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The Role of Gonadotropins in Alzheimer's Disease

Potential Neurodegenerative Mechanisms

Anna M. Barron, Giuseppe Verdile, and Ralph N. Martins

Centre for Aging and Alzheimer's Disease, Edith Cowan University, Joondalup, Australia; School of Psychiatry and Clinical Neurosciences, The University of Western Australia, Perth, Australia; and Sir James McCusker Alzheimer's Research Unit, Hollywood Private Hospital, Perth, Australia

In addition to the classical role of gonadotropins as a modulator of sex hormone production, it is now becoming apparent that the gonadotropins may have actions within the central nervous system. Evidence is also mounting that age-related increases in levels of the gonadotropin, luteinizing hormone (LH), may exert neurodegenerative effects such as those seen in Alzheimer's disease (AD). LH has been implicated in key cellular and biochemical processes that contribute to the pathogenesis of AD. These processes include the altered metabolism of key proteins in AD pathology, beta amyloid (Aβ), and its parent molecule, the amyloid precursor protein (APP). Evidence in the literature suggests that gonadotropins may be involved in processes that contribute to the etiology/pathogenesis of AD such as inflammation, cholesterol homeostasis, and insulin status. Here we examine the potential mechanisms by which gonadotropins could influence neurodegenerative processes. The role of gonadotropins in the brain and potential direct neuropathological effects of elevated gonadotropin levels is an exciting new topic in neuroendocrinology that in turn will lead to the development of novel therapeutic approaches for AD.

Key Words: Luteinizing hormone; beta amyloid; human chorionic gonadotropin; insulin; cholesterol.

Introduction

The most widely recognized function of the gonadotropins, luteinizing hormone (LH), follicle stimulating hormone (FSH), and human chorionic gonadotropin (hCG), is the regulation of gonadal function, including gametogenesis and steroidogenesis. More recently, novel effects of these hormones have been identified in the central nervous system. Evidence is also emerging implicating gonadotropin dysregulation in the development of Alzheimer's disease (AD). AD is a progressive neurodegenerative disorder primarily affecting the elderly. It is initially characterized by memory loss, eventually resulting in a complete loss of higher cognitive functions. Currently there is no effective prevention or treatment, nor can the disease be definitively diagnosed until postmortem. Causal genetic mutations have been identified accounting for a small proportion of familial cases. These mutations have provided insight into potential mechanisms central to AD pathogenesis. A number of non-genetic factors have been identified that have been associated with an increased risk of developing sporadic AD. These factors include age, exercise, diet, and, of particular interest, gender *(1–4)*. Multiple epidemiological studies have found increased prevalence of AD in females *(5–7)*, fuelling investigation into the neural effects of postmenopausal hormonal changes during reproductive senescence. This research has uncovered a potential link between AD and both sex hormone and gonadotropin levels.

Diminished levels of the sex hormones, estrogen and testosterone, have been associated with AD. Although in vitro and in vivo animal studies indicate estrogen and testosterone are very important for normal brain functioning, there has been limited, inconsistent success in the prevention of AD with estrogen replacement therapy *(8–15)*. However, such studies do not take into account the effect of gonadotropin levels on brain functioning, as evidence suggests that gonadotropins can also influence neural function and behavior. Therefore, regulation of gonadotropin levels may be an important new therapeutic approach for AD.

Neural Actions of Gonadotropins

Gonadotropins are large, lipid-insoluble proteins that, in contrast to the steroid hormones, have classically been thought not to cross the blood–brain barrier. However, it has now been experimentally demonstrated that a small percentage of LH and hCG, but not FSH, can cross the blood–brain barrier in rodents *(16,17)*. Lukacs et al. *(17)* showed that 30 min after peripheral injection, 1% of radiolabeled hCG passes the blood–brain barrier into the cerebrospinal

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Author to whom all correspondence and reprint requests should be addressed: Professor Ralph Martins, Centre for Excellence for Alzheimer's Disease Research and Care, Sir James McCusker Alzlheimer's Disease Unit, Edith Cowan University, 100 Joondalup Drive, W.A. 6027, Australia. E-mail: r.martins@ecu.edu.au

fluid (CSF). Radiolabeled hCG was localized to the choroid plexus, brain blood vessels, and hippocampus *(17)*. Early studies demonstrated the presence of gonadotropins in the CSF and synchrony between serum and CSF gonadotropin levels in both primates and humans *(18,19)*. As could be predicted based on Lukacs et al.'s *(17)* experimentally determined rate of gonadotropin passage through the blood–brain barrier in rats, normal concentrations of LH in the CSF represent a very low percentage (2–3%) of total serum concentrations *(19)*. It is therefore evident that gonadotropins are capable of crossing the blood–brain barrier from the periphery into the central nervous system.

The mechanism(s) of gonadotropin passage through the blood–brain barrier remain unclear. The localization of the luteinizing hormone receptor (LHR) in the ependymal cells of the third, fourth, and lateral ventricles and the choroid plexus has led to the suggestion that LH is transported across the blood–brain barrier via receptor-mediated transcytosis *(20)*. This mechanism has been reported in the testes for transport of radiolabeled hCG across the blood–testes barrier *(21)*. There is also evidence for extrapituitary *de novo* synthesis and secretion of gonadotropins in the brain *(22, 23)*. Such phenomena have been reported in vitro for other pituitary hormones such as adrenocorticotropic hormone *(24,25)*. In support of this notion, gonadotropin hormone transcripts have been localized in non-pituitary regions of the brain *(26–28)*. Furthermore, neural LH concentrations persist following removal of the pituitary gland *(29)*. Irrespective of the source of neural gonadotropins, their presence in brain tissue and CSF has been confirmed in both animals and humans *(29–34)*.

The presence of LH in the brain has been elegantly demonstrated and characterized in multiple regions of the rodent brain including the hypothalamus, amygdala, cerebellum, preoptic area, hippocampus, and cerebral cortex *(30,34)*. Measurement of LH levels by radioimmunoassay revealed the greatest abundance in the hypothalamus and small quantities in the remaining brain regions *(30)*. This was further characterized in a later study, finding over half of the assayable LH pool to be of cytoplasmic origin, while the remaining portion was associated with membranes, particularly synaptosomes *(35)*. Immunohistochemical mapping of LH in the rat brain revealed cytoplasmic immunoreactivity in the hypothalamus, while only occasional fiber reactivity was evident in the hippocampus and cerebral cortex *(36)*. The presence of gonadotropins in the hippocampus and cerebral cortex is of particular interest in AD research since these areas are severely affected by the disease.

