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Review Article

Targeting Gonadotropins: An Alternative Option for Alzheimer Disease Treatment

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Recent evidence indicates that, alongside oxidative stress, dysregulation of the cell cycle in neurons susceptible to degeneration in Alzheimer disease may play a crucial role in the initiation of the disease. As such, the role of reproductive hormones, which are closely associated with the cell cycle both during development and after birth, may be of key import. While estrogen has been the primary focus, the protective effects of hormone replacement therapy on cognition and dementia only during a "crucial period" led us to expand the study of hormonal influences to other members of the hypothalamic pituitary axis. Specifically, in this review, we focus on luteinizing hormone, which is not only increased in the sera of patients with Alzheimer disease but, like estrogen, is modulated by hormone replacement therapy and also influences cognitive behavior and pathogenic processing in animal models of the disease. Targeting gonadotropins may be a useful treatment strategy for disease targeting multiple pleiotropic downstream consequences.

BACKGROUND: ALZHEIMER'S DISEASE

Alzheimer's disease (AD), the primarily cause of senile dementia, is characterized by progressive memory loss, impairments in language and visual-spatial skills, episodes of psychosis, aggressiveness, and agitation, and ultimately death (reviewed in [1, 2]). AD is the most prevalent neurodegenerative disease, affecting approximately 4-5 million people in the United States and 15 million people worldwide. Given current population demographic predictions, it is estimated that by 2050, 50 million people will suffer from this devastating disease if no successful treatments are found [3]. The severity and the chronicity of this disease ultimately leads to institutionalization of patients, and thus results in a tremendous cost for the individual families and for society at large. Indeed, in the United States alone, the current cost of caring for patients with AD dementia is estimated at $100 billion per year and this will undoubtedly increase in coming years [4].

Unfortunately, to date, only palliative treatments of the symptoms are available and it is widely accepted that a better understanding of the etiology and disease pathogenesis is crucial for the development of new drugs capable of forestalling the progression of the disease. The leading hypothesis, the amyloid-β hypothesis, which is based on mutations in either the amyloid-β protein precursor (AβPP) or presenilins-1/2 (PSEN1/2) that affect the processing of amyloid-β and contribute to its accumulation in neurons and consequent formation of senile plaques [5], has come under increased scrutiny since manipulation of amyloid-β in cell or animal models does not yield the multitude of biochemical and cellular changes characteristic of the human disease. In fact, it is becoming increasingly evident that amyloid-β deposition may be a consequence rather than an initiator of the pathophysiological cascade [6–9]. Other mechanisms of disease, such as abnormally hyperphosphorylated bundles of tau protein found in neurofibrillary tangles [10], oxidativestress [11], metal ion deregulation [12],

and inflammation [13] also fail to completely explain all the abnormalities found in AD. Moreover, the lack of efficient therapeutic strategies based on such mechanisms only serves to emphasize the fundamental gap in our knowledge of disease.

CELL CYCLE DYSREGULATION: AN ALTERNATIVE HYPOTHESIS FOR ALZHEIMER'S DISEASE

There is increasing evidence for activated cell cycle in the vulnerable neuronal population in AD [14–16]. We suspect that the dysregulation of the cell cycle, in conjunction with oxidative stress, in hippocampal neurons leads to initiation of the pathophysiolological cascade of AD [17]. This hypothesis is supported by several neuronal changes seen in AD including the ectopic expression of markers of cell cycle [18], organelle kinesis [19], and cytoskeletal alterations such as tau phosphorylation [20]. Importantly, and suggestive of this pivotal effect, mitotic alterations are not only one of the earliest neuronal abnormalities found in AD but are also related to the majority of the pathological hallmarks, such as hyperphosphorylation of tau, amyloid-β accumulation, and oxidative stress (reviewed in [21]). To this end, a near identical phosphorylation of tau also occur when cells are mitotically active and phosphorylation is driven by cyclin-dependent kinases (CDKs) [22–25]. Therefore, one possibility is that cell cycle alterations could lead to tau phosphorylation and subsequent neuronal degeneration. In support of this hypothesis, several reports in the literature indicate that cell cycle markers are abnormally expressed in nerve cells with filamentous tau deposits. These markers include proteins cyclin D and Cdk4/Cdk6, involved in the G_0/G_1 transition, retinoblastoma protein, and the CDK inhibitors p15, p16, p18, and p19 [26–32]. Other markers such as cyclin E and Cdc25A, usually associated with G_1/S transition, have also been shown to be abnormally expressed in degenerating neurons [33–35]. Importantly, colocalization of different cell cycle markers with phosphorylated tau protein has also been demonstrated. In this regard, colocalization of cyclin B, Cdc2, Cdc25B, Polo-like kinase, Myt1/Wee1, and p27Kip1, all regulators of the G2/M transition, and some mitotic epitopes, such as phosphorylated histone H3, phosphorylated RNA polymerase II, PCNA, Ki67, and MPM2, has been demonstrated [27, 33, 34, 36–45]. Importantly some of these markers appear to precede the phosphorylation and aggregation of tau protein, suggesting a possible cause-and-effect relationship [37, 46, 47]. Moreover, these in vivo findings are supported by studies in cell models showing AD-like phosphorylation of tau protein in mitotically active cells [48–50] and also by the phosphorylation of recombinant tau by CDKs in vitro [51]. While cell cycle changes often precede tau phosphorylation, in a *Drosophila* model, cell cycle abnormalities appear to follow tau alterations [52]. Also, of relevance, experimental studies have established that inappropriate reentry into the cell cycle results in nerve cell death and reactivation of the cell-cycle machinery likely plays an important role in the apoptotic death of postmitotic neurons [53, 54]. Taken together, these findings indicate that cell cycle is intimately associated with AD and tau phosphorylation. However, as stated above, the chronology and mechanistic origin of tau phosphorylation remain to be clearly characterized.

