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Gonadotropin-releasing hormone receptor system: modulatory role in aging and neurodegeneration

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Abstract

Receptors for hormones of the hypothalamic-pituitary-gonadal axis are expressed throughout the brain. Age-related decline in gonadal reproductive hormones cause imbalances of this axis and many hormones in this axis have been functionally linked to neurodegenerative pathophysiology. Gonadotropin-releasing hormone (GnRH) plays a vital role in both central and peripheral reproductive regulation. GnRH has historically been known as a pituitary hormone; however, in the past few years, interest has been raised in GnRH actions at non-pituitary peripheral targets. GnRH ligands and receptors are found throughout the brain where they may act to control multiple higher functions such as learning and memory function and feeding behavior. The actions of GnRH in mammals are mediated by the activation of a unique rhodopsin-like G protein-coupled receptor that does not possess a cytoplasmic carboxyl terminal sequence. Activation of this receptor appears to mediate a wide variety of signaling mechanisms that show diversity in different tissues. Epidemiological support for a role of GnRH in central functions is evidenced by a reduction in neurodegenerative disease after GnRH agonist therapy. It has previously been considered that these effects were not via direct GnRH action in the brain, however recent data has pointed to a direct central action of these ligands outside the pituitary. We have therefore summarized the evidence supporting a central direct role of GnRH ligands and receptors in controlling central nervous physiology and pathophysiology.

Keywords

Gonadotropin releasing hormone; hypothalamic-pituitary-gonadal axis; brain; GnRH receptors; Alzheimer's disease; amyloid precursor protein; neuron

INTRODUCTION

Hypothalamic gonadotropin-releasing hormone (GnRH) is a decapeptide that plays a crucial role in the regulation of reproduction as well as controlling many other functions related to reproductive activity outside the hypothalamic-pituitary gonadal (HPG) axis [1]. GnRH is released in synchronized pulses from nerve endings into the hypophyseal portal system every 30–120 minutes to stimulate the biosynthesis and secretion of luteinizing hormone (LH) and

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follicle-stimulating hormone (FSH) from pituitary gonadotropes. GnRH-I (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂) was the first GnRH isoform discovered in the mammalian brain. At least two, and usually three, forms of GnRH are present in most vertebrate species. Amongst these, is a form originally isolated from the chicken, *i.e.* chicken GnRH II (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH₂) that was found to be universally present and uniquely conserved between from jawed fish to humans [2–6]. With respect to the effects of GnRH and its analogues upon the central nervous system (CNS), considerable evidence has accumulated for a role of LH in promoting alterations in neurophysiological activity. For example, epidemiological support for a role of LH/GnRH in Alzheimer's disease (AD) is evidenced by a reduction in neurodegenerative disease among prostate cancer patients receiving GnRH agonistic therapy [7,8]. We will discuss the evidence supporting the role of the GnRH hormonal receptor system in modulating the biochemical, pathological, and cognitive changes associated with aging and age-related neurodegenerative disorders. An examination of the potential widespread actions that this traditional reproductive hormone exerts may lead to the generation of novel therapies and provide fresh insight into the therapeutic tackling of complex disorders that affect multiple aspects of peripheral and central nervous tissue function.

HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN AGING-RELATED PHYSIOLOGY

In humans, aging is inexorable and is the eventual summation of multiple interacting physiological or pathophysiological effects. “Aging” and the related word “senescence” are commonly used to refer to post-maturational processes that lead to diminished homeostasis and increased organismic vulnerability. This is typically associated with a progressive decline in physical and cognitive functions. There are at least five notable supporting common characteristics of aging in mammals: increased mortality with age after maturation; changes in biochemical composition in tissues with age; progressive decrease in physiological capacity with age; reduced ability to respond adaptively to environmental stimuli with age and an increased susceptibility and vulnerability to disease [9]. Age-related disorders can be separated into those that develop through normal physiological processes (and are often universally presented to some extent in aged populations), *e.g.* menopause or the decline in renal function, and those that are associated with age-related pathophysiological aging, *e.g.* AD. Alzheimer's disease is an example of pathophysiological aging and is therefore not universal to all elderly people. This approach to aging can utilize a conceptual framework that identifies intrinsic (developmental-genetic) versus extrinsic (stochastic) causes. Accumulating evidence increasingly stresses the impact of age-dependent endocrine changes on the dynamics of neuronal behavior, neurodegeneration, cognition, biological rhythms, sexual behavior, and metabolism. Disrupted metabolic homeostasis in the elderly is likely exacerbated by age-dependent molecular alterations in components of signal transduction pathways and declining production of sex steroids, which determines the response to exogenous influences and thereby increases the predisposition to illness and death. Attempts at understanding the causes of aging have been limited by its complexity. However, considerable evidence has suggested that reproductive activity is a major pacemaker of aging and death. A putative integrative site for these regulatory actions is the neurosecretory system, particularly the HPG axis [10,11]. In humans, reproduction is controlled primarily by the HPG axis hormones, found in both central and peripheral locations. Classically, the prime source of GnRH is considered to be the hypothalamus, where it is released into the hypophyseal portal system to activate GnRH receptors on pituitary gonadotropes, to facilitate the synthesis and release of LH and FSH. GnRH can also be produced and act peripherally in many reproductive tissues (prostate, breast, gonads) and non-reproductive tissues (pancreas, immune system) [12–14]. These peripheral organs, and especially the gonads, also generate important HPG feedback hormones such as the sex steroids themselves, inhibins, activins and follistatin. The levels of each of these

hormones are regulated by multiple complex feedback loops between the gonads, and the hypothalamus and anterior pituitary [15].

The HPG axis undergoes a number of changes throughout the reproductive lifecycle that are responsible for the development, pubertal changes, and eventual senescence of reproductive systems. While the ligand members of the HPG axis are the direct regulators of reproduction, each member of the axis is also likely impacted by other hormonal factors that would be altered under adverse or favorable reproductive and aging conditions. During this natural progression, levels of HPG axis hormones and reproductive tissue activity can be modulated by higher neuronal activities, and in turn also affect these neural networks via feedback loops. Age-related declines in reproductive function results in an imbalance of this hormonal axis, and lead to menopause- and andropause-related pathophysiology in the central nervous system (CNS) and periphery. Eventually, reductions in gonadal sex steroid production lead to a disruption of hypothalamic feedback inhibition, disturbing normal GnRH and gonadotropin production. In women, the loss of this negative feedback by estrogen and inhibins [16] results in significant and long-lasting increases in serum LH and FSH levels [17,18]. Post-menopause gonadotropin concentrations eventually decline but never decrease to levels seen during the reproductive period. Men experience a more gradual and minimal loss of reproductive function, with a corresponding progressive increase in gonadotropin levels. This ultimately leads to a greater than 2- and 3-fold increase in LH and FSH levels, respectively [19]. Following reproductive life, menopause/andropause-mediated changes in serum and neuronal concentrations of HPG axis hormones may significantly alter neuronal signaling mechanisms. It is difficult to ascribe structural and functional changes during development, adulthood and senescence to a single HPG hormone, but the imbalance effects of this altered neuronal signaling on the structure and function of the brain is likely to be connected the development of pathophysiology in multiple neurodegenerative disorders.

DISRUPTION OF HYPOTHALAMIC-PITUITARY-GONADAL AXIS HORMONES IN THE CENTRAL NERVOUS SYSTEM

Epidemiological and biochemical studies have indicated an association between hormones of the HPG axis and cognitive senescence. For example, changes in HPG hormones following menopause/andropause are involved in the cognitive and neuropathological changes observed in familial AD. Aging-mediated increases in neuronal LH have been associated temporally with the increase of neuronal populations at risk of degeneration and death. Elevations in LH parallel the ectopic expression of cell cycle alteration and oxidative markers, which can precede neuronal degeneration by decades [20,21]. LH also can promote reactivation of mitotic signaling pathways shown to occur early in Alzheimer's pathogenesis [22–24]. The isolation of several forms of GnRH in neural tissue of tunicates and their activation of the gonads [25, 26], suggests that direct regulation of the gonads evolved before the development of a neuroendocrine role in mammals regulating both the pituitary and gonads. Neurons are probably one of the earliest cells in evolution to synthesize and secrete GnRH peptides. Interestingly, the major CNS biochemical and neuropathologic changes reported for AD, *e.g.* alterations in Amyloid Precursor Protein (APP) metabolism, Amyloid- β ($A\beta$) deposition, tau phosphorylation, mitochondrial alterations, chromosomal replication, synapse loss, death of differentiated neurons, may all be the combined result of increased mitotic signaling by gonadotropins and GnRH, decreased differentiative and neuroprotective signaling via sex steroids, and increased differentiation signaling via activins.