Specificity of gonadotropin action is determined by target cell expression of gonadotropin receptors. There are two gonadotropin receptors: the luteinizing hormone receptor (LHR), which also interacts with the LH homolog, hCG, and the follicle stimulating hormone receptor (FSHR). Although it was previously thought that gonadotropin receptors were only expressed in the gonads, several studies have demonstrated LHR expression in the brain indicating it may also be a target organ for gonadotropin action *(37–41)*.

LHR expression has been systematically mapped in the rat brain central nervous system, showing receptors in the hippocampus, hypothalamus, amygdala, cerebral cortex, cerebellum, brainstem, and spinal chord *(38,41)*. These receptors were in greatest abundance in the hippocampus, a brain region involved in memory and severely affected in AD. Whether the LHR distribution pattern observed in the rat brain is conserved in the human brain remains to be established, although one study has localized LHR to the microglia and some neuronal cells of the human parietal cortex *(42)*.

LHR expression in the brain is developmentally regulated, with protein expression in rats increasing significantly between 17 and 21 d gestation *(43)*. Al-Hader et al. *(39)* also demonstrated changes in the regional distribution of LHR throughout development. The developmental regulation of LHR expression indicates that gonadotropins may play a key role in neural development. Consistent with this notion, treating fetal neuronal cultures with hCG stimulates neurite outgrowth and reduces apoptosis *(44)*. The structural similarity of the gonadotropin hormones to growth factors and cytokines has stimulated interest into the potential neurotrophic effects of these hormones *(45)* (reviewed in ref. *46*). In fact, hCG has been shown to be neurotrophic in the spinal chord, and has been investigated as a therapy for paraplegia *(47,48)*. Based on this combined evidence, it has been speculated that hCG promotes fetal brain growth and development during gestation *(20,44,45,49,50)*.

In addition to effects on the fetus, hCG is also believed to influence behavior and brain function in the mother. During pregnancy hCG levels have been shown to correlate with the severity of nausea and vomiting experienced *(51)*. Animal behavioral studies have also implicated elevated gonadotropin levels, particularly hCG, with behavioural changes associated with premenstruation, perimenopause, and pregnancy. In rats, both peripheral and intracerebroventricular administration of hCG affects feeding behavior, taste neophobia, sleep–wake phases, and exploratory activity *(17, 52–54)*. These behavioral responses to elevated hCG levels may explain some of the behavioral changes experienced during pregnancy including nausea, fatigue, insomnia, and food preference. More importantly, impaired cognitive function and memory is also associated with pregnancy, termed "maternal amnesia" (reviewed in ref. *55*). This amnesia is primarily thought to be a result of hormonal interaction with the hippocampus *(55)*. However, the individual hormones mediating these effects and the underlying biochemical mechanisms are uncertain. It has long been known that LH has the capacity to modulate neuronal excitability and activity in the rat hippocampus in vivo *(52,56)*. Thus, it is conceivable that elevated hCG levels potentially contribute to decreased memory function during pregnancy. The

specific contribution of the dramatic changes in hCG levels accompanying pregnancy to these behavioral changes will become more apparent as the function(s) of gonadotropins in the brain are clarified.

The discovery of LHR in the brain coupled with evidence that gonadotropins may cross the blood–brain barrier has caused a paradigm shift in the study of gonadotropin action. This has facilitated interest in the neural actions of gonadotropins and, consequently, the body of evidence that gonadotropins may participate in neural development and normal brain function is growing. Study of the classical roles of gonadotropins in the gonads may provide us with clues as to their novel functions in the brain. Classical models of gonadotropin action may also help identify pathological processes resulting from gonadotropin dysregulation associated with aging. In fact, many of the signaling pathways found to be affected by gonadotropins in the brain, such as cAMP signaling and prostaglandin levels *(39,57)*, were initially identified as targets for investigation due to the effects of gonadotropins on these systems in the periphery. Given that LHR is expressed at its highest levels in the hippocampus, a region important in memory function and severely affected in AD, coupled with evidence that gonadotropins can affect hippocampus-related behaviors and functions, further investigation into the role of gonadotropins in the development of AD is warranted.

Gonadotropin Regulation and Reproductive Decline

The non-pituitary and placental gonadotropins are differentially produced and regulated. The placental gonadotropin, hCG, is produced solely during pregnancy in the chorionic villi of the placenta, with synthesis and production controlled by autoregulatory mechanisms *(58)*. Human chorionic gonadotropin can be detected as early as 1 d after embryo implantation, with levels peaking in the first 2–3 mo of gestation (reviewed in ref. *59*). Its main function during pregnancy is to stimulate progesterone production and secretion, maintaining embryo implantation *(58)*.

The pituitary gonadotropins, LH and FSH, are secreted from the anterior pituitary in response to gonadotropin releasing hormone (GnRH) secretion from the hypothalamus. Pituitary gonadotropin and sex hormone homeostasis is maintained via intricate feedback mechanisms at all levels of the hypothalamic–pituitary–gonadal (HPG) axis (Fig. 1). At the onset of menopause there are too few remaining follicles to sustain HPG interactions *(60)*. This is accompanied by diminished sex hormone secretion from the ovaries and correspondingly increased pituitary gonadotropin secretion *(61)*. Men also experience a similar phenomenon during andropause. However, unlike menopause, andropause is not necessarily tightly coupled with the loss of reproductive function, and hormonal changes are gradual with high individual variation *(62–65)*. As a general trend, men between the ages 40 and 70 have significantly decreased testosterone levels and corresponding increases in gonadotropin levels due to a loss of negative feedback *(65)*.

