estradiol or genistein 48 h before addition of Aβ. Data are mean ± SD of three different experiments. *$p < 0.01$ *vs.* control. #$p < 0.05$ *vs.* Aβ. (**B**) P38 MAPK phosphorylation was determined by Western blot analysis in neurons treated for 5 min with 5 μM Aβ 40-1 (C), 5 μM Aβ 1–42 (Aβ), 5 μM Aβ 1–42 + estradiol (0.2 nM) (Aβ + E), or 5 μM Aβ 1–42 + genistein (0.5 μM) (Aβ + G). Neurons were incubated with estradiol or genistein 48 h before addition of Aβ. A representative immunoblot of each protein is shown. Data are means ± SD of 5 independent experiments. *$p < 0.05$ *vs.* control sample. #$p < 0.05$ *vs.* Aβ cells.

lated to the timing of estrogen replacement. In the study conducted by Zandi *et al.* (118), the reduced risk for dementia included women who were current HRT users and women who had used HRT for >10 years. Further research regarding the use of HRT in AD patients is necessary to establish the potential benefits. However, there is considerable evidence to suggest that HRT may have a protective effect against AD development, and may be useful in slowing disease progression. Consideration must also be given to the potential adverse effects of HRT (*e.g.*, carcinogenesis). Phytoestrogens (*e.g.*, soy isoflavones), improve cognitive function in animal studies and in humans, and may offer protection against AD development. Furthermore, phytoestrogens have not been associated with the carcinogenic effect of hormone replacement therapy, and may be a more appropriate alternative in the prevention of AD. Phytoestrogens are a group of biologically active plant substances with a chemical structure that is similar to that of estradiol. This structural similarity accounts for the ability of these compounds to bind to estrogen receptors and exert various estrogenic or antiestrogenic effects. There are three main classes of phytoestrogens: isoflavones, coumestans, and lignans, which occur in either plants or their seeds. A single plant often contains more than one class of phytoestrogen. For example, the soy bean is rich in isoflavones and the major isoflavones are genistein and daidzein. Epidemiologic data indicate that Asian people have lower rates of osteoporotic fractures, cardiovascular diseases, postmenopausal symptoms, and certain cancers than Western populations. These health advantages are notably reduced when Asians adopts a Western lifestyle and eating habits (104). These observations have led researchers to look at the Asian diet for possible answers. Soy is part of the traditional diet in the Asian population and is rich in phytoestrogens such as genistein and daidzein. An increasing number of researchers are investigating the relationship between soy intake and AD, and obviously phytoestrogens are a candidate novel drug for AD. There are a few papers about the beneficial effects of phytoestrogens on memory or the central nervous system (28, 49), but the effects of phytoestrogen on the central nervous system in humans are still poorly understood. Thus, reports about the effects of phytoestrogen (and endogenous estrogen) on the central nervous system, especially on learning and memory, are controversial and will still need further study.

As stated before, we have traced the protection against oxidant production in females to the upregulation of antioxidant genes caused by the interaction between estrogens and the estrogen receptor (8, 9, 108). We found that estrogens protect against normal damage caused by aging by activating the MAP kinase–NFKB pathway. Recent work from our laboratory (submitted for publication) confirms that Aβ increases phosphorylation of the p38–MAP kinase. Incubation of neurons with estradiol or genistein (phytoestrogen) prevents the increase in peroxide levels in cells due to the action of the Aβ. The increase in oxidant levels found in neurons incubated with Aβ was prevented by co-incubation with estradiol or genisteine. This increase in oxidant production resulted in a subsequent activation of the P38 MAP kinase which leads to cell death. Incubation with estradiol or genisteine prevented both the activation of the MAP kinase and the cell death caused by the Aβ (Fig. 5). MAP kinases are members of specific signaling cascades that serve as convergent points for numerous and diverse extracellular signals and thus are critically important integrators of signaling events. Four distinct groups of MAP kinases have been identified: the extracellular signal-regulated kinases (ERKs); the c-jun N-terminal protein kinases (JNKs); big MAP