There is also abundant evidence indicating that oxidative stress and free radical damage play key roles in the pathogenesis of AD [11, 55, 56]. Importantly free radicals, free-radical generators, and antioxidants act as crucial control parameters of the cell cycle [57] and have all been implicated in the development or halting of cell-cycle-related diseases such as cancer [58]. Therefore, one possibility is that oxidative stress and cell cycle dysregulation work synergistically in the development of AD [59]. In support of this notion, during the cell cycle, there is division and redistribution of cellular organelles such that mitochondrial proliferation is evident [60]. Mitochondrial proliferation is imperative for providing the energy needed for cell division, however, in cells where the cell cycle is interrupted or dysfunctional, cells incur a "phase stasis" to serve as a potent source of free radicals and cause redox imbalance [6], especially in those redox reactions involving calcium metabolism [61]. On the other hand, it is known that one pathway for oxidative stress mediated neuronal cell death is cell cycle reentry [62] and antioxidant treatments, most with potent cell-cycle inhibitory properties produce declines in tau phosphorylation [63].

Therapeutic interventions specifically designed to arrest cells at G_0 phase of the cell cycle, halt mitotic signalling cascades, or reduce the levels of endogenous or exogenous mitogens responsible for the aberrant mitosis in senescent neurons could have a tremendous success in AD treatment (reviewed in [21, 64]). Supporting this, nonsteroidal anti-inflammatory drugs (NSAIDs), which also possess antiproliferative properties, are useful to delay the progression of AD [65]. Likewise, antiapoptotic compounds such as resveratrol are well established in aging and AD. Resveratrol, a potent antioxidant of natural origin [66–69], may be of benefit in murine senescence and AD models and in some clinical studies in patients with AD [70]. Studies with animals also demonstrated protective effects of resveratrol against kainite-induced seizures [71] and its protective effects against brain injury due to ischemia/reperfusion in gerbil model [72]. Likewise, flavopiridol, a synthetic flavone closely related to a compound found in a plant native to India, *Dysoxylum binectariferum*, is a potent inhibitor of most CDKs, including CDK1, CDK2, CDK4, and CDK7 [73]. It induces growth arrest at either the G_1 and/or G_2 phases of the cell cycle in numerous cell lines in vitro by acting as a competitive binding agent for the ATP-binding pocket of CDK [73]. One consequence of this inhibition is a decrease in cyclin D1, the binding partner of CDK4 in G_1, by depletion of cyclin D1 mRNA resulting in a decrease in CDK4 kinase activity [74]. Importantly, the drug is in phase I and II clinical trials as an antineoplastic agent for breast, gastric, and renal cancers [75] and recent studies demonstrate its effectiveness on brain cancers such as gliomas [76]. These findings indicate that flavopiridol is a powerful CDK inhibitor as well as a potential therapeutic avenue for AD.

Notably, one powerful endogenous mitogen, luteinizing hormone (LH), a gonadotropin most often associated with reproduction, is particularly increased during aging and AD. Therefore another potential therapeutic option is to target age-related increases of this hormone in AD. The exploration of the link between gonadotropins such as LH and the etiology of AD and its potential value as a therapeutic avenue will be the focus of this review.

ARE SEX STEROIDS INVOLVED IN THE ETIOLOGY OF ALZHEIMER'S DISEASE?