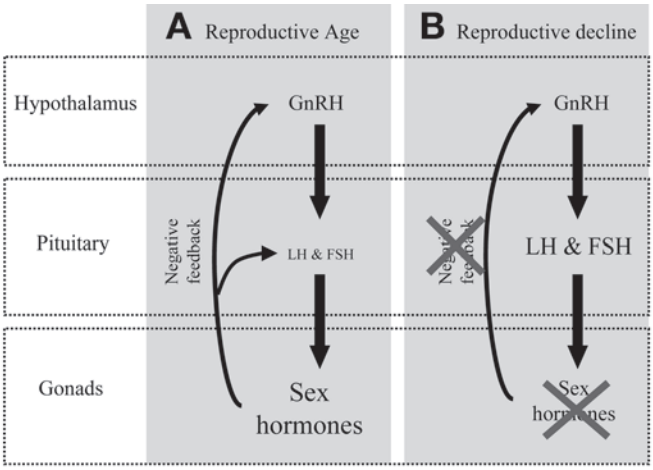

Fig. 1. HPG axis interactions at reproductive age (**A**) and during reproductive decline (**B**). At reproductive age GnRH is secreted from the hypothalamus into the hypophyseal portal capillaries, which convey it to the pituitary. In the pituitary GnRH stimulates gonadotrope function, causing LH and FSH secretion into the peripheral circulation. The gonadotropins affect target organs expressing their specific receptors: LHR and FSHR. In the gonads LH and FSH stimulate sex hormone production, including testosterone, estrogen, and progesterone. The gonadal steroid hormones exert negative feedback on GnRH secretion and thus gonadotropins. The gonadal hormones also provide direct feedback to the pituitary effecting gonadotropin secretion. The hypothalamic–pituitary–gonadal axis integrates behavioral, environmental, and internal factors to maintain reproductive hormonal homeostasis. During reproductive decline (**B**), gonadal failure to produce sex hormones abolishes negative feedback on the hypothalamus and pituitary and gonadotropin secretion rises unchecked. This results in marked increases in LH and FSH levels.

Disinhibition of gonadotropin levels by sex hormones results in elevated postmenopausal and andropausal GnRH, LH, and FSH levels. Additionally, the HPG axis shows insensitivity to negative feedback in postmenopausal women *(66)*. In women, gonadotropin levels begin to rise after age 35 until postmenopause, when serum LH levels are up to 18-fold higher and FSH levels are threefold higher than those observed at reproductive age *(61)*. Similar, although less severe, changes are reported in postandropausal men, with two- to threefold increases in serum levels of LH and FSH compared to levels at reproductive age *(63)*.

Corresponding changes in gonadotropin levels in CSF have also been reported in women. One study reported sixfold and twofold LH increases in serum and CSF levels, respectively, in postmenopausal women compared to women of reproductive age *(67)*. FSH levels were similarly elevated in both serum (eightfold increase) and CSF (threefold increase). This confirms that modest, yet significant, elevations in peripheral gonadotropin levels during menopause

are translated into the central nervous system. Although not confirmed, presumably a similar situation occurs during andropause.

Alzheimer's Disease and Reproductive Decline: Epidemiological Links

Many epidemiological studies to date have focused on sex hormone levels and AD, while gonadotropins have been neglected. Initial interests in these hormones arose due to gender dimorphism in AD prevalence observed cross-culturally *(5–7)*. Interpretation of gender-specific AD prevalence is in itself controversial and is complicated by gender differences in life expectancy *(68,69)*. Supporting the putative role of sex hormones in AD, significantly lower levels of sex hormones have been reported in AD subjects compared to age matched controls *(70)*. Furthermore, in vitro and in vivo animal studies indicate that sex hormones are neuroprotective (reviewed in ref. *71*), yet clinical studies have had inconsistent success in AD prevention *(8–11)*. More recently, epidemiological studies have correlated both elevated central and peripheral LH levels with declining cognitive function and increased AD prevalence *(72,73)*.

Bowen and colleagues, who put forward the "gonadotropin hypothesis" of AD, have been at the forefront of research into the association between gonadotropins and AD, and have identified many interesting epidemiological trends that indicate that gonadotropins may contribute to gender dimorphism in the prevalence of AD *(74,75)*. Interestingly, Down syndrome men, who exhibit elevated gonadotropin levels and normal testosterone levels *(76,77)*, have a higher prevalence and earlier onset of AD than Down syndrome women *(68)*. This represents a reversal of AD gender prevalence trends observed in the general population, revealing an association between elevated gonadotropin levels and AD risk independent of sex hormone status. This association needs to be systematically examined to determine the extent of correlations between AD and pituitary dysfunction independent of sex hormone levels within the Down syndrome population. Given pituitary dysfunction is common in the Down syndrome population, presenting as early as infancy *(77)*, examination of this population may prove very insightful into the effects of prolonged exposure to elevated gonadotropin levels.

Bowen et al. *(74)* conducted the first epidemiological study into gonadotropin levels and dementia in a small cohort of male subjects. They reported significantly higher LH and FSH levels, coupled with significantly lower testosterone levels in dementia subjects compared to control subjects with dementia. In 2001, a larger study was conducted including both male and female AD, fronto-temporal dementia and control subjects *(75)*. Although unable to confirm the trend in men, significantly elevated LH levels in female AD subjects compared to age-matched cognitively normal subjects was reported *(75)*. Estrogen and testosterone levels were not examined in this study; however it is likely that subjects with high gonadotropin levels also exhibit low sex hormone levels, as was observed by Bowen et al. *(74)*. As with all epidemiological studies, establishing cause and effect relationships proves to be very difficult, especially so in this case since the regulation of gonadotropins and sex hormones is interdependent. This issue will continue to hamper research efforts; however, animal models and in vitro studies will prove invaluable in resolving the relative contributions of gonadotropins and sex hormones to AD.

An independent group has recently reported significantly elevated LH and FSH levels coupled with decreased free testosterone levels in male AD subjects compared to age-matched control subjects *(78)*. The elevated gonadotropin levels in the AD subjects were found to be dependent on age, demonstrating a positive correlation between gonadotropin levels and age in the AD subjects *(78)*. An initial study published by this group examining a smaller cohort reported no significant difference in gonadotropin levels between AD affected males and controls, although a trend of higher gonadotropin levels in older AD cases was noted *(79)*. The effect of age may play an important role in the outcome of studies into the association between AD and elevated gonadotropin levels. Hence age effects may prove to be an important factor to consider in the design and interpretation of future studies into the relationship between gonadotropins and AD.

More recently, our laboratory has studied a cohort of 650 elderly women, comparing gonadotropin levels with cognitive function assessed by CAMCOG performance *(80)*. This study revealed an association between high LH levels and significantly decreased cognitive function, while no association with estradiol levels was noted. In this study, the possibility that declining cognitive function may be the result of secondary effects of gonadotropins on sex hormones was negated. However, epidemiological studies examining links between gonadotropins and AD are proving to be contentious, as some other recent studies have not reported such associations *(81,82)*. Comparison of gonadotropin and sex hormone levels between a small cohort of female AD subjects, subjects with dementia and cognitively normal subjects, revealed no significant difference in gonadotropin levels *(82)*. It could be argued that the cohort size was insufficient to provide the necessary power to observe the differences previously reported. However, another study conducted in a much larger cohort of subjects was consistent with these negative findings *(81)*. This emphasizes the difficulty in establishing whether associations between AD and elevated gonadotropin levels indicate a cause and effect relationship or are merely a secondary event.

