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Forum Review

Reproductive Hormones Modulate Oxidative Stress in Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease characterized by gradual cognitive decline, impairments in speech and language, and dysfunction in the sensorimotor systems, culminating in complete reliance on nursing care. Oxidative stress, caused by an imbalance in the pro-oxidant/antioxidant mechanisms in the body, has been implicated in AD pathogenesis, as in many other age-associated diseases such as atherosclerosis, Parkinson disease, and amyotrophic lateral sclerosis. Although the hormones estrogen, progesterone, testosterone, and luteinizing hormone are best known for their roles in reproduction, many studies show these hormones have other roles, including neuroprotection. Changes in the levels of these hormones that occur in reproductive senescence are hypothesized to increase risk of AD, as a result of reduced protection against oxidative insults. The Aβ peptide, overproduction of which is thought to be a key pathogenic event in the development of AD, is neurotoxic, most likely due to its ability to promote oxidative stress. The reproductive hormones are known to influence Aβ metabolism, and this review discusses the beneficial and detrimental effects these hormones have on Aβ production and oxidative stress, and their relevance in potential AD therapies. *Antioxid. Redox Signal.* 8, 2047–2059.

INTRODUCTION

ALZHEIMER'S DISEASE (AD) is the most common form of age-related dementia (59). It is a complex disorder, with many molecular triggers synergistically culminating in a final common pathway. Examination of the atrophied AD brain reveals a vast range of pathological changes including widespread neuronal loss, particularly in the hippocampus and frontal lobes (59). Histopathologically, the AD brain is characterized by extracellular senile plaques, intracellular neurofibrillary tangles, and congophilic amyloid angiopathy (59). Senile plaques are comprised mostly of aggregated deposits of a 39–42 amino acid peptide termed beta amyloid (Aβ), and are often surrounded by activated microglia and degenerating neurons (reviewed in Ref. 126). Aβ is also found in deposits within and around blood vessels, and these deposits comprise the congophilic amyloid angiopathy (59). Neurofibrillary tangles are abnormal intracellular deposits of hyperphosphorylated and polymerized forms of the cytoskeletal protein tau. The AD brain is under oxidative stress and Aβ is thought to play a major role in promoting oxidative damage (24, 76, 79). This review will describe the role of reproductive hormones in the modulation of Aβ levels and oxidative stress in AD.

OXIDATIVE STRESS IN AD

Oxidative stress is recognized as a major neuropathological feature of AD, and recent evidence indicates that it may

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indeed be one of the earliest pathological events in the disease process (89). For example, elevated levels of oxidative stress markers have been reported in subjects with mild cognitive impairment, who exhibit a high conversion rate to AD (61, 98). Oxidative stress has also been linked to several other age-associated conditions such as atherosclerosis, certain cancers, Parkinson's disease, and familial amyotrophic lateral sclerosis. The first report that oxidative stress plays a role in AD was the discovery of elevated glucose-6-phosphate dehydrogenase activity in AD brains (76); this enzyme is known to help defend against oxidative stress. Since this initial discovery, evidence of elevated levels of lipid peroxidation, as well as protein and DNA oxidation, have been reported in AD brains by several laboratories (reviewed in Ref. 97). Studies of transgenic animal models of AD support the concept that oxidative stress in AD, as increased levels of oxidative stress markers have been found in these animals, coinciding with amyloid deposits (113). The challenge now lies in characterizing the molecular mechanism(s) that drive these oxidative changes in AD and finding therapeutic strategies to prevent and overcome these changes.

REPRODUCTIVE HORMONES MODULATE OXIDATIVE STRESS

Inherent gender differences in responses to oxidative stressors suggest that reproductive hormones have the potential to modulate the pro-oxidant/antioxidant balance, and thereby modulate the susceptibility to oxidative stress. Women have been found to have lower lipid peroxide levels than men of the same age (77) and animal studies have indicated that the female brain is more resistant to oxidative insults mediated by ischemic injury (48). These gender differences in oxidative stress susceptibility have been attributed to reproductive hormones. Comparisons between pre- and postmenopausal women have detected age-independent increases in levels of lipid peroxidation markers coupled with decreases in levels of antioxidants such as glutathione peroxidase, ascorbic acid, and α-tocopherol (77, 110, 129). Similarly, elevated lipid peroxide levels have been reported in women who have undergone surgically induced menopause via ovariectomy, corroborating the hypothesized role of the reproductive hormones in oxidative stress (132).

GENDER DIFFERENCES IN AD

Gender differences have also been reported in the incidence, clinical presentation, and pathology of AD. Whilst gender differences in AD prevalence are difficult to interpret due to gender differences in life expectancy, incidence studies indicate that women are at greater risk of AD, particularly in the older population (5, 100), and slightly more severe cognitive deficits have been reported in women compared to men (54). Gender differences in levels of antioxidants and oxidative stress indicators have also been reported in AD. Levels of the antioxidant enzymes superoxide dismutase and glutathione peroxidase are elevated in AD brains when compared to controls, and additionally, when compared to their male counterparts, female AD subjects have significantly greater levels of these enzymes than male AD subjects (105). The elevated antioxidant levels observed in the female AD brains were hypothesized to reflect a compensatory mechanism offsetting elevated pro-oxidant species (105). These gender differences in AD presentation and pathology may therefore reflect gender differences in vulnerability to oxidative insult, perhaps mediated by gender differences in the levels of the reproductive hormones following menopause compared to andropause (male reproductive senescence).

HORMONE REGULATION AND REPRODUCTIVE SENESCENCE

Steroidal sex hormones are predominantly synthesized in the gonads under the regulatory control of the gonadotropin hormones. Relatively small quantities of steroidal sex hormones are also produced in the adrenal glands, placenta, heart, bone, adipose tissue, and brain (reviewed in Ref. 94). The gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), are synthesized in and secreted from the pituitary, and act on the ovary and testes to stimulate gonadal sex hormone production (Fig. 1). Following reproductive senescence (menopause), the gonads fail to synthesize and secrete steroidal sex hormones. Compensatory increases in nongonadal synthesis of steroidal sex hormones have been recorded (85), nevertheless estrogen, progesterone, and testosterone deficiencies occur following reproductive senescence (26). Gonadotropin production and secretion also increase markedly following reproductive senescence, in a compensatory measure to stimulate sex hormone production; however, this is inevitably unsuccessful (26, 78, 88, 122).

