The Contribution of Luteinizing Hormone to Alzheimer Disease Pathogenesis

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Several hypotheses have been proposed that attempt to explain the pathogenesis of Alzheimer Disease (AD) including theories involving senile plaque and neurofibrillary tangle formation, increased oxidative stress, and cell cycle abnormalities, since evidence for each of these pathological phenomena have been well documented in AD. Recent epidemiological and experimental data also support a role for the gonadotropin luteinizing hormone in AD. Paralleling the female predominance for developing AD, luteinizing hormone levels are significantly higher in females as compared to males, and furthermore, luteinizing hormone levels are higher still in individuals who succumb to AD. Luteinizing hormone, which is capable of modulating cognitive behavior, is not only present in the brain, but also has the highest receptor levels in the hippocampus, a key processor of cognition that is severely deteriorated in AD. Furthermore, we recently examined cognitive performance in a well-characterized transgenic mouse that over-expresses luteinizing hormone and found that these animals show decreased cognitive performance when compared to controls. We have also found that abolishing luteinizing hormone in amyloid- β protein precursor transgenic mice (Tg2576) using a potent gonadotropin-lowering gonadotropin-releasing hormone agonist, leuprolide acetate, resulted in improved hippocampally-related cognitive performance and decreased amyloid- β deposition. These findings, together with data indicating that luteinizing hormone modulates amyloid- β protein precursor processing *in vivo* and *in vitro*, suggest that luteinizing hormone may contribute to AD pathology through an amyloid-dependent mechanism. These promising findings support the importance of luteinizing hormone in AD and bring to the forefront an alternative, and much needed, therapeutic avenue for the treatment of this insidious disease.

Key words: Alzheimer Disease; Amyloid- β ; Cognition; Luteinizing hormone

Alzheimer Disease (AD) is the most prevalent neurodegenerative disease affecting more than 15 million people worldwide¹ and is characterized by selective neuronal degeneration resulting in progressive memory loss.² AD attacks several different regions of the brain including the cerebral cortex which is involved in conscious thought and language, the basal forebrain which is important in memory and learning, and the hippocampus which is essential to memory storage. Clinical manifestations of AD are varied but can include memory loss, difficulty performing familiar tasks, disorientation to time and place, misplacing things, and changes in mood or behavior.³ Several hypotheses have been proposed in attempts to explain the pathogenesis of AD

and include theories involving senile plaque and neurofibrillary tangle formation, increased oxidative stress, and cell cycle abnormalities, since evidence for each of these pathological phenomena have been well documented in AD.⁴

The senile plaque, which is primarily composed of the 4.2 kDa amyloid- β polypeptide, is produced from the proteolytic cleavage of the larger amyloid- β precursor protein (A β PP) and is the extracellular hallmark of AD. To date, three genes involving A β PP or its cleavage product have been identified as containing fully penetrant, mutations resulting in early-onset AD. These mutations can be found on genes encoding A β PP itself and also on presenilin-1 and presenilin-2 genes.⁵ While these mutations are known to

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dramatically increase susceptibility to AD at early ages, only a small percentage of total AD cases can be attributed to these rare mutations, suggesting that there is another, non-mutation-based amyloid- β -dependent mechanism contributing to AD pathogenesis.

Neurofibrillary tangles comprise the major intracellular protein component in AD and are primarily composed from a hyper-phosphorylated form of the microtubule-associated protein called tau.^{6,7} The high level of tau phosphorylation seen in AD is thought to cause a destabilization of neuronal microtubular dynamics which ultimately results in neuronal dysfunction.^{8,9} While the kinases responsible for tau phosphorylation in AD are not completely characterized, hyperphosphorylation of tau also occurs in healthy, mitotically active neurons where phosphorylation is driven by cyclin-dependent kinases (CDK).¹⁰⁻¹⁴ Of note, CDKs such as CDK2 and CDK5, as well as cdc-kinases and mitogen-associated protein kinases, are increased in AD in a topographical manner that overlaps with hyperphosphorylated tau.^{15,16} *In vitro* assays have demonstrated these kinases also cause the hyperphosphorylation of tau.¹⁷⁻²¹ This suggests that tau hyperphosphorylation in AD may involve kinases also responsible for normal physiological tau phosphorylation.