Currently very little is known about brain gonadotropin levels in humans and how this relates to peripheral serum levels. CSF gonadotropin levels are much lower than peripheral levels; however, even CSF levels may not reflect tissue levels as brain gonadotropin production, degradation, and

penetration into tissue is unknown. Supporting this idea, comparisons of LH levels between serum and brain extract revealed no correlation *(30)*. The discrepancies between epidemiological studies may reflect inconsistencies between peripheral and central gonadotropin levels. Thus, the direct measurement of LH in brain tissue levels would prove more useful. Confirming epidemiological studies, a twofold elevation in LH immunoreactivity has been demonstrated in the hippocampus of AD brains compared to age-matched control brains *(32)*. LH immunoreactivity was localized in the cytoplasm of pyramidal neurons and co-localized with neurofibrillary tangles in the AD brains *(32)*. Given this is the first study to describe LH immunoreactivity in non-pituitary regions of the human brain, additional investigation is required to confirm and further characterise this novel finding. The results of Bowen et al.'s *(32)* study are consistent with the epidemiological trends identified, providing strong evidence of an association between elevated gonadotropin levels and AD.

Potential Neurodegenerative Mechanisms of Gonadotropins

Potential interactions between elevated gonadotropin levels and key neurodegenerative mechanisms implicated in AD will be examined in this review, including neurotoxicity mediated by beta amyloid (Aβ), chronic inflammation, and oxidative stress. The gross effects of these neurodegenerative processes are apparent upon inspection of the AD brain, typified by atrophy of the hippocampus, amygdala, and cerebral cortices *(83)*. At a microscopic level the diseased brain is characterized by widespread neuronal loss and abnormal depositions of Aβ and the cytoskeletal protein tau. Plaques consisting mainly of Aβ are surrounded by activated microglia and dystrophic neurites. Examination of the roles of gonadotropins in the periphery reveals potential mechanisms by which gonadotropins could influence these neurodegenerative mechanisms. Furthermore, we discuss potential secondary effects of gonadotropin–insulin interaction, which may also have implications for understanding the epidemiological trends observed in AD.

Bowen and colleagues have pioneered the notion that gonadotropins play a role in the pathogenesis of AD *(74)*. They postulate that higher gonadotropin concentrations elicit mitogenic pathways in postmitotic neurons, resulting in neuronal dysfunction and death *(84–87)*. This subject will not be reviewed further here as it has previously been comprehensively addressed *(32,72–75,86,88–92)*.

Modulation of APP Processing

A key characteristic of an AD affected brain is the deposition of amyloid in the form of senile plaques. The major protein component of these plaques is a small peptide termed beta amyloid (Aβ). The cause of the accumulation of Aβ in the more common sporadic form of AD is largely unknown, be it due to failure of degradative pathways or overproduction of the protein. Aβ dimers and oligomers are neurotoxic both in vivo and in vitro, contributing to oxidative stress, excitotoxicity, and inflammation (reviewed in ref. *93*). The "amyloid hypothesis" purports Aβ to be the toxic principle eliciting neurodegeneration cascades in AD. Aβ is a product of the proteolytic processing of its parent molecule, the amyloid precursor protein (APP) (reviewed in refs. *94* and *95*). The APP molecule undergoes proteolytic processing by two competing pathways, the non-amyloidogenic and amyloidogenic pathways (Fig. 2). In the non-amyloidogenic pathway, APP undergoes cleavage by enzymes termed α-secretase and γ-secretase to form a secreted form of APP (α-APPs) and non-amyloidogenic fragments, respectively. The amyloidogenic pathway generates Aβ through the sequential cleavage of APP by two enzymes termed beta-site APP cleaving enzyme (BACE) and γ secretase (Fig. 2) *(96)*.

In vitro studies have provided important evidence that gonadotropins can alter APP processing in neuronal cells *(91)*. Bowen et al. *(91)* showed that treatment of a neuronal cell line with concentrations of LH similar to those observed following menopause alters APP processing, resulting in elevated secretion of Aβ. Currently, the molecular mechanism by which LH modulates Aβ production is unknown. In the gonads, low concentrations of LH/hCG activate the cyclic adenosine monophosphate (cAMP) signaling pathway through LHR, while high concentrations activate the phospholipase C (PLC) signaling pathway *(97–100)*. There is evidence of induction of cAMP signaling pathway in response to LHR activation in neuronal cells *(57)*. However, the relevance of the PLC pathway to gonadotropin action in the brain is unexplored. Because the cAMP signaling cascade has been implicated in Aβ production *(101)*, the described effects of LH on Aβ levels could potentially be mediated through LHR/cAMP. If high LH levels similarly potentiate Aβ production in vivo, this could exacerbate neurodegenerative processes in AD.

The relationship between serum LH and Aβ levels has been examined in a small group of elderly demented men *(102)*. Although Aβ levels significantly correlated with testosterone and sex hormone binding globulin (SHBG) levels, no correlation was observed with LH levels. Because the cohort was large enough to establish a statistically significant relationship between Aβ and testosterone/SHBG levels, it seems unlikely that the size of the cohort can explain the discrepancy with the in vitro research. Additional examination of LH levels to determine if they were outside of the normal range in men of reproductive age may provide an explanation, because aging-related changes in gonadotropin levels in men are minor compared to those observed in women. It is possible that the effect was too small to be detected in these men, or perhaps the effects of testosterone on Aβ levels masked any effects of LH. Examination of the relationship between Aβ and LH levels in women should provide more conclusive evidence.