REPRODUCTIVE HORMONES AND AD

A large body of evidence indicates that estrogen, progesterone, and testosterone are neuroprotective, mediated at least in part by antiamyloidogenic, antioxidative, and anti-inflammatory mechanisms. Consistent with the neuroprotective effect of the steroidal sex hormones, an association between AD and depleted levels of estrogen in women, and testosterone in men, has been reported (55, 75, 91). Consequently, it has been hypothesized that depleted sex hormone levels confer neurodegenerative susceptibility to AD. It has also been speculated that the increased prevalence of AD in women is the result of depleted estrogen levels following reproductive senescence (reviewed in Ref. 73). Although superficially this may seem paradoxical since men also experience testosterone depletion following reproductive senescence, the loss of gonadal function and resulting sex hormone depletion generally occurs abruptly in women, whereas men experience a gradual depletion of testosterone over several decades (43, 78, 88, 122). Testosterone depletion in males is also highly variable between individuals. Sex hormone depletion may also play an important role in the development of other neurodegenerative diseases that display similar gender predilections including

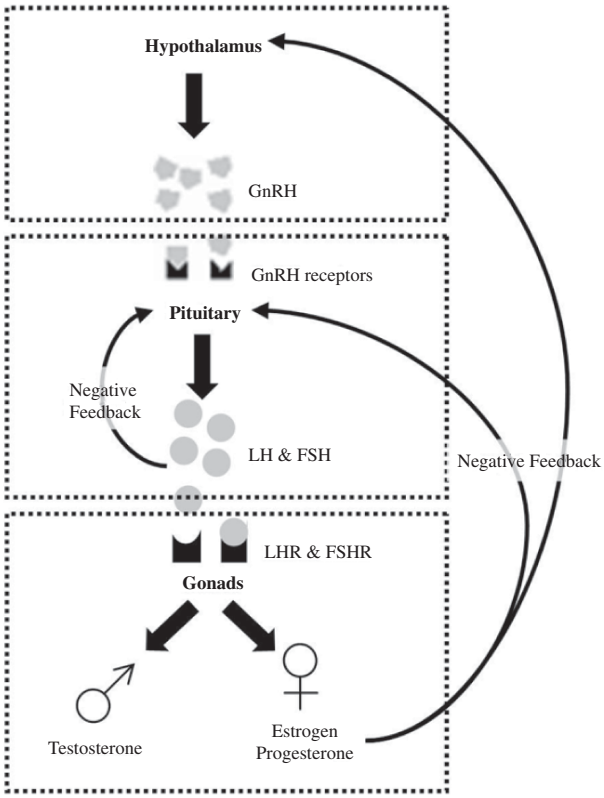

FIG. 1. Reproductive hormone homeostasis: the hypothalamic pituitary axis. Gonadotropin releasing hormone (GnRH) secreted from the hypothalamus binds with GnRH receptors on the pituitary activating production and secretion of LH and FSH. LH and FSH interact with their respective receptors (LHR and FSHR) on the gonads to stimulate gonadal sex hormone production (estrogen and progesterone from the ovaries, testosterone from the testes). The gonadal sex hormones exert negative feedback on the hypothalamus and pituitary to inhibit further GnRH and gonadotropin secretion. LH and FSH also exert negative feedback on the pituitary to inhibit further gonadotropin production. Following reproductive senescence, the gonads fail to produce significant quantities of the gonadal sex hormones, abolishing the negative feedback mechanisms keeping gonadotropin secretion in check.

amyotrophic lateral sclerosis, Parkinson disease, multiple sclerosis, and AIDS-induced dementia (29).

Hormone replacement therapy (HRT) has been examined as a potential preventative and therapeutic strategy in the treatment of AD. Initial clinical trials of testosterone replacement therapy in older men have reported selective improvements in cognitive function (8, 57, 111), whilst testosterone treatment was found to improve the ability to perform daily tasks and reduce frailty in men with multi-infarct dementia (8). Another study has also reported that testosterone improves cognitive function in a small cohort of subjects with AD (117). Surprisingly, clinical trials have found HRT to be of no benefit to female AD patients (87, 130). Even the potential preventative benefits of HRT are contentious. Whilst many small studies have reported improved cognition and reduced prevalence of AD amongst HRT users (9, 60, 118), the recent Women's Health Initiative clinical trial reported HRT to be detrimental to cognitive function and increase AD risk (32, 109). It has been argued that the long postmenopausal delay prior to HRT initiation in the Women's Health Initiative study, with all participants 65 years and older, may explain the detrimental effects observed (73). In light of this, another HRT clinical trial (Kronos Early Estrogen Prevention Study) has been initiated in perimenopausal women to re-address the potential cardioprotective effects of HRT when administered without a long postmenopausal delay (maximum 36 months following final menses) (50). Other factors have also been identified for consideration in interpretation of the WHI study including the source of hormone (equine estrogens as compared to synthetic human forms of these hormones) and mode of delivery (cyclic vs. continuous) (73).

The neuroprotective properties of progesterone have been most widely studied for the treatment of spinal chord and traumatic brain injury. Although animal studies indicate that natural progesterone is neuroprotective, the synthetic progesterone, medroxyprogesterone acetate (MPA), is not neuroprotective and has been shown to antagonize the neuroprotective effects of estrogen (112). This is particularly interesting since MPA was used in the WHI clinical trials; therefore, it is clear there are a number of issues to be addressed before a definitive conclusion regarding the preventative benefit of HRT against AD can be determined.

More recently, elevated postmenopausal and andropausal LH have also been identified as potential neurodegenerative factors in AD. Supporting this notion, significantly elevated serum LH levels have been reported in dementia and AD subjects (19, 108). Elevated LH levels have also been confirmed immunohistochemically in the hippocampus of AD brains when compared to control brains, and the hippocampus is a brain region particularly vulnerable to degeneration in AD (18). Accordingly, it has been hypothesized that elevated LH confers susceptibility to neurodegeneration in AD (19).

