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# The Gonadotropin Connection in Alzheimer's Disease

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**Although not traditionally thought of as regulators of neuronal function, the hypothalamic–pituitary–gonadal (HPG) hormones luteinizing hormone (LH), gonadotropin-releasing hormone (GnRH), and activins possess neuronal receptors. These receptors are found throughout the limbic system on a number of different cell types, and, like reproductive tissues, the expression of these receptors is regulated by hormonal feedback loops. These hormones and their receptors regulate structure and a diverse range of functions in the brain. Therefore, it is not surprising that the dysregulation of the HPG axis with menopause and andropause (leading to elevated LH, GnRH, and activin signaling but decreased sex steroid signaling) might promote alterations in both the structure and function of neuronal cells. To date, most evidence has accumulated for a role of LH in promoting neurodegenerative changes. LH is known to cross the blood–brain barrier, receptors for LH are most concentrated in the hippocampus, that region of the brain most vulnerable to Alzheimer's disease (AD) and LH is significantly elevated in both the serum and the pyramidal neurons of AD subjects. LH promotes the amyloidogenic processing of the amyloid-β precursor protein in vitro, and the antigonadotropin leuprolide acetate decreases amyloid generation in mice. Moreover, leuprolide acetate improves the cognitive performance and decreases amyloid-β deposition in aged transgenic mice carrying the Swedish AβPP mutation. Therefore, the elevation of LH with the dysregulation of the HPG axis at menopause and andropause is a physiologically relevant signal that could promote neurodegeneration. Epidemiological support for a role of LH/GnRH in AD is evidenced by a reduction in neurodegenerative disease among prostate cancer patients a group known to GnRH agonists. Clinical trials are underway for the treatment of AD using GnRH analogs and should provide further insights into the gonadotropin connection in AD.**

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## Introduction

Epidemiological and biochemical studies indicate an association between hormones of the hypothalamic–pituitary–gonadal (HPG) axis and cognitive senescence. In particular, these studies indicate that changes in HPG hormones following menopause/andropause are involved in the cognitive and neuropathological changes observed in Alzheimer's disease (AD). Although most attention has focused on the sex steroids as mediators of the disease, the decrease in the production of gonadal sex steroids (estrogens and androgens) and inhibin with the loss of reproductive function (menopause/andropause) results in alterations in the serum concentration of *all* hormones of the HPG axis (Fig. 1). This leads to a net change in neuronal signaling such that there is an increase in gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and activin signaling together with the well-described decrease in sex steroid signaling in the limbic system of the brain. We have termed this dysregulated HPG hormone signaling "dyotic signaling" *(1)*. This review will discuss the evidence supporting HPG hormones in modulating the biochemical, pathological, and cognitive changes associated with AD, and which hormones of the axis studied thus far have the greatest influence on degenerative processes.

## Alzheimer's Disease

AD is a neurodegenerative disorder of the elderly that leads to progressive memory loss; to impairments in behavior, language, and visuo-spatial skills; and ultimately to death. The disease is invariably associated with and defined by neuron and synaptic loss and the presence of extracellular deposits of amyloid-β (Aβ, a 39–43 amino acid protein) and intracellular neurofibrillary tangles [(NFTs) composed primarily of phosphorylated tau] in the parenchyma of the brain *(2)*.

### *Neuronal Aβ Generation and Deposition*

The Aβ protein is derived from the larger amyloid β protein precursor (AβPP) that can be processed by two com-

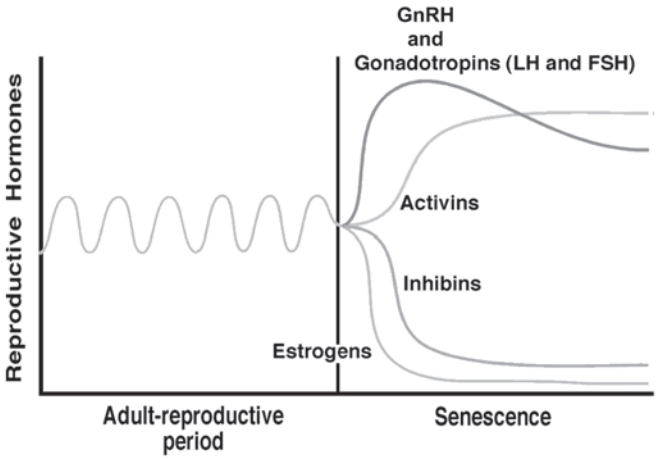


**Fig. 1.** Representative figure illustrating HPG hormonal profiles following menopause and during andropause. Reproductive changes result in a net change in cellular signaling induced by the increase in serum concentrations of gonadotropin-releasing hormone, luteinizing hormone, and activin, and decrease in serum sex steroids.