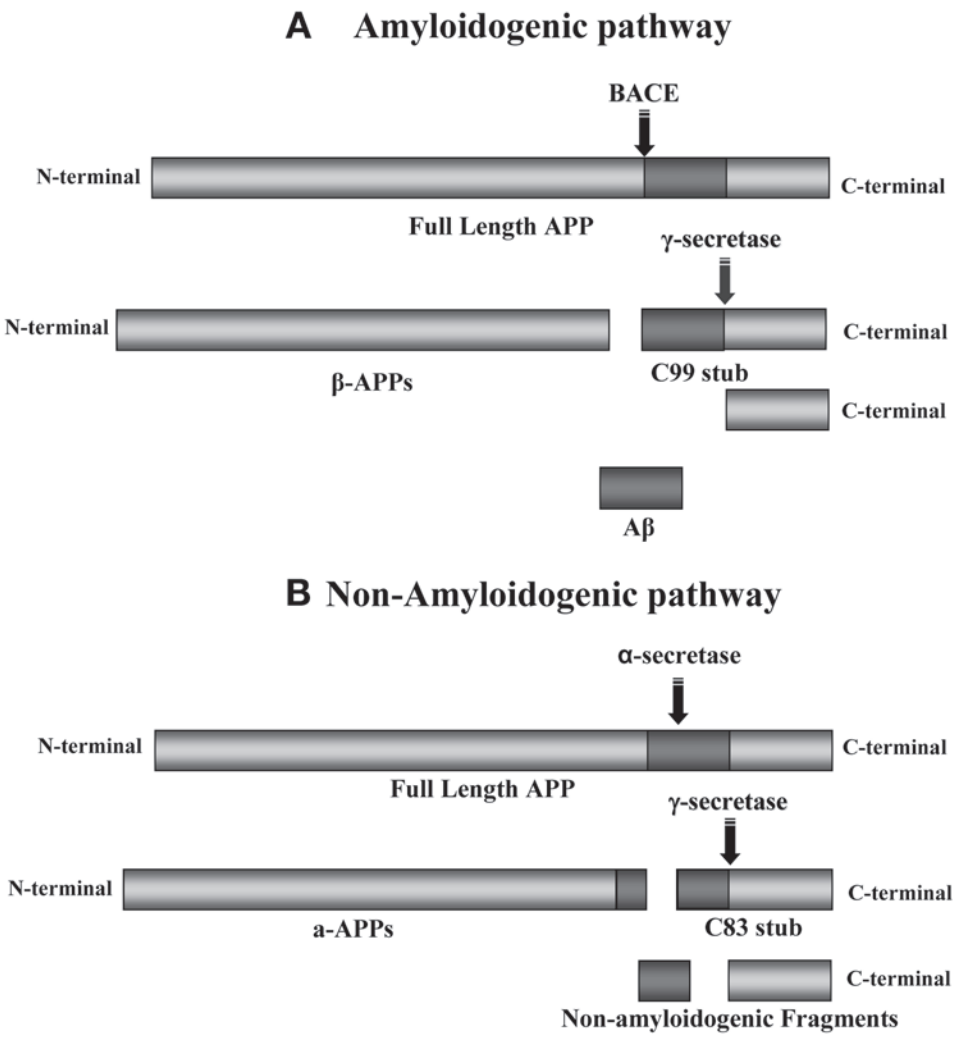

Fig. 2. Amyloidogenic and non-amyloidogenic APP processing pathways. (**A**) Amyloidogenic APP processing. Membrane-bound full-length APP is cleaved by BACE releasing a soluble N-terminal fragment (β-sAPP). The C-terminal fragment (C99 stub) is then cleaved by γ-secretase releasing the Aβ peptide. (**B**) Non-amyloidogenic APP processing. α-Secretase cleaves the full-length APP fragment in the Aβ domain releasing a soluble N-terminal APP fragment (α-APP). The remaining C-terminal fragment (C83 stub) is then cleaved by γ-secretase yielding the non-amyloidogenic fragments. (Adapted from ref. *180*.)

There is some evidence that gonadotropins can modulate other key proteins in AD pathogenesis, particularly the presenilins (PS1 and PS2). The presenilins are critical components of the multisubunit γ-secretase enzyme complex, and it is thought that they comprise the catalytic component of this enzyme (reviewed in ref. *103*). Changes in the expression of PS1 and PS2 have been reported in cultured human granulosa cells in response to treatment with LH, FSH, and forskolin *(104)*. This study examined the expression of a wide range of oncogenes and proteins implicated in disease. The authors found a significant increase in PS1 expression in response to both LH and FSH treatment, while PS2 expression was significantly elevated in response to LH treatment. Conversely, in a human neuroblastoma cell line expressing LHR, no effect of LH on PS1 protein expression was observed *(91)*. The cells were treated with LH reflecting pre- and postmenopausal serum levels. However, as the presenilins have other important roles in cellular functioning, including involvement in notch signaling *(105–107)*, the physiological relevance of these studies remains to be determined. In addition, the different results from the two studies may reflect cell type differences. Further investigation is clearly warranted to determine whether LH acts to regulate presenilin expression and metabolism in the brain.

Inflammation

Activation of immune and inflammatory responses plays a key role in AD (reviewed in ref. *108*). Activated microglia and astrocytes are typically found within and in close proximity to neuritic plaques in AD brains. Activated microglia secrete neuroprotective substances, such as glia-derived neurotrophic factor, as well as neurotoxic cytokines and free radicals including nitric oxide. Secreted neuroinflammatory molecules from chronically activated microglia are potentially toxic and can induce oxidative stress, contributing to neurodegeneration *(109,110)*. In the periphery, gonadotro-

pins, particularly LH, have been implicated in inflammatory processes *(111,112)*. LH stimulates inflammation in the gonads; indeed ovulation, which is under the regulatory control of gonadotropins, has been described as an inflammatory process *(111,113,114)*. Furthermore, gonadotropins have been implicated in the regulation of immune responses during pregnancy. Because inflammation is thought to play an important role in AD, elevated postmenopausal levels of LH may potentially affect AD progression by aggravating inflammatory responses, in turn causing oxidative stress and neurodegeneration.

Interaction between components of the HPG axis and immune responses has been well established. Interestingly, differences have been noted between men and women in the risk of development and recovery from a range of inflammatory neurological disorders including multiple sclerosis, stroke, Parkinson's disease, and amyotrophic lateral sclerosis *(115–117)* (reviewed in ref. *118*). Estrogen has anti-inflammatory properties, regulating the expression of inflammatory cytokines (reviewed in ref. *118*) and in turn, cytokines have also been implicated in the regulation of all the hormones within the HPG axis including GnRH, gonadotropins, and sex hormones *(115,119)*. Pro-inflammatory cytokine levels are increased in menopausal women and decrease following estrogen replacement therapy *(120,121)*. Evidence points toward estrogen as the mediator of these effects, although it is possible that elevated gonadotropin levels also contribute to this phenomenon.