Sex hormone regulation and LH homeostasis are inextricably linked. These hormones also play a role in the modulation of Aβ peptide production (overproduction of which is thought to be a key event in AD pathogenesis) and inflammatory processes, both of which may result in the generation of oxidative stress.

AMYLOID PRECURSOR PROTEIN (APP) AND Aβ METABOLISM IN AD

Causative genetic mutations have been identified that account for <5% of AD cases (127). These include mutations in the parent molecule of Aβ, the amyloid precursor protein (APP), and mutations in genes coding for the presenilin proteins that are involved in the proteolytic processing of APP (67). These mutations lead to the increased production of Aβ, particularly the longer, more amyloidogenic form Aβ1–42 (67). In AD, Aβ peptides aggregate into insoluble fibrils, and over time these deposit in the brain to produce the characteristic amyloid plaques. These studies, as well as many *in vitro* and transgenic mice studies, have led to the "amyloid hypothesis," which states that Aβ accumulation is central to AD

pathogenesis (67). Although plaques and neurofibrillary tangles are the recognized neuropathological hallmarks of the disease, evidence suggests that in the early stages of the disease, the "toxic principle" of AD may consist of Aβ dimers or small soluble oligomers of the peptide, that have been shown in many *in vitro* studies to have neurotoxic and oxidative stress-inducing properties (82).

APP PROCESSING AND OXIDATIVE STRESS

Many studies support the theory that the Aβ peptide itself is responsible, at least in part, for the AD-related oxidative stress. It has been shown to be neurotoxic in many *in vitro* models, most likely via the peptide's ability to induce oxidative damage (51). Aβ can induce the overproduction of superoxide radicals by interaction with vascular endothelial cells (121). The peptide can also induce the intracellular accumulation of hydrogen peroxide in cultured neuroblastoma and hippocampal neurons, and conversely antioxidants can attenuate Aβ-mediated neurotoxicity (17, 41, 42). In studies of synaptosomes and cultured cortical cells, Aβ has also been found to induce oxidative damage including lipid peroxidation (17). Aβ also impairs mitochondrial function, potentially increasing levels of free radicals generated via respiratory oxidative phosphorylation (1). Of relevance in the later stages of AD, studies have found that amyloid plaques are a focus of cellular and molecular oxidation (reviewed in Ref. 79).

The Aβ peptide is a product of the proteolytic processing of its much larger parent molecule APP (126). This transmembrane APP molecule undergoes proteolytic processing by two competing pathways, the nonamyloidogenic and amyloidogenic pathways (Fig. 2). Sequential cleavage of APP by α-secretase and γ-secretase, respectively, thought to occur at the cell surface, results in the secretion of soluble APP (α-APPs) and nonamyloidogenic fragments. Alternatively, the cleavage of APP by beta-site APP cleaving enzyme (BACE) and γ-secretase, thought to occur following endocytosis of cell-surface APP, results in the production of a different set of cleavage products including the amyloidogenic Aβ peptide (Fig. 2) (reviewed in Ref. 126).

It has been hypothesized that Aβ accumulation increases free radical generation beyond neural antioxidant capabili-

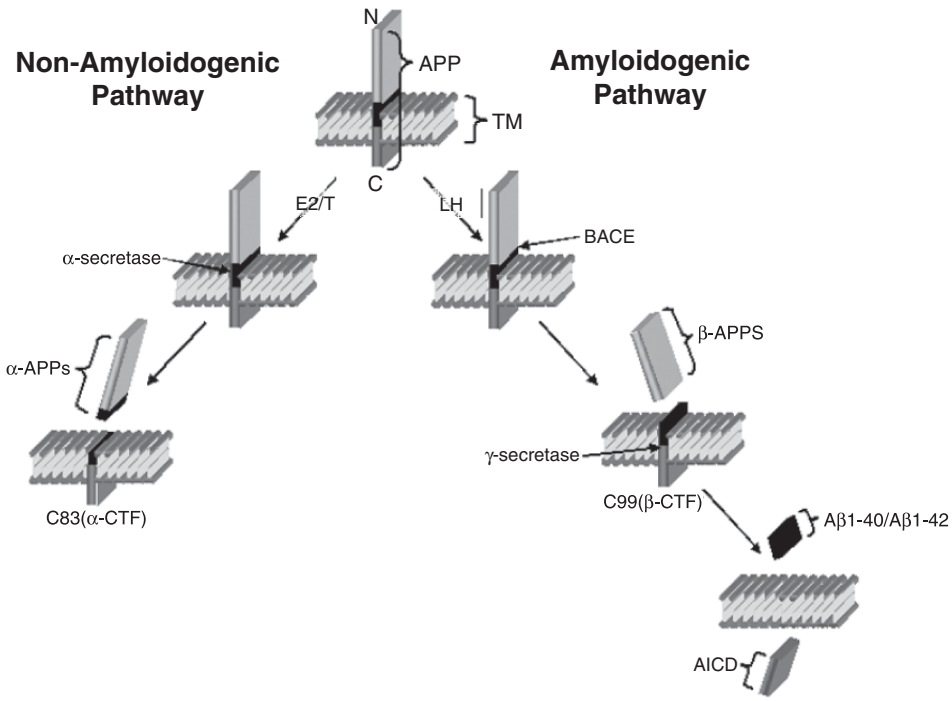

FIG. 2. APP proteolytic processing pathways. Two competing pathways, the nonamyloidogenic and amyloidogenic pathways, proteolytically cleave the majority of APP within the cell. In the nonamyloidogenic pathway, APP is cleaved with in the Aβ domain by α-secretase to liberate a secreted form of APP (α-APPs). A C-terminal fragment (C83/αCTF) is left embedded in the membrane for further cleavage into nonamyloidogenic fragments. In the amyloidogenic pathway, APP is first cleaved by BACE to liberate β-APPs. The C-terminal fragment (C99/β-CTF) left embedded in the transmembrane (TM) is cleaved by the γ-secretase enzyme, which consists of four proteins (PS1, Nicastrin, APH-1, and PEN-2) that interact with each other in a high molecular weight complex. This cleavage event liberates the Aβ40/Aβ42 peptides. It is thought that another fragment is also released termed the APP intracellular domain (AICD), that can translocate to the nucleus and activate gene transcription. Both pathways can be regulated by reproductive hormones. Evidence suggests that high levels of estradiol (E2) or testosterone (T) drive APP processing towards the nonamyloidogenic pathway and formation of the neuroprotective and neurotrophic α-APPs metabolite. In contrast, high levels of luteinizing hormone (LH) are thought to drive APP processing towards the amyloidogenic pathway and Aβ formation.