Multiple cell surface receptors for hormones of the HPG axis, including the GnRH receptor, that regulate reproductive function are expressed throughout the brain, and in particular the limbic system and hippocampus, two sets of neurons which are vulnerable to AD pathology. Changes in receptor expression and concentration of peripheral circulating hormones related

to aging affect their signaling transduction, as well as neuronal structure and function. Hippocampal GnRH receptor expression has been shown to be increased in old rats [27], and also after castration, which partially mimics age-dependent reproductive decline [28]. LH is known to cross the blood-brain barrier and LH receptors are expressed in the brain with the highest expression in brain regions susceptible to AD neuropathology [23]. Additionally, aging also leads to a reduction in hippocampal estrogen or androgen receptor expression in mice and rats [29–32]. Evidence supporting a role for gonadotropins in the etiology of AD includes the two-fold elevation in serum gonadotropin concentrations in AD patients, compared to age-matched control subjects [33,34]. Epidemiologic studies indicating a female predominance of the disease (2:1, female:male) are also consistent with the earlier loss of reproductive function, and an earlier increase in serum gonadotropin concentrations in women [35–37]. Based on these epidemiological and biochemical studies, steroidal hormone-replacement therapy was, until recently, viewed as a major factor in the prevention of AD. However, a recent randomized clinical trial revealed that estrogen hormone replacement therapy may actually exacerbate the incidence of dementia, when administered to elderly women [38]. These contradictory reports have cast doubt on the role of estrogen in disease pathogenesis and led us to consider an alternate hypothesis that would be consistent with both observations. Specifically, we suspect that hormones of the hypothalamic pituitary gonadal axis, such as gonadotropins, as well as GnRH, are involved in the pathogenesis of AD. For example, LH is significantly elevated in both the sera and brain tissue of patients with AD and leads to an increased production of $A\beta$, one of the toxicity-inducing factors of AD. Distribution and expression levels of neuronal receptors for LH corresponds to the populations of neurons that degenerate during the course of this disorder. Evidence that pathogenesis can be mediated outside of gonadal steroids has led to a therapeutic shift to GnRH-based therapies, not only for the treatment of AD, but also for a wide variety of other aging-related disorders.

GnRH and GnRH RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

GnRH neurons are distributed in a loose array along the ventral medial forebrain from the posterior olfactory bulbs to the arcuate nucleus. GnRH neurons are found in the vicinity of the olfactory placode during prenatal development, after which GnRH-producing cells migrate through the nasal system into the forebrain [1,39,40]. Therefore, differences in GnRH-producing areas within the brain appear to result from greater or lesser penetration along the olfactory-forebrain-hypothalamus continuum to the median eminence. The GnRH family currently includes 25 isoforms, 14 and 11 from representative vertebrate and invertebrate species, respectively [41]. To date, three forms of GnRH (GnRH I, II and III) has been identified in many vertebrates (*e.g.* bony fish and amphibians), but in reptiles, birds and mammals only GnRH I and II are apparently present in addition to a truncated form of GnRH I [42]. Therefore, all vertebrates possess a functional second major ligand in the GnRH system, also known as chicken GnRH-II (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH₂), due to its initial species isolation. As we have mentioned previously, the primary amino acid sequence of GnRH-II has remained unchanged from cartilaginous fish through mammals, which would suggest an immensely strong selective pressure for its conservation. Surprisingly however, its specific molecular physiological role in the CNS is currently poorly understood [41,43,44]. The expression pattern of GnRH-II varies from species to species, but is usually highly expressed in the brain and then to a lesser extent in numerous peripheral reproductive and non-reproductive tissues [41,45]. In humans and primates, GnRH-II is particularly abundant in the caudate nucleus, hippocampus and amygdala [46]. GnRH-II expression in extrahypothalamic regions is often not coincident with expression of the GnRH-I peptide, suggesting unique regulation and functional activities for this conserved peptide. As a caveat to this assumption, there are species though in which GnRH-II is found in the hypothalamic regions and acts at the pituitary for stimulating gonadotropin function [47]. It has been repeatedly proposed