LHR has been immunohistochemically localized to macrophages in the parietal cortex of human brains, suggesting LH and/or hCG may influence the activity of these cells *(42)*. In the periphery, gonadotropins are thought to play a role in the regulation of immune responses during pregnancy. High concentrations of hCG alter production of the inflammatory cytokine interleukin (IL-8) from monocytes under in vitro conditions *(112)*. Gonadotropins may similarly effect the production of cytokines in microglia. Interestingly, IL-8 levels are elevated in AD brains, and it has been demonstrated that IL-8 enhances inflammatory responses to Aβ in human microglia *(122)*. LH has also been demonstrated to dose-dependently increase secretion of reactive oxygen species such as nitric oxide, superoxide, and hydrogen peroxide from leukocytes *(111)*. This is proposed to play an instrumental role in luteolysis and ovulation. Consistent with this, reactive oxygen species in women during the periovulationary period were found to be significantly elevated despite unchanged blood leukocyte levels *(111)*. Whether gonadotropins elicit similar inflammatory responses in the brain is yet to be determined. There is no apparent purpose for such responses during normal neural function, although postmenopausal elevations in gonadotropin levels could elicit abnormal signaling pathways. Studies have indicated that postmenopausal women have altered immune responses that can be corrected with hormone replacement therapy *(119)*. Whether these responses are directly due to the addition of exogenous estrogen or due to a subsequent reduction in gonadotropins remains to be determined and warrants further investigation.

In immortalized hippocampal cells hCG activation of LHR results in an upregulation of the enzyme 5'lipoxygenase (5'LOX) *(57)*. This enzyme stimulates the formation of inflammatory leukotrienes from arachidonic acid *(123)*. 5' LOX is expressed at particularly high levels in the hippocampus and the cerebellum *(124)*. As the hippocampus is susceptible to AD neurodegeneration and coincidently expresses high LHR densities, this may provide another link between gonadotropin levels and AD. Dysregulation of 5' LOX levels is associated with aging, as aging rat brains express elevated transcript and protein levels *(125–127)*. Furthermore, elevated brain 5'LOX expression correlates with neuronal susceptibility to excitotoxicity, an important factor in neurodegenerative processes *(128,129)*. In light of the age-related increase in 5'LOX expression, LOX inhibitors have been identified in the literature as a potential AD treatment *(130,131)*. The 5'LOX inflammatory signaling pathway represents a potential neuronal target for gonadotropins in vivo, which may be overstimulated in postmenopause/andropause by elevated gonadotropin levels. This could confer susceptibility of areas expressing high LHR levels to excitotoxic neurodegeneration and elevated inflammatory response.

Cholesterol Homeostasis

Abnormalities in central nervous system cholesterol levels are typically observed in AD. Retrospective studies indicated that subjects using cholesterol-synthesis inhibitors have reduced risk of developing AD, a finding now confirmed in prospective studies *(132–135)*. How elevated cholesterol levels increase the risk of AD is not known, although animal and cell culture studies indicate that elevated cholesterol levels increase Aβ production and decrease secretion of neuroprotective soluble α-APPs *(136–138)*. Elevated cholesterol levels are also believed to shift APP metabolism toward the amyloidogenic processing pathway resulting in an upregulation of Aβ *(139,140)*. Gonadotropins play an important role in the regulation of cholesterol in the gonads, as cholesterol is the substrate for sex hormone synthesis. Because the brain is a steroidogenic organ, it may also follow that gonadotropins play a role in the regulation of cholesterol and steroidogenesis in the central nervous system.

Supporting the potential role of gonadotropins in the regulation of cholesterol homeostasis, multiple studies have found significantly altered peripheral cholesterol levels that coincide with changes in HPG homeostasis; an effect attenuated by hormone replacement therapy and thus suggesting a link between HPG axis dysregulation and elevated cholesterol *(141–143)*. Unfortunately, it is very difficult to dissect effects of sex hormones from those of gonadotropins following hormone replacement therapy, as gonadotropin and sex hormone changes are intertwined. Given the impor-

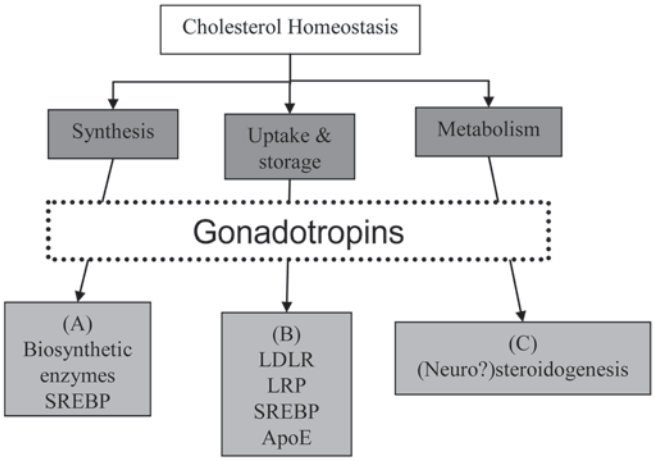

Fig. 3. Model of gonadotropin interaction with cholesterol homeostatic processes in the gonads. Mechanisms of cholesterol regulation in the periphery may also be applicable to the central nervous system. Cholesterol homeostasis can be modified by altering cholesterol synthesis, uptake, and storage or metabolism. In the periphery, gonadotropins have been implicated in the modulation of each of these three pathways. (**A**) Gonadotropins can influence cholesterol synthesis through the regulation of cholesterol biosynthetic enzymes and other cholesterol synthesis regulatory factors (SREBP). (**B**) Gonadotropins affect the expression of multiple proteins involved in the uptake and storage of cholesterol including low-density-lipoprotein receptor (LDLR), low-density-lipoprotein receptor–related protein (LRP), sterol regulatory binding proteins (SREBP), and apolipoprotein E (ApoE). (**C**) Gonadotropins influence the usage of cholesterol through the regulation of steroidogenesis in the gonads. It is not known if gonadotropins play a role in neurosteroidogenesis. These processes represent candidate targets for examining how gonadotropins may influence cholesterol homeostasis in the central nervous system.

tant role gonadotropins have in the regulation of gonadal cholesterol synthesis and metabolism, further investigation into their putative role in brain cholesterol homeostasis is warranted.