ties, thus initiating oxidative stress-stimulated neurodegenerative cascades (24). The accumulation of Aβ can also elicit secondary neurodegenerative cascades such as inflammation, thereby indirectly contributing more oxidative stress (96). Interestingly, oxidative stress may itself stimulate the formation of the more toxic insoluble Aβ aggregates, thereby exacerbating the neurotoxic activity of Aβ and potentially leading to a self perpetuating cycle of Aβ accumulation and free radical generation (65). Accordingly, it has been postulated that decreasing Aβ production and/or improving its clearance may lower oxidative stress and prevent the development of the neurodegenerative cascades thought to lead to AD (128).

In contrast to studies of Aβ, studies of secreted α-APPs have shown this protein to have antioxidant and neuroprotective properties, as it can attenuate free radical generation and suppress Aβ toxicity. *In vitro* studies have shown that the production of α-APPs stabilizes cellular calcium homeostasis and protects neurons from metabolic, excitotoxic, and oxidative insults (41, 80). This protective effect of α-APPs extends to *in vivo* studies, which have reported that the nonamyloidogenic APP metabolite reduces damage to hippocampal neurons following forebrain ischemia in rats (114).

REPRODUCTIVE HORMONES AND APP METABOLISM

The reproductive hormones, estrogen, testosterone, and LH, all influence the metabolism of both the neurotoxic Aβ peptide and the neuroprotective APP fragment, α-APPs. Estrogen has been reported to increase the secretion of α-APPs (56, 131), and to cause a decrease in Aβ production in cultured human neuroblastoma and cerebrocortical neurons (56). In addition to promoting the nonamyloidogenic APP processing pathway, estrogen has also been shown to inhibit APP overexpression following ischemic injury (106), in turn reducing substrate for APP processing and subsequent Aβ production. Apart from its effects on Aβ production and accumulation, estrogen also directly inhibits the neurotoxicity of the peptide (16, 40, 47). *In vivo*, estrogen depletion (induced via ovariectomy) results in significantly elevated Aβ levels, particularly the more toxic Aβ1–42 species, an effect that is partially reversed following estrogen replacement (68, 95, 137). Estrogen may also influence Aβ accumulation via nonestrogenic receptor-mediated mechanisms since the biologically inactive 17α-estradiol can similarly reduce Aβ levels (68). It is conceivable that estrogen reduces Aβ accumulation in the intact animal through modulation of both Aβ production and clearance. In support of this, estrogen has been found to stimulate Aβ clearance and degradation by microglial phagocytosis (52, 69). Correspondingly, increased Aβ burden and impaired microglial Aβ clearance has been reported in an estrogen-deficient transgenic mouse model of AD (133). In this study, estrogen deficiency was induced by crossbreeding transgenic mice overexpressing APP with aromatase knockout mice, aromatase being an enzyme responsible for converting testosterone to estrogen. However, in direct contradiction, another recent study has reported that estrogen depletion induced by ovariectomy had no significant effect on Aβ burden in a transgenic mouse model of AD (45). This discrepancy may result from differences in neural estrogen status, since Yue *et al.* (133) found that ovariectomy was insufficient to deplete estrogen levels in brain homogenate, whereas the aromatase knockout mice exhibited significantly depleted central and peripheral estrogen levels. It is interesting to note that although ovariectomy is the commonly used experimental model of estrogen deficiency, it appears that at least in some circumstances ovariectomy does not induce estrogen depletion in the central nervous system. High estrogen activity has been demonstrated in the brain of ovariectomized mice relative to other body regions using the estrogen-responsive element-luciferase mouse model, which has been engineered to express the nonmammalian luciferase protein in response to classical estrogen receptor activation (27). This confirms that central and peripheral estrogen homeostasis is not necessarily synergistic, this is not a new concept, and this observation was the first evidence for *de novo* synthesis of estrogen and other hormones in the brain (reviewed in Ref. 84). Hence, peripheral estrogen status may not be an accurate indication of central nervous system estrogen status. Depleted estrogen levels have in fact been reported in brain homogenate of female AD patients (133).

Testosterone has also been implicated in the metabolism of APP and Aβ production (39, 44). In a manner similar to estrogen, testosterone treatment increases secretion of soluble α-APPs and simultaneously decreases Aβ production (39). In this study, the duration (but not the dose) of testosterone treatment was found to correlate negatively with Aβ production (39). Since testosterone can be converted to estrogen by aromatase in the brain, it is possible that estrogen synthesized from the testosterone may be responsible for such effects on APP metabolism. Aromatase inhibitors and dihydrotestosterone are being used to investigate whether testosterone has a direct role in the modulation of Aβ metabolism. Estrogen and testosterone depletion induced via chemical castration in men undergoing prostate cancer treatment is accompanied by a significant increase in plasma Aβ levels (4), and in men with impaired memory function, free testosterone levels have been found to correlate negatively with plasma Aβ levels (38). Interestingly, no correlation was found between Aβ levels and estradiol levels in this study, implying that testosterone is not influencing Aβ metabolism entirely via its conversion to estrogen. Nevertheless, human studies such as these substantiate the purported roles of both estrogen and testosterone in the regulation of Aβ burden.

Unlike estrogen and testosterone, LH has been implicated in the promotion of amyloidogenic processing of APP (20). LH treatment of cultured human neuroblastoma cells results in dose-dependent increases in the accumulation of Aβ and amyloidogenic APP C-terminal fragments, whilst decreasing secretion of soluble α-APPs (20). Leuprolide treatment, which inhibits both gonadotropin and estrogen production, has been used to study the effect of LH suppression on Aβ production in female mice (20, 25). LH suppression was found to decrease brain Aβ levels, particularly the longer, more toxic Aβ1–42 species (20, 25). This result was surprising since estrogen depletion is known to be associated with elevated Aβ levels, yet despite estrogen depletion induced by chemical gonadoectomy, Aβ levels were reduced. Casadesus

et al. (25) argued that this demonstrates LH has a much greater capacity to modulate Aβ levels than the sex hormones. If so, the gonadectomy model of reproductive senescence should be reconsidered since LH and sex hormone homeostasis are intrinsically linked. Ideally, new models need to be developed to discern the individual contributions of LH and each of the gonadal sex hormones in neurodegenerative processes.