however that GnRH II has a neurotransmitter-neuromodulatory role, based on the wide distribution of GnRH II in the central and peripheral nervous system [48,49].

The actions of GnRH in mammals are mediated by stimulation of the Type I GnRH receptor (GnRHR), which belongs the rhodopsin-like G protein-coupled receptor (GPCR) superfamily. In earlier-developed species, such as sea-squirts, up to three different GnRH receptor isoforms exist. This diversity progressively reduces with more recently evolved animals, even those very closely related in time. For example, humans possess only one functional type of GnRH receptor (Type I) while small pro-simians, such as marmosets, possess two functional GnRH receptors (Type I and Type II receptors). The mammalian Type I GnRH receptor is unique among rhodopsin-like GPCRs, as it has a relatively short third intracellular loop, and lacks an intracellular carboxyl terminus (C-terminus). This C-terminus deletion facilitates the pre-ovulatory LH surge in mammalian reproduction and prevents rapid agonist-induced receptor internalization [50,51]. In rhodopsin-like GPCRs, this C-terminus typically acts as a substrate for G protein-coupled receptor kinase phosphorylation that initiates the desensitization process of generic rhodopsin-like GPCR, such as the beta-adrenoceptor. GnRH-mediated activation of the Type I GnRHR typically induces $G_{\alpha q}/G_{11}$ GDP-GTP exchange, which then stimulates increased phosphoinositol turnover by activating phospholipase C (PLC). This enzyme leads to the generation of several second messengers [52,53], among these, diacylglycerol (DAG) and inositol 1, 4, 5-tris-phosphate (IP3) are critically important. DAG leads to activation of protein kinase C (PKC), and IP3 releases Ca^{2+} from intracellular pools [54]. Both events, in the anterior pituitary gonadotrope cells, result in gonadotropin synthesis and release [55,56]. In recent years the true range of GnRH signal transduction pathways that are elicited upon receptor activation has been dramatically expanded. Ligand activation has been shown to mediate: increases in cAMP [57], activation of multiple members of the mitogen-activated protein kinase (MAPK) family [48,58], inhibition of glycogen synthase kinase-3 [59], stimulation of the non-receptor tyrosine kinases including c-Src [60] and proline-rich tyrosine kinase 2 (Pyk2) [61], and also diacylglycerol kinases [62].

The presence of GnRH II in all vertebrates initially suggested the probable existence of cognate Type II GnRH receptors. This was supported by the presence of GnRH receptors in amphibian sympathetic ganglia that were selective for GnRH II [63]. It was thought that only non-mammalian GnRHRs possessed functional C-termini until the discovery of a mammalian Type II GnRH receptor with a functional C-terminus [48]. However, as yet, a human Type II receptor that selectively responds to GnRH II has not been cloned. A human type II receptor pseudogene homolog carries a frame-shift and a premature stop codon [48,64]. It is likely that the function of the non-mammalian Type II GnRH receptors has become subserved by the Type I receptor in humans thus creating the potential for differential ligand activation of this GPCR [58]. The expression of the Type I GnRHR in multiple cellular contexts has, in-part, revealed that signaling diversity for GnRH I at the Type I receptor can be created.

