CURRENT OPINION

Drugs R D 2006; 7 (3): 187-193
1174-5886/06/0003-0187/$39.95/0

The Estrogen Myth

Potential Use of Gonadotropin-Releasing Hormone Agonists for the Treatment of Alzheimer's Disease

Gemma Casadesus ,[1] *Matthew R. Garrett**,[1] *Kate M. Webber*,[1] *Anthony W. Hartzler*,[1] *Craig S. Atwood*,[2] *George Perry*,[1] *Richard L. Bowen*[3] and *Mark A. Smith*[1]

1 Department of Pathology, Case Western Reserve University, Cleveland, Ohio, USA
2 School of Medicine, University of Wisconsin and William S. Middleton Memorial Veterans Administration, Madison, Wisconsin, USA
3 Voyager Pharmaceutical Corporation, Raleigh, North Carolina, USA

Abstract

Estrogen and other sex hormones have received a great deal of attention for their speculative role in Alzheimer's disease (AD), but at present a direct connection between estrogen and the pathogenesis of AD remains elusive and somewhat contradictory. For example, on one hand there is a large body of evidence suggesting that estrogen is neuroprotective and improves cognition, and that hormone replacement therapy (HRT) at the onset of menopause reduces the risk of developing AD decades later. However, on the other hand, studies such as the Women's Health Initiative demonstrate that HRT initiated in elderly women increases the risk of dementia. While estrogen continues to be investigated, the disparity of findings involving HRT has led many researchers to examine other hormones of the hypothalamic-pituitary-gonadal axis such as luteinising hormone (LH) and follicle-stimulating hormone. In this review, we propose that LH, rather than estrogen, is the paramount player in the pathogenesis of AD. Notably, both men and women experience a 3- to 4-fold increase in LH with aging, and LH receptors are found throughout the brain following a regional pattern remarkably similar to those neuron populations affected in AD. With respect to disease, serum LH level is increased in women with AD relative to non-diseased controls, and levels of LH in the brain are also elevated in AD. Mechanistically, we propose that elevated levels of LH may be a fundamental instigator responsible for the aberrant reactivation of the cell cycle that is seen in AD. Based on these aforementioned aspects, clinical trials underway with leuprolide acetate, a gonadotropin-releasing hormone agonist that ablates serum LH levels, hold great promise as a ready means of treatment in individuals afflicted with AD.

Alzheimer's disease (AD) is the leading cause of dementia among people aged >65 years and the most prevalent neurodegenerative disease. Dementia affects approximately 24 million people worldwide. Importantly, given current population demographic predictions, if no successful treatments are found, it is estimated that over 80 million people will be affected by this devastating disease by

Drs Casadesus and Garrett contributed equally to this work.

2040.[1] The disease is characterised by a gradual decline in cognitive function, beginning with impaired memory, progressing to changes in behaviour and personality, and ultimately leading to death.[2] Current treatments such as the acetylcholinesterase inhibitors can improve cognitive function and provide temporary relief, but do not stop disease progression.[3] As such, therapies targeted to the underlying cause of the disease are greatly needed. While the exact aetiology of AD remains unclear, here we examine a relatively new mechanism of disease pathogenesis that could explain some previously enigmatic observations regarding gender differences in AD and the controversial effects of hormone replacement therapy (HRT) on disease. Specifically, we will present evidence for a novel role for luteinising hormone (LH) in the development and progression of AD. The evidence presented represents a synthesis of published materials (available through PubMed) together with our own experimental data.

1. Pathology and Pathophysiology of Alzheimer's Disease (AD)

Pathologically, AD is characterised by diffuse atrophy of the brain with characteristic microscopic lesions, namely senile plaques, neurofibrillary tangles and amyloid angiopathy. Neurofibrillary tangles are primarily composed of highly phosphorylated tau, a protein with a role in cytoskeletal organisation, whereas senile plaques and amyloid angiopathy are composed of amyloid-β (Aβ) protein.