There are multiple levels at which gonadotropins could potentially be involved in the regulation of cholesterol homeostasis: synthesis, cellular uptake and storage, and metabolism (Fig. 3). Gonadotropins have been implicated in the regulation of cholesterol in all three of these pathways in the periphery; the potential relevance of these pathways to the central nervous system and neurodegenerative mechanisms will be examined in the ensuing sections.

Synthesis

Cholesterol cannot cross the blood–brain barrier, thus endogenous cholesterol synthesis is very important in the central nervous system. Despite this, it has been experimentally demonstrated that peripheral and central cholesterol levels are not independent and little is known regarding the regulatory mechanisms of central nervous system cholesterol synthesis. Animal experiments have implicated gonadotropins in the regulation of cholesterol biosynthesis in the gonads *(144)*. Rats treated with hCG express upregulated levels of cholesterol biosynthetic enzymes including mevalonate kinase and 3-hydroxy-3-methylglutaryl-coenzyme A in the ovaries. Human chorionic gonadotropin can also upregulate the expression of the 1c-isoform of the sterol regulatory-binding proteins (SREBP), which are membrane-bound transcription factors controlling the synthesis and uptake of cholesterol and fatty acids *(145)*. The SREBP-1c isoform is predominantly involved in fatty acid synthesis; however, its biological significance is poorly understood *(145,146)*. If gonadotropins contribute to the regulation of CNS cholesterol production, elevated gonadotropin levels may increase cholesterol synthesis and thus potentially indirectly contribute to Aβ deposition and aggregation.

Uptake and Storage

Cellular uptake and storage of cholesterol is a cholesterol homeostatic mechanism in which intracellular cholesterol reserves are generally compartmentalized into free cholesterol and cholesterol esters. When cholesterol and lipoproteins are bound to lipid-transport molecules, such as apolipoprotein E (apoE), they can be internalized into cells through receptor-mediated endocytosis by receptors such as the low-density-lipoprotein receptor (LDLR) and low-density-lipoprotein receptor–related protein (LRP) *(147)*. ApoE is the major cholesterol transporter in the brain and the ε4 allele is a significant risk factor for the development of AD. LRP may also play an important role in the pathogenesis of AD because selected polymorphisms increase the risk of AD *(148–150)*.

Gonadotropins have been implicated in the regulation of both apoE and LDLR/LRP in the periphery. In cultured interstitial cells, LH can elevate apoE secretion, thereby modulating cholesterol availability and sex hormone production *(151)*. Gonadotropins also modulate lipoprotein receptor expression and function in human and animal granulosa cells *(104,152)*. Furthermore, FSH and hCG regulate surface LDLR expression and LDLR ligand metabolism *(104)*. In cultured human granulosa cells, LH, and to a lesser extent FSH, increases LRP transcription *(152)*. This leads to the possibility that gonadotropins may similarly regulate apoE and LDLR/LRP expression in the brain. Presuming gonadotropins can influence the expression of these proteins in the brain, high gonadotropin levels could potentially modify cholesterol transport, uptake, and storage. Furthermore, there is evidence that apoE and LRP interact with Aβ and APP, facilitating intracellular Aβ accumulation and aggregation *(153,154)*. Therefore, if gonadotropins influence the neuronal expression of apoE and LDLR/LRP, they may indirectly increase Aβ accumulation and neurotoxicity *(74,75)*.

Metabolism

Cholesterol is the substrate for steroid hormone synthesis. Neurally acting steroid hormones, or neurosteroids,

directly act on neurotransmitter receptors to effect psychological processes and play a role in development, effecting neural modeling and axon growth (reviewed in ref. *155*). These effects were initially believed to be mediated by peripherally synthesized steroid hormones; however, it is now known that the brain is a steroidogenic organ and can independently synthesize neurosteroids (reviewed in refs. *156* and *157*). Experimental evidence suggests that there are parallels between neural and gonadal steroid hormone biosynthetic mechanisms *(158)*. Classical steroidogenic enzymes including the cytochrome P450 and hydroxysteroid dehydrogenase (HSD) enzymes have been localized in astrocytes, oligodendrocytes, and neurons throughout the brain *(159)*. The important role that gonadotropins play in the regulation of steroidogenesis in the periphery raises the possibility that gonadotropins may also serve a similar purpose in neurosteroidogenesis.

In the gonads, the expression of steroidogenic enzymes such as the P450s and HSDs is regulated by LH through its receptor *(160)*. The regulatory mechanisms involved in neurosteroidogenesis have thus far remained elusive; although the co-localization of LHR and side chain cleavage P450 (P450scc) has led to speculation that gonadotropins may play a role *(37)*. P450scc converts cholesterol into pregnenolone, which is the precursor of all sterdoigenic sex hormones. The role of gonadotropins in the regulation and dysregulation of neurosteroidogenesis in the brain remain tantalizingly unresolved; however, given the important function of neurosteroids in the brain, gonadotropin dysregulation may also result in neurosteroid hormone imbalances. This may also have implications for cholesterol homeostasis within the central nervous system, as cholesterol is the precursor of sex hormones.

Hormone-Hormone Interactions - Insulin Status

Metabolic factors such as insulin, which orchestrates energy storage and requirements throughout the body *(161)*, are important in the regulation of the HPG axis and reproductive homeostasis *(162–164)*. Consequently, metabolic disturbances are often accompanied by reproductive dysfunction stemming from altered gonadotropin levels. Diseases such as diabetes mellitus and polycystic ovary syndrome (PCOS) are characterized by dysregulation of both insulin and gonadotropin homeostasis *(165,166)* (reviewed in ref. *167*). In vitro and in vivo evidence indicates that insulin acts on the hypothalamus to stimulate GnRH release, and thus indirectly stimulates gonadotropin release, while insulin insensitivity in the central nervous system is associated with decreased gonadotropin levels *(161–164)*. However, how this experimental evidence translates to normal physiological conditions remains to be determined. The HPG axis also provides regulatory feedback on insulin homeostasis. Low sex hormone levels, both testosterone and estrogen, are associated with altered insulin levels and increased insulin resistance *(168–170)*, which are consistent with higher incidences of insulin resistance associated with menopause *(171)*.

Insulin dysfunction such as insulin resistance has been implicated in a range of neurodegenerative disorders including AD, vascular dementia, Parkinson's disease and Huntington's disease *(172–176)*. Apart from critical effects upon glucose homeostasis, insulin dysregulation exacerbates chronic inflammation and influences Aβ accumulation and tau phosphorylation, all of which contribute to AD pathogenesis *(177)*. It is therefore possible that elevated gonadotropin levels observed in AD cases may be driven by high insulin levels, and thus it could be argued that the gonadotropins themselves may not play a direct role in the development of AD, but instead may be a secondary factor. Alternatively, both elevated gonadotropin and insulin levels may co-contribute to neurodegeneration through separate mechanisms.