peting pathways that involve different proteolytic enzymes that are termed secretases (α-, β-, and γ-secretases). These pathways either promote the generation of Aβ (amyloidogenic pathway) or preclude its production [non-amyloidogenic pathway *(3)*]. Aβ is released as a soluble product from AβPP *(4,5)* via a series of metabolic cleavage steps through the combined actions of β- and γ-secretases [*(6)*; see ref. *7* for a recent review]. Although Aβ is a normally soluble and constitutive protein found in biological fluids and tissue *(8,9)*, Aβ aggregates to form diffuse amorphous deposits as well as dense, focal, extracellular deposits in AD [*(10)*; see ref. *11* for a review).

Recent evidence has helped provide a more coherent understanding of the properties and functions of this protein. Aβ possesses both neurotrophic as well as neurotoxic properties *(12)*. These activities appear to be regulated by differentiation-associated changes in tau and cyclin-dependent kinase 5 (Cdk-5) such that Aβ is not toxic to undifferentiated neurons, but is toxic to differentiated neurons *(13–16)*. Our recent finding that Aβ production increases only when a neuron commits to death indicates that Aβ generation is a late event in the life cycle of a neuron (Verdile et al., submitted). Aβ released at this late stage is more likely involved in facilitating rather than preventing the death of differentiated neurons. Released Aβ also likely plays a role in maintaining the integrity of the degenerating region *(17)*, while deposited Aβ is a signal for microgliosis and the phagocytosis of dying neurons (reviewed in ref. *18*). Since Aβ has neurotrophic properties toward undifferentiated neurons and has been shown to increase neurogenesis of neural stem cells, released/deposited Aβ may then act as a mitotic signal to replace the dying neuron *(19)*. Taken together, these results imply that while the toxic expression of Aβ may be directed toward the dying neuron, the trophic properties of the protein may be directed toward maintaining the structural integrity of the region and promoting neurogenesis. Indeed, the increase in Aβ in astrocytes of the developing fetal brain *(20)* may be involved in apoptotic patterning, and that released Aβ is neurogenic to neighboring neural stem cells during the growth and development of the brain.

### *Tau Phosphorylation and Neurofibrillary Tangle Formation*

The major protein component of the NFT, the major intracellular pathology of AD, is a highly phosphorylated form of the microtubule-associated protein tau *(21–23)*. Tau's major cellular role is thought to involve regulation of neuronal microtubule assembly and stabilization of microtubules against depolymerization in vivo *(24–26)*. Disassembly of the rigid microtubule structure of neurons for neuronal division is accomplished by removing the microtubule stabilizing protein tau via its phosphorylation. The lack of dephophorylation leads to neurofibrillary tangle formation as seen in AD.

Phosphorylation of the microtubule-associated protein tau normally occurs during metaphase of neuronal division, and during differentiation hyperphosphorylated tau is observed in neurons of the fetal brain *(14,27)*. This high and residue-specific phosphorylation of tau in mitotically active neurons is driven by Cdks *(27–31)*. Several Cdk's are associated with phosphorylated tau in AD and in vitro phosphorylate tau in a manner similar to that found in AD *(32–36)*. A number of other kinases such as glycogen synthase kinase-3β (GSK3β) also appear to play a pivotal role in neuron tau phosphorylation *(37)*. Because increased phosphorylation and altered microtubule stability are coincident during progression through the cell cycle *(38,39)*, it is likely not coincident that there are microtubular abnormalities associated with AD *(40)*.