COMPLEX SIGNALING PATTERNS THROUGH THE GnRH RECEPTORS

With respect to the signaling output from GnRH receptors, it is clear that at the level of the G protein the Type I receptor can interact with multiple G-proteins such as $G_{\alpha q}/11$, $G_{\alpha i}$, and $G_{\alpha s}$ in order to mediate various biological actions. A 'cycle of signaling' that may decode a GnRH pulse can already be detected at the G-protein level. GnRH increases the mRNA levels of RGS2 (regulators of G-protein signaling), a large family of proteins that modulate G-protein activity by interacting with active G_{α} -subunits to accelerate their intrinsic GTPase activity and limit their half-life [65]. On the other hand, GnRH activates PKC, which can then phosphorylate RGS2 leading to its inhibition and hence may reduce the deactivation by RGS2 of the G_{α} -subunits. In addition to interaction with multiple G proteins, the Type I GnRHR can also interact functionally with other receptor systems such as the androgen receptor [66] and in a manner

reminiscent of the prototypic GPCR, the β 2-adrenoceptor, with the epidermal growth factor receptor [67–70]. Indicative of the pluripotent signaling nature of the Type I GnRHR, GnRH receptor stimulation may mediate different, even opposite, responses depending the expression context of the receptor, as well as the agonistic stimulation mode, *e.g.*, signal responses can be qualitatively diverse at different doses. It has been shown that pulsatile GnRH stimulates more sustained extracellular signal-regulated kinase (ERK) activity (more than 8h), whereas continuous infusion of gonadotrope α T3-1 cells with GnRH stimulates short-term (2h) ERK activity [71]. Also, GnRH treatment can stimulate cAMP production at nanomolar concentrations, but has an inhibitory effect at micromolar concentrations [72]. It should be pointed out that the nanomolar concentration range (0.01–1nM) corresponds to the physiological circulating level, and the effects caused by this dose range may represent the physiological functions of GnRH [73,74]. At the low concentrations (0.1–10nM) GnRH stimulates cell proliferation, migration and invasion in a dose-dependent manner whereas high concentrations (100nM to 1 μ M) inhibit these functions [75–77]. Moreover, the same dose of GnRH can elicit completely opposite responses in cells derived from the same tissue. In two human ovarian cancer cell lines OVCAR-3 and SKOV-3, GnRH-I and GnRH-II induce invasion of OVCAR-3 cells, but inhibit the invasiveness of the SKOV-3 cells [76]. Similar differences have been found in the effects of GnRH on cell proliferation and cell migration in the prostate carcinoma cell lines TSU-Pr1 and DU-145 [78]. The observation that GnRH-I and GnRH-II have no significant effects on cell lines with type I GnRHR depletion, indicates that the type I GnRHR is indispensable for the effects of both GnRH-I and GnRH-II [76,78].

Functional differences of GnRHR output may, in-part, be due to the fact that the receptor interacts with different G-proteins and other signaling factors in a ‘cell context’-dependent manner, potentially a generic facet of GPCR signal transduction activity [79]. As we have described, GnRH actions have been shown to be mediated by coupling to different $G\alpha$ -proteins, depending on the mode of ligand exposure [70,78]. In general, it has been noted that $G\alpha_q$ and $G\alpha_s$ are associated with GnRH stimulatory effects [72], whereas $G\alpha_i$ often mediates the antiproliferative and proapoptotic effects of GnRH [81–83]. Low GnRH concentrations appear to primarily promote the coupling of GnRHR to $G\alpha_s$ [72]. It has also been demonstrated that peptidergic GnRH analogs can exert direct anti-proliferative actions upon reproductive tissues, in which $G\alpha_i$ stimulation has been implicated [84–87]. In addition to peptide GnRH analogs and GnRH-I, the role of GnRH-II as an autocrine growth inhibitor has also been demonstrated. Like GnRH-I, treatment with GnRH-II *in vitro* inhibits the proliferation of both non-tumorigenic and tumorigenic ovarian surface epithelial cells in a dose- and time-dependent manner [12,88]. These findings suggest that the intracellular milieu in different tissues can result in differential coupling and diverse phenotypic signaling effects, potentially through the creation of unique substates of the Type I GnRH receptor [58,79].