Numerous hypotheses attempt to explain the pathogenesis of AD. The theory that Aβ itself initiates the disease remains attractive, since mutations in the Aβ protein precursor (*AβPP*) gene, or in presenilin-1 or -2 (enzymes involved in AβPP processing), are known to cause rare forms of familial AD.[4] However, this hypothesis is at best incomplete since it accounts for only a small minority (<3%) of AD cases. The events that initiate amyloid deposition in the majority of AD patients, with normal AβPP and presenilin genes, remain unknown. Moreover, Aβ fails to elicit pathology in experimental models such as the AβPP transgenic mice, which, despite the deposition of large amounts of Aβ, experience little or no neuronal loss.[5] Additionally, a novel interpretation for the role of Aβ in disease suggests that it may be neuroprotective, having antioxidant and metal-ion sequestration properties.[6-8] In this latter scenario, the increase in Aβ in AD could be explained as an adaptive response to some unknown neurotoxic insult(s),[9] and familial forms of AD would result from the abnormal functioning of a normally protective pathway.

Other hypotheses regarding the aetiology of AD include aberrant tau phosphorylation,[10-12] oxidative stress,[13] metal ion dysregulation,[14] and inflammation.[15] However, while there is substantial evidence for the role of each of these, none provides a holistic narrative suitable to encompass the totality of the disease process.

2. Gender Differences in Alzheimer's Disease

Age, gender and environmental factors are common variables attributed to the development of AD. The majority, though not all, of epidemiological investigations indicate that women have a higher incidence and prevalence of the disease,[16-20] and based upon this gender predilection, hormone-specific hypotheses have found favour among researchers.

Post-menopausal estrogen deficiency could potentiate risk, a notion supported by the observation that higher post-menopausal levels of estrogen are associated with a reduced risk of AD.[21] It has also been shown that women with AD have elevated levels of sex hormone-binding globulin, suggesting decreased levels of estradiol.[22] HRT, then, would be expected to protect against AD. Early case-control and cohort studies did show HRT to be protective,[23-25] and other studies show that estrogen replacement in postmenopausal women is associated with improved cognitive function.[26] A protective role for estrogen has also been investigated in a variety of *in vivo* model systems, including fimbria-fornix lesions and middle cerebral artery occlusion, which suggest neuroprotection via estrogen.[27] Because of the promising findings and observations

regarding the protective nature of HRT in postmenopausal women, a large-scale randomised, controlled study (the Women's Health Initiative [WHI] study) was carried out to examine this phenomenon more rigorously. However, much to the field's surprise, this study, evaluating the incidence of dementia among relatively healthy postmenopausal women taking oral estrogen-progestogen therapy, was discontinued because of increased health risks in the treatment group, including a 2-fold increase in clinically diagnosed dementia.[28] Also, a smaller randomised, controlled trial evaluating estrogen for the treatment of AD showed no benefit.[29]

While the prior studies regarding the protective nature of HRTs were mostly epidemiological/observation studies and thus had limited weight on proof of cause, likely because estrogen has an extensive neuroprotective record in the basic research AD literature,[30] a plethora of hypotheses have been postulated to justify the results of the WHI study. In this regard, aspects related to the form (estradiol vs conjugated equine estrogen) and the route of administration (oral vs transdermal) of estrogen, the choice of progestogen (natural vs synthetic progestogens), the high doses administered, as well as the type of treatment regimen (continuous vs cyclic) have all been speculated factors for these WHI study results.[31,32] However, one additional aspect that has been overlooked, and that could explain these contradicting findings, involves the timing of HRT initiation and the age of the participants of the WHI study. In this regard, HRT protection against AD may only be effective when administered during a 'critical period' at menopause transition or early menopause,[33,34] and ineffective or even harmful when administered years post-menopause, when perhaps latent preclinical stages of AD may be evident.[28,35] In support of this assertion, studies demonstrate that while cognitive decline can be rescued with HRT initiated right after ovariectomy (which mimics menopause), HRT initiated a long time after ovariectomy is ineffective at rescuing cognition.[36,37]

Based on the aforementioned evidence, and while estrogen levels do show potential associations with AD, the relationship remains ambiguous. Even if we assume that a transient perimenopausal estrogen deficiency sets in motion a disease process that cannot then be corrected by HRT, a key question to address is how the disparity in effectiveness between HRT at different ages and the benefits/risks of developing AD occurs from a mechanistic stance. To this end, a link between the data presented in the WHI study and prior observational/epidemiological studies that could also provide a mechanistic role based on the 'critical period' observations, is evident when other hormones of the hypothalamic-pituitary-gonadal axis,[38] which are regulated simultaneously with estrogen by HRT (such as LH, which is also modulated [simultaneously with estrogen] during HRT), are taken into account.[39] Specifically, LH provides a compelling explanation of the array of data. In support of this hypothesis, while the mechanism involved in the time-dependent differential modulation of estrogen on, for example, cognitive decline is unknown, administration of estrogen after short or long intervals after ovariectomy leads to differential sensitivity of LH gene expression and biosynthesis. Therefore, LH levels, regulated indirectly via HRT, could be a key modulator of cognition and neuronal function affected by aging and AD pathogenesis.