While the presence of oxidative stress and free radical damage in AD pathogenesis has been well established,²²⁻²⁴ the mechanism behind free-radical generator/antioxidant disequilibrium in AD has yet to be elucidated.²⁵⁻²⁷ Similarly, although neuronal changes supporting the involvement of cell cycle abnormalities in the etiology of AD, which include the ectopic expression of cell cycle markers,²⁸ organelle kinesin²⁹ and cytoskeletal alterations²⁷ are evident, the events leading to these pathogenic changes are unknown. Nevertheless, mounting evidence for the participation of both oxidative stress and cell cycle abnormalities in the etiology of AD suggests that both of these phenomena play a fundamental role in AD.³⁰ Considering that senile plaques, neurofibrillary tangles, increased oxidative stress and cell cycle abnormalities all likely contribute to AD pathogenesis, it is clear that the events leading up to neuronal death and ultimately dementia in AD are not only highly complex, but also probably are integrated.

Due to the extended course of the disease, the etiologic events leading to the neuronal loss and dysfunction in AD are difficult to determine; however, recent evidence suggesting a gender-based predisposition towards AD in females that is not found in other dementias such as Parkinson's, supports a role for hormones in AD pathogenesis. Epidemiological studies exploring gender differences in AD have resulted in conflicting data, although most studies support the higher prevalence³¹⁻³⁴ and incidence³⁵ of AD in women. Since gender and age are two of the biggest risk factors for AD, it has been hypothesized that hormonal deficiency following reproductive senescence may contribute to the etiology of AD.

Interestingly, the long held notion of the pathogenic effects of decreased sex hormone levels, namely estrogen, on the brain after reproductive senescence is not consistently reflected in studies measuring sex hormone levels in AD compared to age-matched controls. For example, only one of nine recent observational studies comparing estrogen levels in women with AD to controls reported lower estrogen levels in AD,³⁶ with three studies reporting increased estrogen levels in AD³⁷⁻³⁹ and five reporting no significant differences between AD and controls.⁴⁰⁻⁴⁴ The variability in the results of these studies is thought to be caused in part by the sensitivity of the assay used, as studies that used less sensitive assays reported higher total estrogen levels,³⁹ and resulted in overestimation of the impact of low estrogen levels on the study. However, it is worth noting that the majority of these studies were conducted on samples of relatively elderly women who were many years postmenopause and whose current hormone levels do not necessarily reflect levels in premenopausal periods or in the immediate postmenopausal period that appears to be a critical window for the protective effect of estrogen or hormone replacement therapy. A few studies have examined the relationship of endogenous estrogen levels to cognitive decline in healthy women or to risk of AD, although the results have been inconsistent. Several studies have found an association between elevated sex-hormone binding globulin, or low bioavailable estradiol, and cognitive decline or AD, and it is likely that biologically active or free estradiol, rather than total estradiol, may be the active agents in the central nervous system and may be more important in maintaining cognitive performance.⁴⁵ Additionally, there is growing literature on the role of polymorphisms in the estrogen receptor activity on risk of AD.^{46,47} These effects are likely to be independent of hormone levels.

In addition to inconsistent data, studies reporting no improvement in cognitive function with estrogen plus progestin treatment⁴⁸ cast serious doubt on the role of sex hormones in AD pathogenesis. Similarly, a decline in estrogen or testosterone does not explain why males with Down's syndrome are at significantly higher risk of developing AD-type changes and at an earlier age than their female counterparts.⁴⁹ This shift in the normally gender-based predisposition of AD is not the result of decreased levels of estrogen or testosterone in patients with Down Syndrome because the levels of these hormones in Down Syndrome patients are comparable to the general population.