## Current Hypotheses of Alzheimer's Disease

A number of hypotheses have been proposed to explain AD, with the "amyloid hypothesis" gaining most attention due to the findings that early-onset AD result from mutations in the AβPP, or proteins involved in its processing (presenilin-1 and presenilin-2). Other theories that include tau phosphorylation, oxidative stress, mitochondrial alterations, metal ion dysregulation, inflammation, which are all associated with AD, also have gained support as playing a role in the disease process. While it is agreed that these pathological changes are involved in the disease process, the actual upstream cause of these changes in *sporadic* AD has received less attention.

## Role of HPG Axis Hormones in the Normal Structure and Function of the Brain

The influence of hormones of the HPG axis on brain structure (development and maintenance) and function has become well established over the last century. All hormones

of the axis with hippocampal receptors (activins, GnRH, LH/hCG, and sex steroids) have known functions in regulating neuronal development and adult brain structure and function. Receptors for these HPG hormones are concentrated in the limbic system, particularly the pyramidal neurons of the hippocampus, those neurons vulnerable to AD pathology. For a detailed review of the normal functions of these hormones in the brain see Vadakkadath Meethal and Atwood *(41)*. Given the normal functions of these hormones in normal brain structure and function it is perhaps not surprising that menopause/andropause-related changes (dyotic signaling) could lead to alterations in the structure and function of the brain during senescence.

## Dysregulation of the HPG Axis

The age-related decline in gonadal reproductive hormone secretion into the bloodstream results in an imbalance of the HPG hormonal axis, leading to menopause in women and andropause in men. The decline in sex steroid production by the gonads following menopause and during andropause leads to a loss of hypothalamic feedback inhibition that stimulates GnRH and gonadotropin production *(42)* (Fig. 1). In addition, the decrease in gonadal inhibin production at this time *(43)* results in decreased activin receptor inhibition, and together with the increase in bioavailable activin *(44)* leads to a further increase in the secretion of GnRH and gonadotropins *(45–47)*. The lack of negative feedback from the ovary (both estradiol and inhibin) is therefore responsible for the unopposed elevation of GnRH and gonadotropins following ovarian senescence; in women there is a 3- to 4-fold and a 4- to 18-fold increase in the concentrations of serum LH and FSH, respectively *(48)*. Likewise, men also experience a greater than 2-fold and 3-fold, increase in LH and FSH, respectively, as their reproductive function deteriorates during andropause *(49)*. Although it has not been possible to analyze GnRH release in humans, seminal and elegant studies performed by Terasawa and colleagues have demonstrated that the pulsatile release of GnRH from the stalk-median eminence (using a push–pull perfusion method) following menopause is greatly elevated (5–10-fold) in rhesus monkeys *(50)*, and is likely responsible for elevated serum concentrations of LH/FSH following menopause and during andropause *(48,51,52)*. Normal serum concentrations of these hormones in the human during adult reproductive life and post-reproductive life in men and women are presented in Atwood et al. *(53)*.

How changes in peripheral circulating hormones related to aging affect signaling for brain structure and function *will be dependent not only on their concentration but also upon receptor expression and changes in receptor affinity.* The expression of these neuronal receptors is modulated by hormones: hippocampal GnRH receptor expression is increased in old rats *(54)* and following castration *(55)*; low concentrations of gonadal steroids and high concentrations of LH such as is seen with menopause/andropause promotes LH/hCG receptor expression in human neuroblastoma cells *(56)*; ovariectomy induces a rapid increase in pituitary activin receptor II mRNAs in rats *(57)*. Ovariectomy and aging also leads to a reduction in hippocampal estrogen receptor in mice *(58,59)* and hippocampal androgen receptor in rats *(60,61)*. Interestingly, FSH receptors have not been detected in neuronal cell types and appear to be confined to reproductive tissues, although it cannot be ruled out that FSH may signal via another (G-protein coupled) receptor.