NEUROPHYSIOLOGICAL ACTIONS OF GnRH

GnRH acts not only within the pituitary gonadotropes, but also exerts receptor-mediated effects in placenta, gonads, immune cells, breast, prostate, as well as neurotransmitter and neuromodulatory actions in the central and peripheral nervous system [12,89–98]. With respect to the pathophysiology of cognitive disorders such as AD, that often originate in the hippocampus, it is interesting to note that the hippocampus is one of the CNS regions with the higher levels of GnRH receptor expression. The hippocampus is one of the most important integrative central nervous regions for cognitive, endocrinological and behavioral processes [99,100]. Hippocampal pyramidal neurons demonstrate immunoreactive GnRH receptors and GnRH has been detected in human hippocampus extractions [101,102]. Activation of GnRH receptors can induce a long-lasting enhancement of synaptic transmission, mediated by ionotropic glutamate receptors in CA1 pyramidal neurons of rat hippocampal slices. GnRH potentiates the intrinsic neuronal excitability of both hippocampal CA1 and CA3 pyramidal

neurons in the hippocampus. GnRH-mediated hippocampal neuron activation has also been shown to be profoundly modified by estrogen levels in the rat [103]. The effects of elevated post-menopausal GnRH, due to the loss of estrogen negative feedback [104], upon hippocampal neurons may constitute a component of the neurodegenerative pathology that accompanies AD [99]. GnRH not only affects the excitability of hippocampal neurons but also has the potential to regulate the excitability of cortical neurons which are also crucially involved in learning and memory. Low levels of GnRH have been reported in the human cortex [105], in addition, the splicing intermediate of mature GnRH mRNA, which still contains intron A, has also been detected in the rat cortex [106,107]. GnRH receptor immunoreactive neurons in the cerebral cortex are widespread, suggesting that GnRH may act as common neuromodulatory peptide for multiple signaling tracts in the CNS [108,109]. In the rat, GnRH depresses the activity of cortical neurons [110,111] and has been shown to affect neurite outgrowth and neurofilament protein expression in cultured cortical neurons [112]. In this regard, it will be interesting to examine the additional structural variants of the GnRH family (GnRH II and GnRH III) with respect to their role in modulating structure and function in the brain. The complete and universal conservation of GnRH II's amino acid sequence for more than 500 million years suggests that GnRH II may mediate functions vital for most forms of multicellular life. GnRH II appears to have a variety of reproductive and non-reproductive functions. [113,114]. The wide distribution of GnRH II in the central and peripheral nervous systems suggests a neurotransmitter/neuromodulatory role. The first studies of the neuronal actions of GnRH II demonstrated the inhibition of the KCNQ channel (M-current) in the bullfrog sympathetic ganglion which sensitizes neurons to depolarization [115]. In nutritionally compromised musk shrews or female marmosets, GnRH II stimulates reproductive/sexual behavior while interestingly GnRH I is inactive, suggesting that GnRH II is specific for the neurological effects related to behavior [114]. In addition to its role as a generalized neuromodulator in the nervous system, GnRH II is also present in non-neural reproductive tissues, such as the prostate [116]. GnRH binding sites and antiproliferative effects of GnRH analogues have been described in reproductive tissue tumors and their cell lines [58,117]. Interestingly the GnRH binding sites, signaling and pharmacological effects of GnRH ligands in many peripheral reproductive tissues are distinct from typical pituitary Type I GnRH receptors, but show a greater functional similarity to Type II GnRH receptors from other species [48,58]. However as we have described, a full-length Type II GnRH receptor is absent from man, chimpanzee, cow, horse, sheep, rat and mouse [48,118]. The genetic lack of a functional Type II GnRH receptor in specific species is tolerated pharmacologically by the accommodation of GnRH II signaling through a modified 'allotype' Type I GnRH receptor, with a distinctly different pharmacological profile [58,119]. This functional modulation is made by the forced and selective interaction of the receptor in certain tissues with distinct signal transduction systems to those that interact stably with the Type I GnRH receptor in the pituitary. The selective stimulation of this differential type of GnRH receptor can be achieved through the creation of GnRH analogs by extensive chemical alterations of the GnRH I backbone [58,119].