3. Hypothalamic-Pituitary-Gonadal Axis

The production of estrogen and testosterone are under hypothalamic and pituitary control via the hypothalamic-pituitary-gonadal axis. The hypothalamus releases pulses of gonadotropin-releasing hormone (GnRH), stimulating the anterior pituitary to release follicle-stimulating hormone (FSH) and LH, known as the gonadotropins. LH and FSH act on the gonads to stimulate spermatogenesis or follicle development, and to increase production of the sex steroids estrogen and testosterone. The sex steroids, in turn, provide negative feedback to the hypothalamus and pituitary.

At menopause, the ovarian production of steroid hormones fails, leaving the body with small amounts of androgens from the adrenal cortex, and estrogen derived from the peripheral conversion of

these androgens. The negative feedback effect of estrogen on the hypothalamus and pituitary is lost, and LH and FSH levels increase (by 3- to 4-fold and 4- to 18-fold, respectively) in an effort to drive the ovaries to produce more sex steroids.[40] 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.[41] Surprisingly, the effects of increased circulating gonadotropins, which are known to cross the blood-brain barrier, are largely unexplored.

4. Evidence for Gonadotropins as a Cause of AD

Since decreased estrogen levels have been implicated in AD pathogenesis, it is reasonable to expect, and it has been demonstrated, that serum FSH and LH levels are increased in women with AD compared with age-matched controls.[42] Importantly, LH levels also provide an explanation for the gender-reversal in development of AD observed in Down's syndrome, in which the prevalence of AD-like aetiology is higher in males than in females.[40,41,43] Importantly, estrogen levels of individuals with Down's syndrome are equivalent to levels in the general population. Therefore, while the commonly accepted explanation for AD-type changes in individuals with Down's syndrome involves triplication of the *AβPP* gene locus on chromosome 21 and consequent increases in Aβ, such a notion fails to explain why the gender predilection for AD-type changes is reversed in Down's syndrome compared with the general population.

Physiologically, as mentioned earlier, gonadotropins are known to cross the blood-brain barrier,[44] suggesting effects of this hormone outside the reproductive system.[45] Moreover, LH and FSH are present in the cerebrospinal fluid (CSF) of postmenopausal women, albeit at a fraction of serum concentrations (with LH higher than FSH in its CSF to serum ratio).[46] These physiological findings could explain why receptors for LH are present in the brain and, more importantly, found in the greatest density in neurons of the hippocampus (the prime victim of Alzheimer's-related pathology) and with regional expression consistently corresponding to the regional vulnerability exhibited in AD.[47-49] Likewise, LH itself has been found in the cytoplasm of pyramidal neurons of healthy subjects, but in increased concentrations in the AD brain compared with control.[50]

Neurons are thought to be terminally differentiated, yet numerous lines of evidence show cell cycle reactivation in AD-affected neurons.[51-55] Specific findings include expression of cell cycle markers,[56-61] organelle kinesis,[62] activation of the mitotic signaling pathways (mitogen-activated protein kinase [MAPK] and extracellular regulated kinase [ERK]),[11,63] and cytoskeletal alterations including tau phosphorylation.[61] Importantly, mitotic alterations are one of the earliest neuronal abnormalities in the disease,[59,64-66] and may also lead to the remainder of reported pathological changes since all of the major genetic and protein elements dysregulated in AD, including tau, AβPP, presenilin-1 and -2, and, possibly, apolipoprotein E,[67] are also altered during the cell cycle.[68] We suggest interplay between gonadotropins and neuronal cell cycle. LH has been shown to be a potent mitogen and is capable of activating the ERK and MAPK pathways.[69] In addition, increases of LH in the AD brain share spatial and temporal relationships with the appearance of aberrant cell cycle markers[50] (unpublished observations from Webber, Casadesus and Smith).