It is perhaps simplistic to imagine that the alteration in the serum concentration of only one HPG axis hormone is responsible for the entire plethora of changes associated with the aging brain, especially given the presence of receptors for activin, GnRH, LH, as well as the sex steroids, in the brain. Indeed, if the decline in estradiol were solely responsible for dementia, it might be expected that AD-like changes would be observed during the 12–14-yr period prior to pubescence when circulating concentrations of sex steroids are low. This obviously is not the case and suggests other hormones of the axis play a role in AD. Post-reproductive signaling is more akin, but not exactly the same as, the growth and developmental signaling embryonic stem cells receive during the growth and development of the brain (see below).

## The HPG Axis Connection to Alzheimer's Disease

Aside from the logical conclusion that dyotic signaling will impact normal brain structure and function negatively, there is accumulating epidemiological and biochemical evidence that these hormones drive cognitive senescence. Indeed, research into the influence of HPG axis hormones on brain function has largely been driven by epidemiological studies demonstrating gender dependence in the prevalence, symptomatology, and prognosis of AD (2:1 female to male) *(62–64)*. Even taking into account the fact women live longer than men, women appear to have consistently higher age-specific AD death rates *(65)*. Along these lines, one study has shown a higher incidence of amyloid plaques in the brains of women compared with men, together with a greater age-related increase in the incidence rate of plaques *(66)*. This increased prevalence rate in women is consistent with the abrupt earlier loss of gonadal function in females compared with males, and the decrease in serum sex steroids, but increase in serum gonadotropins, at this time *(67)*.

Epidemiological evidence for HPG hormones in mediating AD includes (1) the positive correlation between AD and decreased estrogen levels following menopause in numerous epidemiological studies, i.e., postmenopausal women with higher endogenous estrogen levels (a less dysregulated HPG axis) have a decreased prevalence of AD *(68)*. Likewise, increased serum LH concentrations are negatively correlated with cognition in post-menopausal women, but positively correlated with serum FSH (Rodrigues et al., sub-

mitted). Serum LH and FSH are ~2-fold elevated in individuals with AD compared to age-matched controls *(69–71)*. (2) The decreased incidence *(72)*, and a delay in the onset *(73)*, of AD among women and men on hormone replacement therapy (HRT) following menopause/andropause *(74)*. HRT in postmenopausal women and in men has been demonstrated to improve cognitive and visual-spatial function *(75,76)*. (3) Sex steroids have been shown to be potent neuroprotective agents in a variety of in vivo model systems including fimbria-fornix lesions and middle cerebral artery occlusion *(77–79)*.

Although the recent Women's Health Initiative studies indicate oral estrogens and progestogens are not protective, and by extrapolation that sex steroids do not play a role in AD, it is not possible from these studies to determine if the major female estrogen (17β-estradiol) and progesterone are protective or not. This is because the hormones used in these trials [conjugated equine estrogens (CEE) and medroxyprogesterone acetate (MPA)] are very different in composition and have well-known differences in cell signaling compared to that of the endogenously produced female hormones, 17β-estradiol and progesterone *(80)*, i.e., estrogens extracted from horse urine (predominantly composed of estrone sulfate) are not the major estrogen present in female bodies. Nor is the synthetic progestagin, MPA, synthesized in females; MPA also has very different signaling properties. Interpretation is further complicated by the route of administration and age at which HRT was administered *(80)* and together likely explain the many discrepancies in HRT studies *(81)*. That HRT only reduces the risk of AD among long-term users and only is effective prophylactically may be explained by the temporal changes in the concentrations of GnRH and gonadotropins during the post-menopausal years *(67)*. Immediately following menopause, serum gonadotropin and GnRH levels are extremely elevated but subsequently decline. Thus, early HRT may provide maximal protection against the potentially detrimental effects of these hormones *(67)*. Reasons that the disease is not halted by HRT (CEE/MPA) treatment after clinical diagnosis may include (1) another hormone of the axis, whose concentration is dysregulated following menopause, but whose secretion into the serum is not directly inhibited by HRT, is driving neurodegeneration, or (2) estrone sulfate, the major CEE, may not modulate the serum concentrations of other axis hormones, i.e., gonadotropins and/or GnRH, to pre-menopausal levels. Indeed, CEE's only partially reverse oophorectomy-induced increases in serum gonadotropin levels *(82)*. It is important to note that the only clinical study that has used the natural estrogen 17β-estradiol has shown beneficial effects on cognition *(83)*. Likewise, testosterone-replacement therapy in a small clinical trial in men also has demonstrated improved cognitive and visual–spatial function *(76)*.