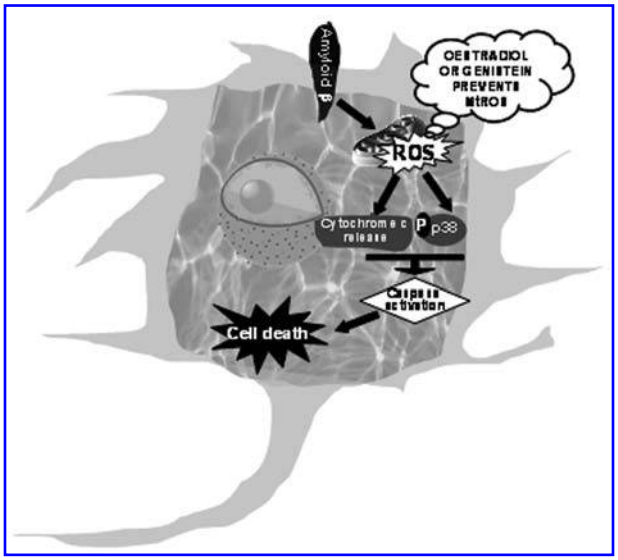

FIG. 6. Protection mechanism of Aβ toxicity by estradiol or genistein.

kinase I (ERK5); and the p38 MAP kinases (75). Over the past several years there has been increasing interest in the p38 group of MAP kinases in the AD research field, as these kinases are involved in inflammation and cell death pathways and therefore could contribute to the pathogenic events that occur in AD brain. Like all members of the MAP kinase families, the p38 family members are activated by upstream dual-specificity kinases called MAP kinase kinases (MKKs). Although the dual phosphorylation motif is conserved in all members of the p38 family, selective activation by specific MKKs has been observed. For example, MKK6 phosphorylates all four p38 isoforms, but MKK3, which is 80% homologous to MKK6, does not efficiently phosphorylate p38β (75). p38 pathways are activated by stress and inflammation (52) and therefore it is not surprising that there is increasing evidence that p38 and its downstream targets are activated in numerous neurological diseases, including in AD (40a). There are also data to suggest that p38 family members can phosphorylate the microtubule-associated protein tau. This is of interest because tau is hyperphosphorylated in AD brain and accumulates to form the intracellular NFTs. *In vitro* p38 and p38β can phosphorylate tau, although not with high efficiency (35, 84). All the findings clearly indicate that p38 inhibition may provide a rationale drug target for the treatment of AD, as well as other neurodegenerative conditions.

CONCLUDING REMARKS

Work from several laboratories has shown that mitochondria are involved in the pathophysiology of AD. Moreover, pioneering work from Perry and Smith showed that oxidant stress is critical in the development of this disease (96).

Work from our own laboratory has shown that mitochondrial oxidative stress is important to determine the different longevity between males and females. We have recently found that direct incubation of mitochondria from young females with soluble Aβ does not cause an increase in oxidant production which occurs when mitochondria from young males are incubated with the peptide. However, mitochondria from old females do indeed show an increase in oxidant production in the presence of Aβ. Thus, there are clear gender and age-related differential effects of the toxicity of Aβ on mitochondria. The fact that Aβ increases the release of oxidants from mitochondria is important, not only because these oxidants cause a direct damage to cellular molecules, but also because they can act as signals which activate cellular pathways that lead to neuronal apoptosis. Activation of the p38 MAP kinase is one of such pathways. Thus, radicals released from mitochondria in the presence of Aβ are critically important in the development of apoptosis in neurons and this is clearly age- and gender-related. Figure 6 is an artistic rendition of the facts reviewed in this paper. Future work should be directed towards elucidating the role of estrogenic substances, natural or otherwise synthetic, which may prevent these effects. Many of these estrogenic substances do not necessarily have to be feminizing, and therefore they could be administered to patients (both men and women) who are at high risk of developing AD, because of familiar natural history, or who develop early symptoms of the disease, like minor cognitive impairment.

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ABBREVIATIONS

ABAD, Aβ-binding alcohol dehydrogenase; AD, Alzheimer's disease; AIF, apoptosis-inducing factor; APP, amyloid precursor protein; Aβ, β amyloid peptide; BACE, β-secretase; CNS, central nervous system; COX, cytochrome c oxidase; HT, hormonal therapy; MtDNA, mitochondrial DNA; ROS, reactive oxygen species; Smac, second-nitochondria derived activator of caspase; SNPs, single nucleotide polymorphisms; SOD, superoxide dismutase.

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