Since Aβ has been identified as a key protein in AD pathogenesis, potentially even playing a role in the initiation of the neurodegenerative process, considerable drug development has focused upon modifying the production and metabolism of this protein (92). Given the antiamyloidogenic properties of estrogen and testosterone, HRT may help minimize Aβ burden and consequently reduce oxidative injury. However, in light of the recent evidence indicating that LH potently facilitates Aβ production, irrespective of gonadal sex hormone status, gonadotropin-suppressing agents such as leuprolide may have greater antiamyloidogenic potential than the gonadal sex hormones (reviewed in Ref. 14). Accordingly, leuprolide has recently been shown to attenuate cognitive decline and Aβ deposition in AD transgenic mice and is currently undergoing clinical trials for the treatment of AD (25).

ANTIOXIDANT PROPERTIES OF THE GONADAL STEROID HORMONES

Estrogens have been coined 'natural antioxidants' since they have been demonstrated to inhibit lipid peroxidation in a variety of biological systems. In microsomal lipid preparations, estrogens have been reported to inhibit iron-induced lipid peroxidation (116). Similar antioxidant properties of estrogens have been reported in tissues of the central nervous system. *In vitro*, 17β-estradiol is a more potent inhibitor of oxidative stress than α-tocopherol in a range of neuronal cell models, protecting against oxidative damage and cell death mediated by the reactive oxygen species, hydrogen peroxide (15, 16). 17β-Estradiol and estriol have also been demonstrated to increase neuronal survival and reduce lipid peroxidation in response to iron-induced oxidative stress (40), and to decrease mitochondrial production of reactive oxygen species in a dose-dependent manner (28). Furthermore, 17β-estradiol inhibits iron induced lipid peroxidation in both rat and human brain homogenates (124).

Although some of the neuroprotective effects of estrogen that have been documented are the result of classical estrogen-receptor mediated signaling pathways, the antioxidant properties of estrogens are mediated by receptor-independent mechanisms (15). The concentrations of estrogens necessary to elicit these antioxidant effects in both the central nervous system and peripheral biological systems far exceed normal physiological concentrations, indicating that these neuroprotective properties are not mediated by the estrogen receptor (15). Supporting this notion, the antioxidant effects of the estrogens have been confirmed in neuronal cell lines that do not express estrogen receptors (16). Furthermore, the antioxidant properties of estrogens cannot be significantly attenuated by co-administration of a competitive estrogen receptor antagonist, or a protein synthesis inhibitor (28, 102, 115). Biologically inactive estrogen forms (with respect to the estrogen receptor) such as 17α-estradiol also exhibit antioxidant activity, again indicating receptor-independent antioxidant activity (15, 102). Comparisons of the antioxidant capacities of estrogens in neuronal cell culture have led to the identification of the hydroxyl group located at position 3 of the phenoxyl ring of estrogen as an important structural feature for estrogenic antioxidant activity (15, 101). The phenolic structure of estrogens enables the direct scavenging of free radicals by the donation of hydrogen to lipid peroxyradicals, thus terminating oxidative chain reactions (115, 116).

In direct contrast to the free radical scavenging capacity of estrogens, some studies have implicated high concentrations of estrogens in metabolic reactions that generate free radicals (70). Estradiol can be enzymatically metabolized into catechol estrogens, which have an additional hydroxyl- or methoxy- group substituted in the ortho- position of the phenolic hydroxyl group of the estrogen (70). The hydroxyl- (but not methylated-) substituted catechol estrogens are readily oxidized, and undergo redox cycling to produce free radicals (70). Elevated levels of estrogens result in increased catechol estrogen redox cycling, thus promoting the formation of the reactive oxygen species (70). These processes have been of interest in cancer research, due to the potential oxidative damage resulting from elevated estrogen levels in tissues that readily catalyze catechol estrogen formation (70, 71). The physiological significance of these metabolic pathways in the brain is not well understood; however, catechol estrogens have been detected throughout the brain (93) and limited oxidative metabolism of catechol estrogens has been demonstrated in brain microsomes (58). Whilst the antioxidant properties of supra-physiological estrogen levels are well documented, we can only speculate about the potential prooxidant properties of estrogen in the brain. The potential long-term effects of supra-physiological estrogen concentrations must be determined prior to considering the clinical use of estrogens as an antioxidant.

The potential antioxidant effects of the other steroidal hormones, progesterone and testosterone, have also been explored. Progesterone was found to be far less effective at inhibiting lipid peroxidation and neural death than either estradiol or estriol (40). However, slight antioxidant properties of progesterone occur in response to iron-induced oxidative stress in neural cells, whilst testosterone offers no neuroprotective benefit (40). Similarly, progesterone but not testosterone reduces iron-induced lipid peroxidation significantly in cultured hippocampal cell homogenates; however, neither progesterone nor testosterone elicited any effect on iron-induced lipid peroxidation in human brain homogenates (124). Progesterone does not improve neuronal survival following hydrogen peroxide treatment (16), yet testosterone exhibits neuroprotective effects in cerebellar granule cells against oxidative insults mediated by the reactive oxygen species, hydrogen peroxide and nitric oxide (NO) (2, 3). These neuroprotective effects were suggested to be mediated by estrogen, following the conversion of testosterone to estrogen by aromatase (7). However, the antioxidant properties of testosterone can be attenuated by

flutamide, an androgen receptor antagonist (2). Furthermore, unlike testosterone, estrogen does not yield any neuroprotective effect in the cerebellar granule cell oxidative stress model (3). Testosterone is now thought to mediate its antioxidant effects by upregulation of the antioxidant enzymes superoxide dismutase and catalase through the androgen receptor, rather than via conversion to estrogen or via direct scavenging activity (2, 3). This is supported by evidence that testosterone treatment results in significant elevations of the superoxide dismutase and catalase enzymes in the cerebellum (2, 3). Testosterone has also been implicated in the regulation of glutathione, with increased levels reported in rat brain homogenate following testosterone treatment (6). Whilst the neuroprotective effect of testosterone is well established, further assessment of the underlying mechanisms is necessary, to determine the degree to which neuroprotection is afforded by its antioxidant properties.