5. Gonadotropin-Releasing Hormone Agonists as AD Pharmacotherapy

The gonadotropin hypothesis is attractive in part because we already possess the means to decrease gonadotropin levels. Leuprolide acetate is a gonadotropin-releasing hormone (GnRH) agonist that, by causing down-regulation of GnRH receptors in the anterior pituitary, leads to suppression of gonadotropin release. In animal models, removal of ovaries, and consequent decreases in sex steroids and increases in gonadotropins, results in increased Aβ deposition. Yet when treated with leuprolide acetate, which blocks gonadotropin release, there is a significant decrease in soluble Aβ and modulation

of AβPP processing towards the amyloidogenic pathway *in vitro*.[70] Even more important is the fact that leuprolide acetate treatment in an animal model of AD led to improvements in cognitive function and decreases in Aβ plaque load.[71] These findings provide direct support for the notion that increased LH may be a key culprit in AD pathogenesis.

In this regard, a recently completed phase II clinical trial[72] indicates that patients treated with high doses of leuprorelin show a stabilisation in cognitive decline (Alzheimer's Disease Assessment Scale-cognitive [ADAS-Cog], AD Cooperative Study-Clinical Global Impression of Change [ADCS-CGIC]) and activities of daily living (Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory [ADCS-ADL]). Phase III trials are currently in the recruiting phase.[73]

6. Conclusions

While there is ample evidence that increases in gonadotropins are associated with AD in women, and that LH itself is a mitogen that could activate cell cycle processes and lead to AD pathology, this hypothesis, like all the others, cannot explain all facets of the disease. Most notably, while lower levels of free testosterone in the serum are associated with AD in men,[74] levels of LH and FSH are found to be no different in AD compared with control.[42,75] AD will likely prove to be a disease of multifactorial origin, with genetics, environment, age and gender all having some influence. We propose the gonadotropins, in particular LH, as one important factor with ready means of treatment.[76,77]

Acknowledgements

Work in the authors' laboratories is supported by Voyager Pharmaceutical Corporation. Drs Atwood, Perry and Smith serve as consultants to and own options in Voyager Pharmaceutical Corporation. Voyager Pharmaceutical Corporation is currently using leuprolide acetate in phase II and phase III clinical trials for the treatment of Alzheimer's disease.