While most research evaluating how differences in gender contribute to AD pathogenesis is primarily focused on the sex hormones, estrogen and testosterone, there are a number of other hormones that regulate reproductive function in conjunction with sex hormones, forming the hypothalamic-pituitary-gonadal (HPG) axis. Like estrogen and testosterone, the other hormones of the HPG axis also undergo drastic changes in their level of expression induced by the onslaught of reproductive senescence. Among these

hormones are gonadotropin-releasing hormone (GnRH) and the gonadotropins, which are heterodimers consisting of a common α -gonadotropin subunit and a gonadotropin-specific β subunit that are non-covalently bound. The gonadotropin family includes luteinizing hormone (LH), human chorionic gonadotropin, follicle-stimulating hormone and thyroid-stimulating hormone; however, only LH and follicle-stimulating hormone are primarily involved in HPG axis regulation as thyroid-stimulating hormone regulates thyroid function, and human chorionic gonadotropin plays an essential role in pregnancy.

Interestingly, receptors for some of these gonadotropin hormones are expressed in many non-reproductive tissues including, most notably, the brain.^{50,51} When this is taken into consideration along with the incomplete protection of hormone replacement therapy, it is evident that gender-specific, age-related changes in GnRH and the gonadotropic hormones may potentially play a role in the pathogenesis of AD.⁵²⁻⁵⁵ Supporting a role for LH, as opposed to estrogen, in cognitive decline after menopause, studies have demonstrated that while cognitive decline can be rescued with estrogen therapy initiated immediately after ovariectomy, hormone replacement initiated after a long interval is ineffective.⁵⁶

Luteinizing Hormone in Alzheimer Disease

There is growing evidence supporting a role for gonadotropins, particularly LH, in AD pathogenesis beginning with the finding of a two-fold increase in circulating gonadotropins in individuals with AD compared with age-matched controls.^{57,58} Since gonadotropin receptors in the brain are found within the hippocampus⁵⁹ and gonadotropins are known to cross the blood brain barrier,⁶⁰ we speculate that elevated gonadotropins, namely LH, may contribute to AD pathogenesis.⁵⁷ In support of this theory, we found significant elevations of LH in vulnerable neuronal populations in individuals with AD compared to age-matched control cases.⁶¹ Notably, such an increase in neuronal LH appears to be a very early change in disease progression serving to predict neuronal populations at risk of degeneration and death. In fact, elevations in LH parallel the ectopic expression of cell cycle and oxidative markers that represent one of the initiating pathological changes and precedes neuronal degeneration by decades.^{62,63}

In another study using the M17 neuroblastoma cell line, LH did not alter A β PP expression; however LH did alter A β PP processing toward the amyloidogenic pathway as evidenced by increased secretion and insolubility of amyloid- β , decreased α -A β PP secretion and increased A β PP-C99 levels.⁶⁴ This same study also reported a 3.5-fold and a 1.5-fold reduction in total brain A β 1-42 and A β 1-40 concentrations, respectively, in C57BL/6J mice treated with leuprolide acetate, a potent GnRH agonist shown to effectively lower serum levels of LH.⁶⁴ Decreased LH levels, as a result of leuprolide acetate administration, were also

shown to significantly improve hippocampally-related cognitive performance and decreased amyloid- β deposition in aged A β PP transgenic mice, an animal model of AD.⁶⁵ These studies are the first to suggest a mechanism of LH-based neurotoxicity, namely the modulation of A β PP processing, and also to demonstrate the potential to reverse these pathological changes by lowering LH levels with leuprolide acetate.

Offering additional evidence for the link between LH and cognition, a recent study demonstrated declines in hippocampally-associated cognitive performance as measured by the Y-maze task⁶⁶ in LH- β overexpressing mice.⁶⁷ Interestingly, this study also showed that mice harboring a disrupted LH receptor (LHRKO) did not differ significantly in the Y-maze task between homozygous and wild-type mice, yet heterozygous mice performed significantly worse than homozygous mice.⁶⁷ This intriguing result provides strong evidence suggesting that LH-induced decreases in cognition are dependent upon the over-stimulation of LH receptor since the lack of an LH receptor in and of itself was not sufficient to evoke decreases in cognition. As previously mentioned, the LH receptor, unlike the follicle-stimulating hormone receptor, is not only expressed in the brain,⁶⁸ but also known to be most highly expressed in the hippocampus,⁵⁹ a region devastated by AD. It should be noted that in this study, neither LH- β overexpressing mice nor LH receptor knockout animals showed declines in spontaneous alternation behavior in the absence of differences in overall exploratory activity. This supports the notion that the declines in Y-maze performance are hippocampal-specific rather than associated with a more general phenomenon such as overall poorer health or tumor development in these animals. This study confirms previous reports revealing that LH is capable of modulating cognitive behavior.⁶⁹ Taken together, these findings suggest that gonadotropins such as LH may play an important role in hippocampally-related cognition and therefore, in the onset and progression of AD.