These epidemiological studies indicate that dysregulation of the HPG axis is pivotal to cognitive decline, but do not distinguish between which hormones of the axis are involved. It should be noted that HRT leads to partial "resetting" of certain HPG hormones such as the gonadotropins to *approximately adult* levels *(82)*. Therefore, the delay in cognitive decline observed in most studies with HRT could just as easily be explained by the reversal of serum gonadotropins to approximately adult levels, as it can to sex steroid supplementation. Indeed, evidence in support of gonadotropins as primary causative agents is given by the fact mentioned earlier that AD-like changes are not observed during the 12–14-yr period prior to pubescence when circulating concentrations of sex steroids are low. Further evidence that gonadotropins may play a role in AD comes from studies of individuals with Down syndrome who have elevated serum concentrations of gonadotropins throughout life and develop AD-like neuropathology if they live into the fourth decade *(84,85)*. In sharp contrast to the general population, males with Down syndrome develop these neuropathological changes earlier and more often than their female counterparts. The reversal in gender predilection cannot be explained on the basis of sex steroid concentrations as there is no difference between Down syndrome individuals and the general population. However, the increase in gonadotropin concentrations is more pronounced and occurs at an earlier age in Down syndrome males than in Down syndrome females *(86)*. Finally, the incidence of dementia in men who underwent prostatectomy for prostate cancer is significantly lower than control groups that underwent other surgeries [cholecystectomy, herniorrhaphy and transurethral prostatectomy *(87)*]. Five years post-procedure, the relative risk for dementia in the control groups is 1.8- to 2.9-fold increased compared to the prostate cancer group (Medicare Inpatient Database). Because GnRH agonist therapy is only administered to men with prostate cancer (approx 40%), the results imply that suppression of gonadotropins is protective of cognitive function. Because GnRH agonists also suppress serum sex steroids, these results do not support the decline in serum sex steroids as being detrimental for cognitive function.

## Gonadotropins, Gonadopause, and Alzheimer's Disease

There is much evidence that LH, hCG (the fetal LH homolog with 83% homology that binds the same receptor), and GnRH modulate brain function *(41)*. LH is known to cross the blood–brain barrier *(88)* and the actions of LH/hCG are likely mediated by their binding to the LH/hCG receptor, which are expressed throughout the brain *(56,89–91)*. LH/hCG receptor expression (mRNA and protein) has been detected in the adult rat brain with the highest density of gonadotropin receptors being found within the hippocampus followed by the hypothalamus, cerebellum, choroid plexus, ependymal tanycytes of third, fourth, and lateral ventricles, cortex, brain stem, and anterior pituitary *(90)*. No dif-

ference has been detected in the distribution of hCG/LH receptors between male and female brains *(92)*. In the hypothalamus, some LH/hCG receptor–containing neurons are colocalized with GnRH–producing neurons *(90)*. All human neuronal cell types (neurons, astrocytes, glia) studied to date possess LH/hCG receptors *(89)*. Immortalized mouse hypothalamic and hippocampal GT1-7 and HN33p cells, human M17 neuroblastoma cells, as well as gonadotropes, contain LH/hCG receptor *(56,91)*.

LH/hCG are thought to play critical roles in brain development, neuron differentiation, and function *(93)*. Because LH and hCG are heterodimeric glycoprotein hormones that contain a cystine knot structural motif (like nerve growth factor), it is not surprising that they have neurotropic effects *(94,95)*. Rat neurons cultured in the presence of highly purified hCG have been shown to respond in a dose-dependent manner by increasing the outgrowth of neuritic processes and total cellular protein and by decreasing apoptosis *(94–96)*.