GnRH AND ALZHEIMER'S DISEASE PATHOPHYSIOLOGY

AD is the most common form of dementia in elderly people. Familial AD is characterized by a marked decline in memory and cognitive performance, including deterioration of language, as well as defects in visual and motor coordination, and eventual death [120,121]. AD involves a loss of neurons, beginning in the entorhinal cortex and later spreading to the neocortex [122]. AD is not only the predominant cause of senile dementia, but is also the most prevalent neurodegenerative disease worldwide [121]. At the molecular level, familial AD is characterized pathologically by the occurrence of intracellular neurofibrillary tangles rich in tau protein and extracellular plaques containing β -amyloid ($A\beta$) peptides [123,124]. These amyloid plaques and neurofibrillary tangles accumulate first in the hippocampal and cortical

regions of the brain [125;126]. In addition, mice transgenically overexpressing A β , or containing genetic mutations that enhance A β aggregation, show many of the symptoms of AD [127–130]. A β is the product of serial cleavage of the amyloid precursor protein (APP), first by β and then by γ secretases, to yield A β peptides of varying lengths, predominantly the 37-, 40-, and 42- residue forms. An increasing ratio of the full-length, 1–42 peptide to the 1–40 form is associated with disease [124], and mutations underlying familial forms of AD either increase this ratio or increase the amount of A β secreted [131]. A β peptides belong to a class of natively unfolded proteins, and as a consequence can adopt a wide variety of tertiary and quaternary structures *in vivo* and *in vitro*, including monomers, oligomers, and fibrils [132, 133]. However, other naturally occurring oligomeric forms of A β are also toxic [133,134], and evidence is accumulating that the capacity of A β , mutant A β , or fragments of A β to aggregate into oligomers is directly related to toxicity [131]. In this regard, the leading hypothesis is that amyloid- β deposition causes the disease [135] since familial forms of AD, resulting from mutations in either the amyloid- β protein precursor or presenilins-1/2, all affect the processing of amyloid- β [136]. However, since perturbation of these elements in cell or animal models does not fully result in the multitude of biochemical and cellular changes found in the human disease [137–139], it is evident that other factors are also involved. In fact, there is now considerable evidence indicating that amyloid- β may be a consequence rather than causative factor in disease pathogenesis [139–141]. Aside from the classical amyloid hypothesis, other theories of AD etiology include: tau phosphorylation; accumulated oxidative stress; mitochondrial alterations; metal ion dysregulation; inflammation. It is clear that these pathologies are involved in the disease process, but the actual upstream cause of these in AD etiology is currently poorly understood. Age-related decline in gonadal reproductive hormones and the integrity of the HPG axis has received considerable attention with regards to their potential role in AD, *e.g.* women who maintain relatively high endogenous estrogen levels and functional HPG axis after menopause exhibit a decreased prevalence of AD [142]. Findings regarding the benefits of hormone replacement therapy (HRT) in AD however have suggested that falling levels of steroid hormones that accompany menopause/andropause cannot sufficiently explain patterns of AD susceptibility [143]. In fact, it is only when one takes into account the role(s) of other hormones and receptor systems of the HPG axis that the susceptibility, onset, and progression of AD can be accurately characterized, *e.g.* the GnRH system that can affect hippocampus and cortical function, during the “critical period” around menopause/andropause. Due to the ability of GnRH to directly regulate LH, FSH and sex steroid production/gametogenesis, GnRH plays a central role in both reproductive function and general hormonal control via precise regulation of the HPG axis. It has been demonstrated that GnRH receptor concentration decreases to low levels during adult reproductive life, before paradoxically increasing in older rats (17 and 21 months of age). This increase in GnRH receptor concentration in post-reproductive animals appears to be due to decreased gonadal hormone production, since castrated male and female rats (high LH/FSH, low estrogen/testosterone) display increased GnRH receptor expression. Linked to this, it has been shown that male and female rats treated with testosterone and estradiol/ progesterone have decreased GnRH receptor expression [28,144]. With regards to the dynamic reproductive status in animals, GnRH receptor concentration is highest in proestrus and significantly lower during estrus [145]. In addition to steroid-induced changes in GnRH receptor expression, the affinity of GnRH receptor for GnRH decreases 18-fold during diestrus I and estrus, compared to ovariectomized animals [145]. In this review, we propose that GnRH, in addition to estrogen, may also be a prime factor in the pathogenesis of AD. Although the concentration of circulating GnRH is very low due to its short half-life [146,147], there is a two fold increase in circulating gonadotropins in individuals with AD, compared with age-matched control individuals [148, 149]. GnRH therefore appears to possess a strong biochemical and electrophysiological action in hippocampal neurons [102,103,144,150]. These actions seem to be connected to and modulated by the presence of estrogen however [103]. The effect of GnRH, with or without the presence of estrogen, on hippocampal pyramidal neurons may constitute an important