Hormones of the hypothalamic-pituitary-gonadal (HPG) axis include gonadotropin releasing hormone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, progesterone, testosterone, activin, inhibin, and follistatin. Each of these hormones is involved in regulating reproductive function by participating in a complex feedback loop. Briefly, this loop is initiated by the secretion of hypothalamic gonadotropin releasing hormone that stimulates the pituitary to secrete the gonadotropins LH and FSH. These gonadotropins are capable of stimulating oogenesis/spermatogenesis as well as the production of sex steroids which complete the feedback loop by reducing the gonadotropin secretion by the hypothalamus into the bloodstream [77].

Menopause and andropause are characterized by a dramatic decline in sex steroids resulting in an increase in the production of gonadotropins. To this end, in women, gonadotropins are considerably increased reaching a 3- to 4-fold increase in the concentration of serum LH and a 4- to 18-fold increase in FSH. Men also experience an increase of LH and FSH, but to a lower degree than those in women, resulting only in a 2-fold and 3-fold increase, respectively [78, 79]. Notably, the link between the HPG axis hormones and AD is not new as it has been hypothesized that the marked reduction in sex hormone levels during postmenopausal states results in various physiological and psychological changes associated with the development and progression of AD. In this regard, several epidemiological studies indicate that women have a higher predisposition to develop AD than do men [80–82] and treatment with hormone replacement therapy (HRT) reduced this risk in women [83, 84]. These gender differences, in addition to the capacity of HRT to reduce this risk in postmenopausal women, led researchers to investigate the role of female sex steroids, namely, estrogen, in the pathogenesis of AD.

To this end, estrogen can act as a neuroprotective agent by lowering the brain levels of amyloid-β [85], by ameliorating the nerve cell injury caused by amyloid-β [86], and/or promoting synaptic plasticity and growth of nerve processes [87]. Moreover, estrogen is also capable of reducing oxidative stress, increasing cerebral blood flow, and enhancing cholinergic function and glucose transport into the brain [88]. All of these effects have a well-known positive impact on the prevention and the amelioration of AD. However, recent prospective studies, including the Women's Health Initiative Memory Study (WHIMS), seem to contradict the previous promising observations regarding HRT. WHIMS, a randomized clinical trial designed to assess the incidence of dementia among relatively healthy postmenopausal women under HRT, showed a substantially increased overall incidence of dementia in postmenopausal women [89–91]. Since hormone therapy is relatively common for menopausal women, these latter findings have raised serious concerns about the long term efficacy and safety of HRT.

HORMONE REPLACEMENT: TIMING IS EVERYTHING

Many hypotheses have been postulated to justify the results of the WHIMS. To date, some aspects related to the form (estradiol versus conjugated equine estrogen (CEE)) and the route of administration (oral versus transdermal) of estrogen, the choice of progestin (natural versus synthetic progestins), the high doses administered, the type of treatment regimen (continuous versus cyclic) might be important points to be considered (reviewed in [92, 93]). For instance, the adverse effects on cognition are mainly attributed to the thromboembolic complications of oral CEE [94]. However, one aspect that has been overlooked and that is tightly linked to the timing of hormone therapy (ie, perimenopausal versus postmenopausal) is the release of, and capacity of, HRT to lower gonadotropins such as LH. In fact, it is only when one takes into account the role of these other hormones of the hypothalamic-pituitary-gonadal axis (reviewed in [77]), during a "critical period" around the onset of menopause and the years beyond that cognitive decline and susceptibility, onset, and progression of AD can be accurately characterized. To this end, chronic elevation of gonadotropins and decline in sex steroids leads to HPG axis shutdown. Therefore, HRT started in older women such as those of the WHIMS [89], while bringing estrogen to premenopausal levels, cannot decrease the levels of gonadotropins such as LH. On the other hand, HRT started during peri-menopause or early menopause, when the HPG axis feedback loop system is functional, does lead to a lowering of LH. Supporting this hypothesis, the levels of gonadotropins including LH are highest during peri-menopause and early menopause [95], when HRT has been observed to be most successful in preventing dementia [96, 97]. Likewise, studies also demonstrate that while cognitive decline can be rescued with estrogen therapy initiated immediately after menopause and ovariectomy (mimics menopause), however, unless subjects are previously primed with estrogen [98], estrogen replacement initiated after a long interval following menopause or ovariectomy is ineffective at rescuing cognition [99–101]. This later finding suggests that by priming, HPG-axis functionality is sustained and thus led to cognitive improvements after HRT. Likewise, estrogen becomes increasingly less effective at modulating LH expression and biosynthesis the longer that HRT is started after ovariectomy [102], a mechanism that is specifically mediated by the gonadotropin-releasing hormone (GnRH) receptor [103, 104]. Importantly, the ovariectomy findings parallel those observed during aging, such that estrogen feedback on LH secretion [105] and GnRH gene expression [106] is decreased. Whether