Interestingly, the highest LH/hCG receptor expression correlates with those brain regions susceptible to AD neuropathology *(92,96,97)*. Surprisingly, we found that pyramidal neurons, those neurons susceptible to AD neuropathology, contain intra-cytoplasmic LH and that these pyramidal neurons contained significantly greater immunoreactivity in AD brains compared with age-matched control brains *(98)*. These results have been confirmed in the aging rat, where LH was recently reported localized to pyramidal neurons of the cerebral cortex and hippocampus of 1- and 2-yr-old rats, but not in young adult (3–5 mo) rats *(99)*. This increase in LH immunoreactivity in the aging brain may be due to increased sequestration of LH from extracellular sources (e.g., blood), decreased intracellular LH degradation, and/or the intriguing possibility that extra-pituitary neurons, like fetal and cancer cells, are capable of synthesizing LH.

### *LH and Cell Division*

The expression of membrane-associated LH, hCG, and their subunits and fragments is a characteristic of cancer cells, and has been demonstrated on numerous human cancer cell lines of different types and origins *(100)*. These findings were corroborated by the presence of translatable levels of hLHβ and hCGβ mRNAs, indicating that the expression of these membrane-associated glycoproteins is a *phenotypic characteristic of human cancer cells.* Indeed, hCG is a common serum marker used to determine the progression or regression of cancer following chemotherapy *(101)*. It is important to note that the presence of translatable levels of hCGβ/hLHβ mRNAs in cultured human fetal cells illustrates the in vivo and in vitro biochemical similarities between fetal and cancer cells *(100)*. It has been well demonstrated that LH/hCG have powerful mitogenic properties in certain reproductive tissues *(102–106)*, and are frequently expressed by tumor cells *(100,107,108)*. Overexpression of LH or hCG increases tumorigenesis potential in the gonads *(109)* and placenta *(110)* and is a major marker in humans of cancer progression. Restoration of LH levels after testosterone and estrogen-induced LH deprivation leads to increased proliferation of Leydig cells in the testis of adult rats *(111)*. Likewise, LH levels are associated with increased T-cell proliferation and activation *(112,113)* and peripheral blood lymphocyte proliferation *(114)*. Finally, LH secretion has been associated with increased proliferation of granulosa cells and activation of MAPKs such as ERK *(115–117)*, and other signal transduction and transcription activators *(118)*; all of which play a major role in cell cycle events *(119)* and, importantly, are associated with AD pathology *(120–124)*.

These results suggest that neurons of the AD brain may be developing characteristics of neoplastic cells. Indeed, there is now much evidence that AD is a disease of aberrant, albeit unsuccessful, re-entry of neurons into the cell cycle resulting in synapse contraction and neuron death (see ref. *53* for an updated review). In this regard, overexpression of wild-type AβPP *(125–127)* and expression of familial AD (FAD) mutants of AβPP *(127–129)* induces apoptosis in primary neurons and cell lines. Recently, Neve and colleagues demonstrated that this cell death in differentiated primary neurons overexpressing wild-type or FAD mutant AβPP was due to the re-entry of neurons into the cell cycle, as demonstrated by the induction of DNA synthesis and cell cycle markers *(130,131)*. Activation of the cell cycle in differentiated neurons is known to promote apoptosis *(132)*. These workers also showed that DNA synthesis occurred prior to neuron death by apoptosis *(131)*. Our recent data showing that Aβ generation occurs only once a cell commits to apoptosis, and not before (Verdile et al., submitted), supports previous evidence showing Aβ generation is associated with neuron death *(133,134)* and explains the well characterized increase in Aβ generation in neurons harboring FAD mutations *(8)*. Taken together, these results suggest that factors that affect AβPP expression, processing, or function not only act to promote cell cycle re-entry of differentiated neurons, but also to promote Aβ generation when a neuron commits to apoptosis. Indeed, it is becoming apparent that Aβ generation is a sensitive marker of neuron re-entry into the cell cycle. An understanding of factors that promote the amyloidogenic processing of AβPP would therefore be candidates for understanding the cause of AD.