In addition to acting as a free radical scavenger, estrogen has also been implicated in the regulation of endogenous antioxidants and enzymes associated with their metabolism (103, 104). Estrogen dose-dependently increases glutathione levels in glial, hippocampal, and cortical neuron cultures (104). Catalase activity is differentially affected by estrogen in glial and hippocampal cultures, estrogen treatment increases catalase activity in glial cells, yet decreases catalase activity in hippocampal cells (103). *In vivo*, ovariectomy has been associated with increased catalase levels yet unchanged glutathione and superoxide dismutase levels (86, 90). Postmenopausal women also have altered levels of antioxidant enzymes compared to women of reproductive age (77, 129) and glutathione peroxidase levels correlate positively with serum estradiol levels in women on HRT (77). Premenopausal women have also been reported to possess significantly higher glutathione peroxidase levels than males of the same age (77). As previously discussed, evidence indicates that compensatory mechanisms have already resulted in significantly elevated glutathione levels in AD brains. It is not known if HRT would increase glutathione levels even further in AD patients, and if so, whether this would elicit any clinical benefit in the treatment of AD.

Clinical research has linked antioxidant therapy to the prevention and delay of AD (reviewed in Ref. 23). The clinical use of estrogens as antioxidants may be limited due to their hormonal actions, including their oncogenic effects on reproductive tissues, and their feminizing effects in men. Furthermore, the high doses of estrogen necessary to elicit antioxidant activity have the potential to increase free radical production through catechol estrogen metabolism when administered long-term. Synthetic estrogens with greater scavenging activity have already been developed for use in cancer treatments, to eliminate potential problems arising from increased estrogenic action through classical signaling pathways (46). Selective estrogen receptor modulators (SERMS) have been developed for the treatment of hormonal driven cancer to antagonize estrogenic effects on reproductive organs, whilst preserving the beneficial actions of estrogen on other organs (120). These compounds may prove clinically useful in antioxidant therapy for the prevention and delay of AD.

NEUROINFLAMMATION AND REPRODUCTIVE HORMONES

Chronic inflammation has been identified as an important neurodegenerative process contributing to AD. For example, inflammatory molecules including cytokines are upregulated in AD brains, and activated astrocytes and microglia are observed within and in close proximity to senile plaques (81). Inflammatory molecules secreted by activated astrocytes and microglia are potentially toxic and provide yet another source of oxidative stress. Microglial activation provokes respiratory burst activity, resulting in the production of the superoxide anion, which can then subsequently be converted to other reactive oxygen species such as hydrogen peroxide, or the very potent hydroxyl radical (10). Activated microglia also secrete the reactive nitrogen species, NO, which is synthesized by inducible nitric oxide synthase (iNOS). Similarly, astrocytes have been implicated in the production of NO, in addition to playing a pivotal role in mediating microglial activation (10). Activation of inflammatory processes in AD may be the result of Aβ deposition (96), a notion supported by evidence that microglia may play a role in the phagocytosis and degradation of this peptide (35). Conversely, there is also evidence that Aβ deposition is the result of inflammation since activated microglia upregulate APP expression resulting in increased production of Aβ (11). Whilst these inflammatory processes may lead to oxidative stress, oxidative stress also triggers inflammatory responses including astrocytic and microglial activation, potentially amplifying neurodegenerative cascades.

It is interesting to note that inflammation can influence reproductive hormone homeostasis, and likewise, the reproductive hormones can influence the regulation of inflammatory reactions (34). For example, altered immune responses have been noted during menopause, and it has been found that HRT can reverse these changes (64). Furthermore, immune suppression during pregnancy is believed to be mediated by the reproductive hormones (72). The monthly cyclic hormone changes in women are also associated with changes in inflammatory markers, including elevated free radical production immediately prior to ovulation (107). The role of estrogen in vascular inflammation has received a lot of attention because of the potential implications for the modulation of cardiovascular disease risk. Similarly, the reproductive hormones have been implicated in the modulation of inflammatory responses in the central nervous system. Evidence indicates that the sex hormones and LH have opposite effects on inflammatory processes—a relationship between these hormones that is now being reported regularly. Estrogen and testosterone have an anti-inflammatory activity, inhibiting the activation of microglia and astrocytes, whilst LH is implicated in pro-inflammatory signaling pathways.

Neurosteroids and gonadal sex hormones modulate inflammatory responses of astroglia following neural injury. The gonadal hormones, 17β-estradiol, progesterone, and testosterone, have all been shown to decrease reactive gliosis in ovariectomized or castrated rats following penetrating brain injury (36). Similarly, 17β-estradiol, progesterone,

dehydroepiandrosterone (DHEA), and pregnenolone have all been found to decrease reactive gliosis in castrated male rats in response to penetrating brain injury, indicating the hormone treatment inhibited astrocyte proliferation and/or migration (36). In this study the neuroactive steroid, DHEA, was found to be the most potent inhibitor of reactive gliosis (36). There is also evidence that brain injury upregulates *de novo* synthesis of estrogen from testosterone in astrocytes (37), indicating that in this model at least some of the effects of testosterone may be due to its conversion to estrogen. Neurosteroids and gonadal sex hormones also have the capacity to induce morphological changes in astrocytes (30). 17β-Estradiol, testosterone, and pregnenolone, but not progesterone, have been found to increase the number of GFAP-immunoreactive astrocyte processes, perhaps indicative of astrocyte arborization (30). It is clear that estrogen not only has the capacity to suppress reactive gliosis following injury, but can also modulate potential neuroprotective activities of astrocytes.