the beneficial AND detrimental effects of HRT are associated with menopause-driven gonadotropin changes is not yet fully known and is currently being examined in our laboratory. However, the above-cited evidence does indicate that timing, pituitary function, and estrogen-gonadotropin influences are more complex than previously thought. These findings may provide the reconciliating link between the contradicting data presented in the WHIMS and prior observational/epidemiological studies. Moreover, these data suggest a potential role for gonadotropins in the CNS, particularly on cognitive decline and AD pathogenesis and, more importantly, places gonadotropins as a potential therapeutic target for the treatment of AD.

EVIDENCE FOR A ROLE OF LH IN ALZHEIMER'S DISEASE

Epidemiological data supports a role of LH in AD. In this regard, and paralleling the female predominance for developing AD [81, 82, 107, 108], LH levels are significantly higher in females as compared to males [97] and LH levels are higher still in individuals who succumb to AD [109, 110]. Also important is the fact that, in Down syndrome, where the prevalence of AD-like etiology is higher in males than in females, that is, a reversal to what is observed in the normal population, males have higher serum LH levels compared to females [111, 112]. Therefore, LH allows an explanation for the reversing of the classical gender-predisposition in AD versus Down syndrome [113].

Like epidemiological data, direct experimental evidence also indicates that LH may be an important player in the development and progression of AD. In this regard, LH is capable of modulating cognitive behavior [114], is present in the brain, and has the highest levels of its receptor in the hippocampus [115], a key processor of cognition affected by aging and severely deteriorated in AD. Furthermore, we have recently examined cognitive performance in a well-characterized transgenic line that overexpresses LH [116–118] and have found that these animals show decreased cognitive performance when compared to controls [119]. Since other hormones in addition to LH are altered in the LH overexpressing mice, we also measured Y-maze performance in a well-characterized LH receptor knockout (LHRKO) strain of mice [120], which also have very high levels of LH, to begin to determine whether cognitive decline could be mediated by a specific LH mechanism (ie, the LH receptor). In this regard, LHRKO (−/−) mice performed indistinguishable from wild-type (+/+) mice. Therefore, the negative effects on cognition affected by high levels of LH were completely attenuated by knockout of the receptor. While comparing the TgLH-β and LHRKO animals should be done with caution (ie, different strain and background), changes in estrogen levels were unlikely to be responsible for the cognitive changes observed in this study since LH overexpressers show elevated rather than diminished levels of estrogen [116–118] and LHRKO mice show decreased levels of estrogen when compared to wild-type littermate controls [120]. On the other hand, both do show high LH but this is obviously a nonissue in the LHRKO animals. These findings support our hypothesis that modulation of cognition by estrogen is interrelated with the status of LH levels and function. Finally, recently we also found that experimentally abolishing LH in the AβPP transgenic mouse, an animal model of AD, using a selective GnRH agonist (leuprolide acetate) that has been shown to reduce LH to undetectable levels by downregulating the pituitary gonadotropin-releasing hormone receptors [121, 122], improved hippocampally related cognitive performance and decreased amyloid-β deposition in these mice when compared to aged-matched controls [123]. These findings, together with data indicating that LH modulates AβPP processing in vivo and in vitro [122], suggest that LH may be a key player in this disease.

Mechanistically, and as alluded to in the previous section, LH could be working via the modulation of cell cycle. LH is known to be a potent mitogen [124, 125] by acting through MAP kinases pathway [126]. In this regard, LH activates ERK [127] and other transcription factors [128] all involved in cell cycle [129], thus suggesting that high levels of this hormone could lead to the aberrant cell cycle reentry of neurons observed in AD.

CAN TARGETING LH BE THE NEW THERAPEUTIC AVENUE?

Findings discussed in this review indicate that targeting the release of LH may indeed be a successful strategy to prevent and forestall the progression of AD. As discussed above, preclinical data using leuprolide acetate leads to modulation of AβPP processing in normal mice [122] and cognitive improvement and decreased amyloid-β burden in AβPP transgenic mice [123]. More importantly, a recently completed phase II clinical trial shows stabilization in cognitive decline in a subgroup of AD patients treated with leuprolide acetate (http://clinicaltrials.gov/ct/show/nct00076440?orden=6). Specifically, female AD patients treated with high doses of leuprolide acetate (http://www.secinfo.com/d14D5a.z6483.htp, pages 56–64) showed stabilization in cognitive function and activities of daily living. These promising findings support the importance of LH in AD and give way for an alternative and much needed therapeutic avenue for this insidious disease.

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