### *LH and AβPP Metabolism*

We have recently reported molecular evidence that gonadotropins modulate the processing of AβPP and the generation of Aβ in vitro and in vivo *(56)*. Treatment of C57/Bl6 mice with the GnRH agonist leuprolide acetate, which suppresses the serum concentrations of both sex steroids and gonadotropin, reduced total brain Aβ1-42 and Aβ1-40 concentrations by 3.5-fold and 1.5-fold, respectively, after 2 mo of treatment. These results indicate that alterations in

AβPP processing were due to the suppression of LH *(56)* because decreased brain Aβ followed treatment with leuprolide acetate (i.e., ablation of LH and sex steroids) but not following suppression of sex steroids alone *(135,136)* with ovariectomy, which results in a well-documented increase in serum LH and FSH concentrations in the mouse *(14)* and other species. Moreover, we have recently shown that suppression of gonadotropins with leuprolide acetate improves cognitive performance and decreases amyloid-β deposition in aged transgenic mice carrying the Swedish AβPP mutation *(137)*. Supporting data from studies on human neuroblastoma cells indicated that LH promotes AβPP processing toward the amyloidogenic pathway as evidenced by the increased generation and secretion of amyloid-β, decreased secretion of AβPP, and increased AβPPCT100 production *(56)*. In this regard, the expression of presenilin-1 and -2 gene, members of the γ-secretase complex involved in the processing of AβPP, have recently been shown to be upregulated by gonadotropins in human granulosa cells *(138)*, although we did not observe a similar increase in presenilin-1 expression following LH treatment in neuroblastoma cells *(56)*. Although LH alters AβPP processing, it has no affect on AβPP expression.

Previous in vitro and in vivo studies that have modulated the levels of sex steroids either with treatment or castration have implied that sex steroids alter neuronal AβPP processing toward the non-amyloidogenic pathway. 17β-Estradiol at concentrations between 2 and 2000 n*M* certainly increases sAβPPα production and reduces the generation of Aβ in both mouse and human cell lines and primary cultures of rat, mouse, and human embryonic cerebrocortical neurons *(135, 139–141)*. Likewise, testosterone treatment (200–2000 n*M*) of mouse neuroblastoma cells and rat primary cerebrocortical neurons increases secretion of sAβPPα and decreases the secretion of Aβ *(136)*. Ovariectomy, which suppresses serum estrogen levels, also has been shown to increase total Aβ concentrations in guinea pigs *(142)* and AβPP transgenic mice *(143)*. Conversely, 17β-estradiol treatment was shown to partially and totally reverse the effects of ovariectomy in guinea pigs *(142)* and AβPP transgenic mice [Tg2576 and Tg2576 x mutant PS1 *(143)*], respectively. However, because physiological concentrations of serum estrogen are no greater than 1 n*M* in women and serum testosterone concentrations are no greater than 35 n*M* in men *(144)*, the interpretation of these results is difficult. This is because physiologically relevant concentrations of 17β-estradiol (1 n*M*) decrease the expression of LH/hCG receptor in neuroblastoma cells, and it is likely that the high concentrations of sex steroids used in previous in vitro studies (approx 2–2000 n*M* 17β-estradiol and approx 200–1000 n*M* testosterone) downregulated neuronal gonadotropin receptors. Thus, suppression of LH/hCG receptors by high concentrations of estrogen in vitro would have the same effect as elevated serum estrogen in vivo, suppressing gonadotropin signaling and diverting AβPP processing to the nonamyloidogenic pathway with a resultant decrease in Aβ generation. In support of estrogens affect being dependent upon LH, Manthey and colleagues have reported for mouse hippocampal HT22 and human neuroblastoma SK-N-MC cells that the enhancement of cellular sAβPPα release was independent of estrogen receptor expression *(141)*. Therefore, the marked increases in serum LH following menopause/andropause may be a physiologically relevant signal that could promote Aβ secretion and deposition in the aging brain.

### *LH and Tau Phosphorylation*

Although there is no direct evidence that LH promotes tau phosphorylation, LH has been shown to increase both the expression and kinase activity of Cdk5, an enzyme known to hyperphosphorylate tau in Leydig TM3 cells *(14)*. Supporting LH-induced Cdk5 expression, a significant decrease in Cdk5 expression and activity has been noted in rat testis after hypophysectomy *(145)*. Recently, LH has been shown to modulate the expression of numerous genes involved in cytoskeletal organization *(117)*.