component of the neurodegenerative pathology that accompanies AD [104]. It is notable that expression of hippocampal spinophilin, a reliable dendritic spine marker, linked to cognitive status that functionally interacts and scaffolds GPCRs, can be directly regulated by the GnRH system [151]. The hippocampal GnRH system is itself acutely sensitive to both age and reproductive status, as hippocampal GnRH receptor expression is increased in aged and castrated rats [28,103].

If the pathology of AD is linked to disruption of the HPG axis, another strategy to suppress the increase in the gonadotropins following menopause/andropause is to use GnRH analogues (agonists and antagonists), which can decrease serum gonadotropin levels and also potentially have direct neuroprotective CNS actions. It has been demonstrated that administration of leuprolide acetate to C57BL/6 mice produced significant decreases in brain A β [23] and was also shown to improve cognitive function in a transgenic mouse model of AD that correlated to decreases in the A β burden [152]. The latter study used mice at a very advanced stage of cognitive decline, indicating a profound therapeutic efficacy of GnRH-based therapies, even in advanced stages of AD pathophysiology. Therefore, despite often complex age-dependent alterations in the GnRH system, targeting this receptor-ligand system may well be an effective strategy to combat the incidence as well as the progression of AD.

CONCLUSION

Following early reproductive life, changes in serum and neuronal concentrations of HPG axis hormones (increased gonadotropins, increased activins, increased GnRH and decreased sex steroids) associated with menopause/andropause have the potential to profoundly alter qualitatively and quantitatively neuronal signaling mechanisms. In particular, alterations in the GnRH-regulated LH release promotes biochemical and cellular changes consistent with the neurodegenerative changes observed in the AD brain [153]. These findings support the premise that GnRH receptor-based therapeutics could be a potential therapeutic target for the treatment of AD. Several double-blind placebo controlled phase II clinical trials are currently underway to conclusively make this determination. A reconsideration of the potential widespread actions that this traditional reproductive hormone exerts may lead to the generation of novel therapies and provide insight into the dynamic temporal alterations of GnRH signaling in normal and pathological aging. To ensure tissue specificity, the rational design of new therapeutics should take into account recent advances in our understanding of GnRH and generic receptor signaling mechanisms [58,79,154–157]. Taken together, the multiple findings of GnRH functionality in cognition may facilitate the future creation of novel selective GnRH analogs with potential for wider and more specific application to multiple neurodegenerative diseases.

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ABBREVIATIONS

HPG	The hypothalamic-pituitary-gonadal
AD	Alzheimer's disease
GnRH	Gonadotropin-releasing hormone
GPCR	G protein-coupled receptor
Aβ	beta-amyloid peptide
cAMP	cyclic adenosine monophosphate

DAG	diacylglycerol
ERK	extracellular signal-regulated kinase
FSH	follicle stimulating hormone
G-protein	guanine nucleotide binding protein
GnRH	gonadotropin-releasing hormone
GnRHR	gonadotropin- releasing hormone receptor
GTP	guanosine triphosphate
Gα	G-protein α subunit
G$\beta\gamma$	G-protein $\beta\gamma$ subunits
IP3	inositol 1, 4, 5-trisphosphate
JNK	jun-N-terminal kinase
LH	luteinizing hormone
LPA	lysophosphatidic acid
MAPK	mitogen-activated protein kinase
PIP2	phosphatidylinositol 1-4-5-bisphosphate
PL	phospholipase
PLA2	phospholipase A2
PLCβ	phospholipase C β
PLD	phospholipase D
PKC	protein kinase C
RGS	regulator of G-protein signaling
HRT	hormone replacement therapy
APP	the amyloid precursor protein
MAPK	mitogen-activated protein kinase

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