Potential Therapeutic Relevance

Although the normal functions of gonadotropins in the brain remain largely unresolved, clear links have been made between elevated gonadotropins and AD. Indeed mice treated with the gonadotropin-reducing agent, leuprolide, over a period of 8 wk have significantly reduced brain Aβ levels *(91)*. Leuprolide is a GnRH agonist that, when chronically administered, causes the downregulation of GnRH receptors and subsequently reduced gonadotropin secretion *(178)* and is consequently used in the treatment of prostate cancer, endometriosis, and infertility *(178,179)*. Clinical trials to assess the efficacy of leuprolide as a potential therapeutic agent for AD are currently underway in the United States. If elevated gonadotropin levels do contribute to biochemical disturbances in AD, gonadotropin lowering agents such as leuprolide may potentially slow neurodegeneration and prevent loss of cognitive function in AD.

Conclusions

There is substantial evidence in the literature that gonadotropins have potential roles in the central nervous system and may affect behavioral and cognitive functions of the brain. Furthermore, the dysregulation of the HPG axis and the associated increases in gonadotropins have implications for the etiology and pathogenesis of neurodegenerative diseases such as AD. In these early stages of research into the role of gonadotropins in brain function and AD, the effects mediated by gonadotropins in the periphery provide us with a model to study gonadotropin action in the brain. Here we have identified a number of potential candidate neurodegenerative mechanisms, which may be influenced by gonadotropins, including Aβ production, inflammation, and cholesterol homeostasis (Fig. 4). One of the major tasks ahead involves identifying and validating how gonadotropins negatively impact on cognitive function, and hopefully concurrently gain further insight into the normal function of gona-

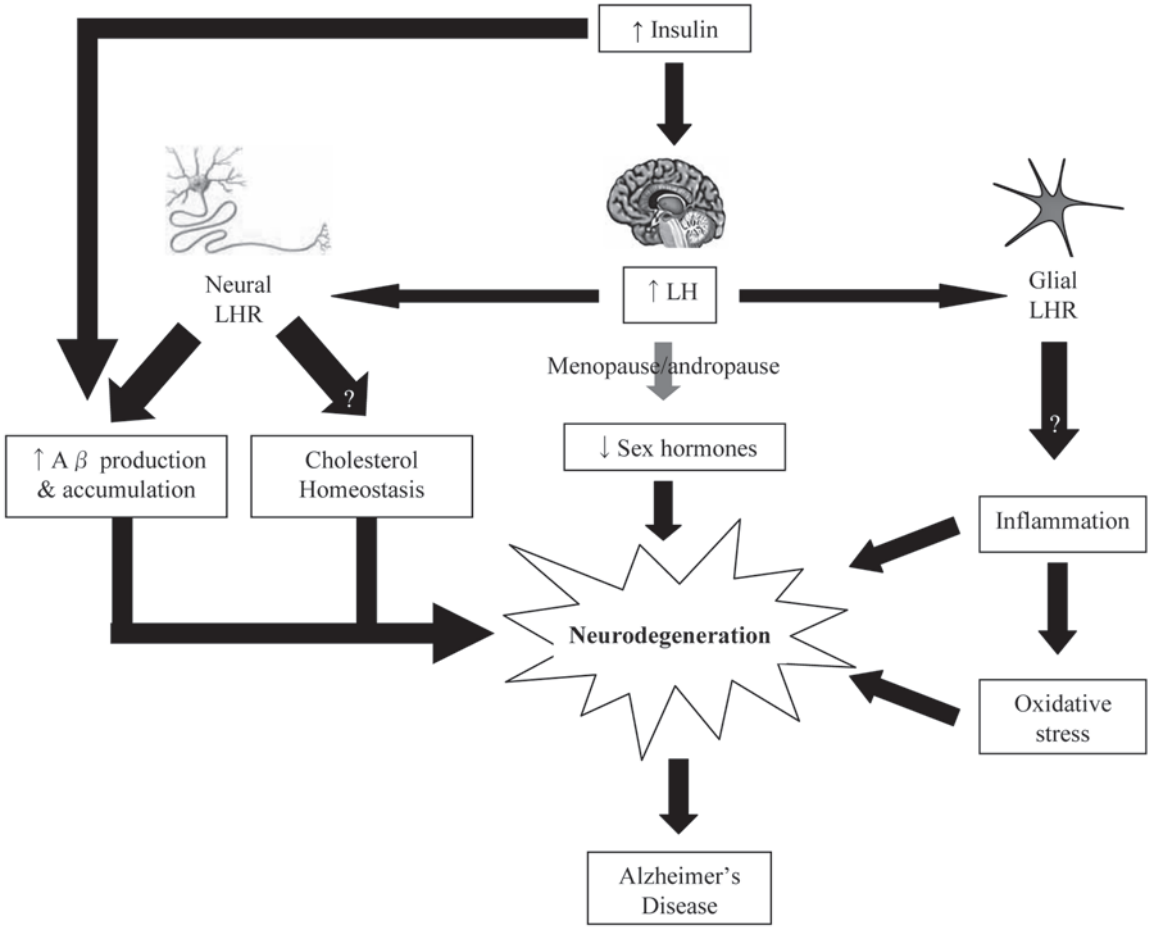

Fig. 4. Putative role of elevated LH in Alzheimer's disease. Elevated postmenopausal/andropausal LH levels could act through LHR to potentially modify cholesterol homeostasis and/or stimulate Aβ production in neural cells. Increased central levels of LH may also activate LHR on glia, stimulating inflammation and oxidative stress. Activation of any of these three pathways would converge in neurodegenerative changes typical of AD. Insulin dysregulation, which is independently associated with AD, may contribute to elevated gonadotropin levels and increased Aβ production concomitantly. Depleted sex hormones levels following menopause/andropause also have an important role in neurodegenerative changes.

dotropins in the brain. The influence of gonadotropins on the central nervous system may also help reconcile apparent discrepancies in the literature regarding the role of sex hormones in AD. Finally, gonadotropins may represent a target for therapeutic intervention for diseases such as AD. Further investigation into the role of gonadotropins in AD will hopefully focus upon examining these pertinent issues.

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