Novel AD Therapies

In large scale clinical trials, hormone replacement therapy has not been shown to be effective in the treatment of AD,⁷⁰⁻⁷² although at least one small study did find positive effects.⁷³ The reason for the lack of efficacy for hormone replacement therapy is likely due to the fact that it does not restore the HPG axis to its premenopausal state since the gonadotropin-stimulating effect of activin is still increased due to the loss of inhibin after menopause. In fact, it was recently shown that hormone replacement therapy, in the form of estrogen plus progestin, administered as a therapeutic agent in a WHI Memory Study was shown to increase the risk for probable dementia in postmenopausal women age 65 years or older.⁷⁴

It is only when the role of the other hormones of the HPG axis during the climacteric years and beyond is taken into account

that the results of the WHI Memory Study can be fully and accurately explained. For instance, it is crucial when interpreting the results of this study to recognize that the hormones of the HPG axis have been in disequilibrium for decades in all women who participated in the WHI Memory Study, so if a lack of estrogen does indeed play a role in AD pathogenesis, these women have been exposed to this disease-promoting hormonal environment for years, if not decades, by the time the estrogen/progestin treatment was administered. This is evidenced by the fact that reports of probable dementia appeared within the first year of the study in both the treatment and placebo groups. Therefore, it is somewhat predictable that the administration of estrogen/progestin in these women was not only unable to restore the proper functioning of the HPG axis, but that the influx of exogenous hormones have served to exacerbate the disease process, as the consequence of increased estrogen in compromised (i.e., old) neurons may be deleterious.⁷⁵

One way to revert the HPG axis to a premenopausal-like state is to lower LH using leuprolide acetate, a GnRH agonist which is known to suppress LH to undetectable levels by down regulating pituitary GnRH receptors. While the use of leuprolide in premenopausal women has resulted in memory loss and depression,⁷⁶ these adverse reactions are due to the secondary abrupt loss of estrogen production, since memory deficits returned to normal after hormone replacement.⁷⁷ Since women with AD are postmenopausal, having already lost their ability to produce estrogen, leuprolide acetate would be predicted to have no effect on their estrogen production. In this regard, a recently completed phase II clinical trial showed stabilization in cognitive decline and activities of daily living in a subgroup of female AD patients treated with high doses of leuprolide acetate coupled with an acetylcholinesterase inhibitor (<http://clinicaltrials.gov/ct/show/nct00076440?orden=6>). The necessity of concomitant leuprolide acetate treatment with an acetylcholinesterase inhibitor in order to observe an effect is likely reflective of the inability of leuprolide acetate to undo the neuronal damage caused by years of elevated LH. However, lessening LH-induced neurotoxicity by leuprolide acetate administration appears to be sufficient to abrogate declines in acetylcholinesterase inhibitor efficacy in this clinical trial.

It should be noted that males with AD were not as responsive to the effects of leuprolide acetate as were females with AD in initial clinical trials. There are a number of potential confounding issues that need consideration prior to drawing any definitive conclusions. First and foremost, leuprolide acetate will deplete endogenous sex steroids, which is clearly a bigger issue for men than postmenopausal women. While testosterone supplementation was used in this initial trial, compliance and dose were clearly confounding issues. However, it has been well documented that changes associated with reproductive senescence occur at very different rates between genders and include highly gender-specific symptoms.⁷⁸⁻⁸⁰ With this in mind, the earlier

chronology increasing LH in women may have pathogenic consequences that are not replicated in men whose LH only increases much later in life.⁸¹ While the results of ongoing clinical trials for the use of leuprolide acetate in the treatment of AD are eagerly awaited, studies investigating LH-induced pathogenesis provide a more complete paradigm of hormonal changes following reproductive senescence that contribute to AD.

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