Estrogen has also been implicated in the regulation of a wide range of microglial functions, including expression of cytokines, cell surface molecules, apoptotic signaling pathways, and free radical generation (31). Cultured microglial cells exhibit respiratory burst activity, phagocytic activity, iNOS expression, and subsequent NO production in response to inflammatory stimuli, all of which can be inhibited by estrogen in a dose-dependent manner (127, 129). These studies suggest that *in vivo* production of reactive oxygen and nitrogen species by activated microglia could be inhibited by estrogen treatment, thereby reducing the oxidative burden caused by chronic inflammatory responses. Similarly, estrogen has been implicated in the modulation of NO production from peripheral macrophages (53). Preincubation of microglial cultures with DHEA also decreases NO secretion, but not iNOS expression (12). The inhibitory effects of estrogen on microglial activation are dependent upon the estrogen receptor, since the biologically inactive 17α-estradiol has no effect on microglial responses (21) and estrogen receptor antagonists abolish the inhibitory effects of estrogen (125). It has been hypothesized that the estrogen receptor acts through the mitogen activated protein kinases (MAPKs) to moderate microglial responses, since MAPK inhibitors also attenuate the effects of estrogen on microglial activation (21). Interestingly, estrogen was found to attenuate microglial activation only when estrogen treatment was given to the cultures *prior* to inflammatory insult, indicating estrogen does not have the capacity to modulate inflammatory reactions once microglial activation has been initiated (125).

The LH receptor has been found to be expressed on a diverse range of immune cells throughout the body, suggesting that the immune system is a target for the gonadotropin hormones. Immune cells that express LH receptors include blood leukocytes (72, 135), follicular and endometrial macrophages (136), and macrophages of the human brain (22). A disparate range of effects of the gonadotropin hormones on immune responses in the periphery has been described. Much research has focused upon the role of human chorionic gonadotropin (hCG) in inflammatory responses due to its role in immunosuppression during pregnancy. The effects of hCG on peripheral inflammatory processes may provide insight into potential effects of LH, as both hormones mediate their biological actions through a common receptor, the LH receptor. HCG has been reported to dose dependently and reversibly inhibit lymphocyte proliferation in response to inflammatory stimuli (49). Furthermore, *in vitro* investigations have found that high concentrations of hCG stimulate the production of the anti-inflammatory cytokines interleukin-8 and interleukin-2 from blood leukocytes (63). In contrast to hCG, however, LH has been implicated in pro-inflammatory and pro-oxidant generation, which is believed to play an important role in luteolysis and ovulation. For example, LH dose-dependently increases secretion of reactive oxygen and nitrogen species from human blood leukocytes (107). Gonadotropin-mediated increased production of reactive nitrogen species may be the result of iNOS upregulation, since hCG has been demonstrated to upregulate iNOS expression in macrophages (62). It is evident that in the periphery the gonadotropins have pluripotent effects on inflammation, differentially potentiating or suppressing inflammation depending on conditions and tissue type.

Whilst researchers have suggested LH has a potential role in the regulation of inflammatory reactions in the brain (13), there has been limited investigation. Although LH receptors have been detected on brain macrophages, no studies have investigated the effect of LH on these cells. In immortalized hippocampal cultures, binding to the LH receptors mediates the upregulation of 5′-lipoxygenase, an enzyme important in pro-inflammatory signaling pathways (134). Lipoxygenases are lipid peroxidizing enzymes that metabolize arachidonic acid, generating free radicals and inflammatory leukotrienes. Further investigation is required to confirm if LH receptor-dependent upregulation of 5′-lipoxygenase induces the classical lipoxygenase signaling pathways, leading to the production of pro-inflammatory and pro-oxidant species. LH has been found to activate similar signaling pathways in gonadal tissue, where LH receptor-dependent cAMP/protein kinase A phosphorylation subsequently activates the lipoxygenase/arachidonic acid signaling pathway (83, 119). 5′-Lipoxygenase is expressed at particularly high levels in the hippocampus and the cerebellum (66), and age-related dysregulation of 5′-lipoxygenase transcription results in increased 5′-lipoxygenase expression in aged animals (99, 123). Therefore, it may be conceivable that elevated LH levels that occur following reproductive senescence may increase 5′-lipoxygenase signaling in the LH receptor-rich hippocampus, stimulating inflammation and oxidative stress. In light of the age-related increase in 5′-lipoxygenase expression, lipoxygenase inhibitors have been recognized as potentially useful in the treatment of AD (74).

The risks associated with long-term HRT diminish the potential for the use of estrogen as an anti-inflammatory agent in the treatment of AD. Since estrogen mediates its anti-inflammatory activity via the estrogen receptor, the synthetic estrogens–selective estrogen receptor modulators–will not mimic the anti-inflammatory properties of estrogen. Furthermore, given that estrogen cannot alter microglial activation once an inflammatory response has been initiated, the anti-inflammatory benefits of estrogen may be limited to prevention rather than treatment.

CONCLUSIONS

A diverse and complex network of inextricably linked neurodegenerative processes contribute to the generation of oxidative stress in AD. It is likely that multiple factors combine to create a neural environment that facilitates oxidative injury; although in some instances a single initiating factor may be sufficient to trigger the neurodegenerative cascade. According to the popular AD hypothesis, the "amyloid hypothesis," oxidative stress and inflammation are closely linked but secondary to the primary cause, which is the accumulation of Aβ. However, oxidative stress induced by other factors can also contribute to inflammation and amyloidogenesis, initiating a vicious cycle.

Dysregulation of the reproductive hormones, estrogen, progesterone, testosterone, and LH, has been associated with AD etiology and pathology. Regulation of these hormones is tightly linked, and following reproductive senescence gonadal sex hormone depletion is coupled to elevated LH levels. Given the neuroprotective mechanisms of gonadal sex hormones described here, including antioxidant, anti-inflammatory, and anti-amyloidogenic properties, it seems surprising that the recent Women's Health Initiative clinical trial has found HRT to be detrimental to cognitive health in women (32, 109). If the clinical neuroprotective benefit of HRT is to be definitively clarified, dosage and duration of treatment will need to be offset against risks of potential negative effects associated with long-term HRT, including increased risk of breast cancer, pulmonary embolism, and stroke. Further, since estrogen has been identified as a potential proconvulsive, the cost–benefit of estrogen use should be individually assessed in patients with seizure disorders (reviewed in Ref. 33). Whilst the potential benefits of HRT may be revisited to resolve issues associated with the age at initiation of HRT, the amyloidogenic and potentially inflammatory properties of LH may also in part help explain the inconsistencies in the effects of HRT on AD risk. It is conceivable that the combined effects of the sex steroids and gonadotropins may influence the susceptibility to and progression of neurodegeneration. The research reviewed here indicates that elevated sex hormone levels combined with low cycling LH levels during the reproductive years may promote antioxidant activity, suppress inflammation, and reduce Aβ accumulation (Fig. 3). Following reproductive senescence, the depleted sex hormones combined with elevated LH levels promote Aβ production and inflammation, and potentially exacerbate oxidative stress (Fig. 4). Given the diverse actions of the reproductive hormones, combinational hormone therapy may prove to be more efficacious in the prevention of AD, though this notion needs to be tested in further studies.