17β-Estradiol has been demonstrated to increase the expression of tau and microtubule associated protein-2 (MAP-2) in pituitary lactotrophs of adult female Wistar rats, and promote their association with the membrane rough endoplasmic reticulum *(146)*. Similar expression results were not found in the hippocampus and the frontal cortex of ovariectomized adult rats for tau, although MAP-2 expression increased in the hippocampus *(147)*. While the expression of tau is regulated by 17β-estradiol, 17β-estradiol does not prevent the heat shock–induced hyperphosphorylation of tau in rats *(148,149)*. In this vein, GSK3β, one of the main kinases that phosporylates tau *(37)*, is transiently activated by 17β-estradiol, leading to increased tau phosphorylation in the adult female rat hippocampus and in cultured hippocampal neurons *(150)*. Following this activation, however, there is a sustained inhibition of GSK3β and a decrease in tau phosphorylation in neurons *(150)*. In this respect, 17β-estra-diol has been shown to increase the binding of GSK-3β with tau *(151)* and beta-catenin, and elements of the PI3 kinase complex *(150)*. Cardona-Gomez and colleagues also demonstrated a novel complex between estrogen receptor alpha, GSK3, and beta-catenin, where the presence of 17β-estradiol removed beta-catenin from the complex. Conversely, testosterone does prevent the heat shock–induced hyperphosphorylation of tau in male rats *(148,149)* by inhibiting the heat shock–induced overactivation of GSK-3β *(152)*. Neither gonadal steroid affects tau dephosphorylation. However, these studies, like those on AβPP, are difficult to interpret because sex steroids are known to modulate receptor levels for the other HPG axis hormones, especially the gonadotropin receptors *(56,153)*.

### *LH and Mitochondria*

Changes suggestive of mitochondria replication have been reported in pyramidal neurons, those vulnerable to AD neu-

ropathology *(154)*. Pyramidal neurons of the AD brain contain threefold elevated levels of cytoplasmic mitochondrial DNA and increased COX-1 expression, indicative of *de novo* mitochondrion synthesis that would be expected during cell division to meet the energy demands of the newly created daughter cells *(155)*. Unlike division-competent neurons, it remains to be determined if such alterations in mitochondrial metabolism in differentiated neurons are responsible for an imbalance in energy metabolism (as observed in the AD brain). Interestingly, COX-2 expression and prostaglandin synthesis in granulosa cells are upregulated by LH/hCG *(156)*. It will be interesting in future studies to determine if gonadotropins have a role in mitochondrial biogenesis besides their well-known role in mitochondrial steroidogenesis.

## GnRH and Alzheimer's Disease

As mentioned previously, the GnRH agonist leuprolide acetate decreases the Aβ protein (a marker of AD neuropathology). However, despite its anti-gonadotropin effects, this GnRH analog also is known to bind to GnRH receptors present throughout the limbic system of the brain (see ref. *41* for a review). Although the concentration of circulating GnRH is very low due to its short half-life *(157,158)*, GnRH signaling via the GnRH receptor in extrapituitary tissues *(165)* of the brain appears to play an important role in neurotransmission. Experiments using leuprolide acetate have demonstrated that activation of GnRH receptors using leuprolide acetate induces a long-lasting enhancement of synaptic transmission mediated by ionotropic glutamate receptors in CA1 pyramidal neurons of rat hippocampal slices *(159,160)*. This suggests that GnRH can increase the intrinsic neuronal excitability of CA1 (and CA3) pyramidal neurons in the hippocampus, an important integrative region for reproductive process, both endocrinologically and behaviorally. The high densities of GnRH receptor in the limbic system in areas concerned with the regulation of behavioral functions *(161)* help explain changes in sex behavior in rats and other species following peripheral or central injections of GnRH *(162–164)*.

## Conclusion

There is now sufficient evidence to indicate that the changes in HPG hormonal signaling with menopause/andropause (*dyotic signaling*) promote biochemical changes similar to those that characterize the AD brain. In particular, LH promotes biochemical and cellular changes consistent with the neurodegenerative changes observed in the AD brain. These findings support the premise that GnRH agonists could be a potential therapeutic agent for AD. Double-blind placebo-controlled phase II clinical trials are currently underway to conclusively make this determination.

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