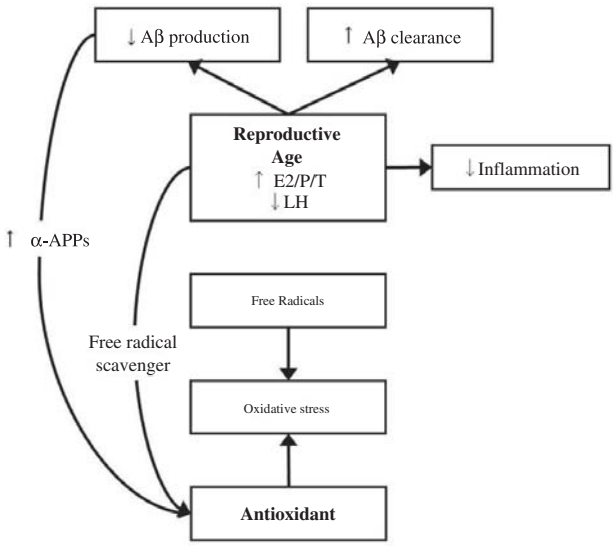

FIG. 3. Hormonal interactions with oxidative stress pathways whilst reproductively functional. High estrogen (E2) and progesterone (P) in women and testosterone (T) in men promotes Aβ clearance by microglial phagoctytosis and decreases Aβ production, thereby reducing oxidative burden offered by the Aβ peptide. Reduced Aβ production is coupled with increased α-APPs production, which contributes neuroprotective and antioxidant properties. Estrogen can also act directly as an antioxidant; however, normal physiological concentrations are likely to be too low to offer any benefit. Estrogen and testosterone may also suppress potentially neurodegenerative chronic inflammatory processes, preventing overproduction of free radicals and other neurotoxic inflammatory products.

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ABBREVIATIONS

α-APPs, secreted protein produced by cleavage of transmembrane APP with APP α-secretase enzyme; Aβ, amyloid peptide produced by proteolytic cleavage of APP by BACE and γ-secretase; AD, Alzheimer's disease; APP, amyloid precursor protein; BACE, β-site APP cleaving enzyme; DHEA, dihydroepiandrosterone; GnRH, gonadotropin releasing hormone; HRT, hormone replacement therapy; GFAP, glial fibrillary acidic protein; hCG, human chorionic gonadotropin; iNOS, inducible nitric oxide synthase; LH, luteinizing hormone; MAPK, mitogen activated protein kinase; MPA, medroxyprogesterone acetate; NO, nitric oxide; SERMS, selective estrogen receptor modulators; WHI, Women's Health Initiative.

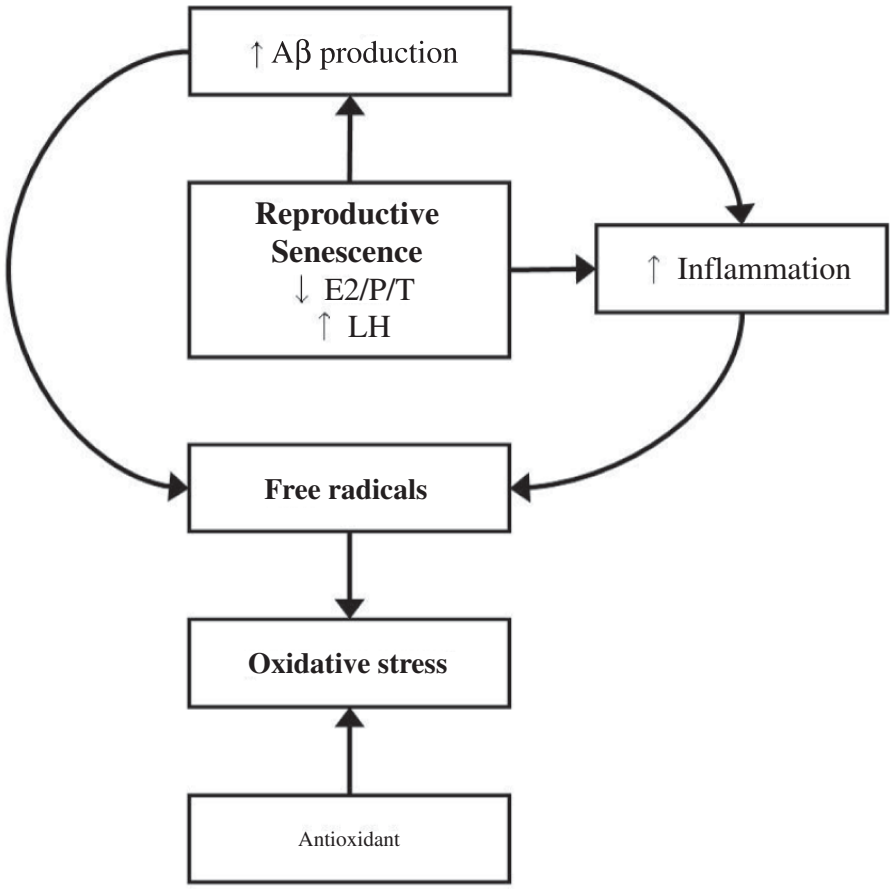

FIG. 4. Hormonal interactions with oxidative stress pathways during reproductive senescence. Following reproductive senescence estrogen (E2), progesterone (P) and testosterone (T) levels become depleted; therefore, they offer little neuroprotective benefit. Elevated LH levels stimulate Aβ production, that can subsequently lead to both oxidative stress and inflammation. It is also possible that LH may stimulate pro-inflammatory signaling pathways in the brain, which also can lead to the generation of free radicals and thus oxidative stress.

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