References

1. Ferri CP, Prince M, Brayne C, et al. Global prevalence of dementia: a Delphi consensus study. Lancet 2005; 366: 2112-7
2. Smith MA. Alzheimer disease. Int Rev Neurobiol 1998; 42: 1-54
3. Marlatt MW, Webber KM, Moreira PI, et al. Therapeutic opportunities in Alzheimer disease: one for all or all for one? Curr Med Chem 2005; 12: 1137-47
4. Selkoe DJ. Alzheimer's disease: genotypes, phenotypes, and treatments. Science 1997; 275: 630-1
5. Irizarry MC, Soriano F, McNamara M, et al. Abeta deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. J Neurosci 1997; 17: 7053-9
6. Perry G, Nunomura A, Raina AK, et al. Amyloid-beta junkies. Lancet 2000; 355: 757
7. Obrenovich ME, Joseph JA, Atwood CS, et al. Amyloid-beta: a (life) preserver for the brain. Neurobiol Aging 2002; 23: 1097-9
8. Rottkamp CA, Atwood CS, Joseph JA, et al. The state versus amyloid-beta: the trial of the most wanted criminal in Alzheimer disease. Peptides 2002; 23: 1333-41
9. Lee HG, Casadesus G, Zhu X, et al. Challenging the amyloid cascade hypothesis: senile plaques and amyloid-beta as protective adaptations to Alzheimer disease. Ann N Y Acad Sci 2004; 1019: 1-4
10. Trojanowski JQ, Clark CM, Arai H, et al. Elevated levels of tau in cerebrospinal fluid: implications for the antemortem diagnosis of Alzheimer's disease elevated levels of tau in cerebrospinal fluid: implications for the antemortem diagnosis of Alzheimer's disease. J Alzheimers Dis 1999; 1: 297-305
11. Zhu X, Lee HG, Raina AK, et al. The role of mitogen-activated protein kinase pathways in Alzheimer's disease. Neurosignals 2002; 11: 270-81
12. Avila J. Tau aggregation into fibrillar polymers: taupathies. FEBS Lett 2000; 476: 89-92
13. Perry G, Castellani RJ, Hirai K, et al. Reactive oxygen species mediate cellular damage in Alzheimer disease. J Alzheimers Dis 1998; 1: 45-55
14. Perry G, Sayre LM, Atwood CS, et al. The role of iron and copper in the aetiology of neurodegenerative disorders: therapeutic implications. CNS Drugs 2002; 16: 339-52
15. Atwood CS, Huang X, Moir RD, et al. Neuroinflammatory responses in the Alzheimer's disease brain promote the oxidative post-translation modification of amyloid deposits. In: Iqbal K, Sisodia SS, Winblad B, editors. Alzheimer's disease: advances in etiology, pathogenesis and therapeutics. Chichester: John Wiley & Sons Ltd, 2001: 341-61
16. Breitner JC, Silverman JM, Mohs RC, et al. Familial aggregation in Alzheimer's disease: comparison of risk among relatives of early-and late-onset cases, and among male and female relatives in successive generations. Neurology 1988; 38: 207-12
17. Jorm AF, Jolley D. The incidence of dementia: a meta-analysis. Neurology 1998; 51: 728-33
18. Jorm AF, Korten AE, Henderson AS. The prevalence of dementia: a quantitative integration of the literature. Acta Psychiatr Scand 1987; 76: 465-79
19. McGonigal G, Thomas B, McQuade C, et al. Epidemiology of Alzheimer's presenile dementia in Scotland, 1974-88. BMJ 1993; 306: 680-3
20. Rocca WA, Hofman A, Brayne C, et al. Frequency and distribution of Alzheimer's disease in Europe: a collaborative study of 1980-1990 prevalence findings. The EURODEM-Prevalence Research Group. Ann Neurol 1991; 30: 381-90
21. Manly JJ, Merchant CA, Jacobs DM, et al. Endogenous estrogen levels and Alzheimer's disease among postmenopausal women. Neurology 2000; 54: 833-7

22. Hoskin EK, Tang MX, Manly JJ, et al. Elevated sex-hormone binding globulin in elderly women with Alzheimer's disease. Neurobiol Aging 2004; 25: 141-7
23. Tang MX, Jacobs D, Stern Y, et al. Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. Lancet 1996; 348: 429-32
24. Henderson VW, Paganini-Hill A, Emanuel CK, et al. Estrogen replacement therapy in older women. Comparisons between Alzheimer's disease cases and nondemented control subjects. Arch Neurol 1994; 51: 896-900
25. Kawas C, Resnick S, Morrison A, et al. A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging. Neurology 1997; 48: 1517-21
26. Jacobs DR, Pereira MA, Meyer KA, et al. Fiber from whole grains, but not refined grains, is inversely associated with all-cause mortality in older women: the Iowa women's health study. J Am Coll Nutr 2000; 19: 326S-30S
27. Shi J, Zhang YQ, Simpkins JW. Effects of 17beta-estradiol on glucose transporter 1 expression and endothelial cell survival following focal ischemia in the rats. Exp Brain Res 1997; 117: 200-6
28. Shumaker SA, Legault C, Rapp SR, et al. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA 2003; 289: 2651-62
29. Mulnard RA. Estrogen as a treatment for Alzheimer disease. JAMA 2000; 284: 307-8
30. Inestrosa NC, Marzolo MP, Bonnefont AB. Cellular and molecular basis of estrogen's neuroprotection: potential relevance for Alzheimer's disease. Mol Neurobiol 1998; 17: 73-86
31. Gleason CE, Cholerton B, Carlsson CM, et al. Neuroprotective effects of female sex steroids in humans: current controversies and future directions. Cell Mol Life Sci 2005; 62: 299-312
32. Baum LW. Sex, hormones, and Alzheimer's disease. J Gerontol A Biol Sci Med Sci 2005; 60: 736-43
33. Zandi PP, Carlson MC, Plassman BL, et al. Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. JAMA 2002; 288: 2123-9
34. Gibbs RB, Gabor R. Estrogen and cognition: applying preclinical findings to clinical perspectives. J Neurosci Res 2003; 74: 637-43
35. Resnick SM, Henderson VW. Hormone therapy and risk of Alzheimer disease: a critical time. JAMA 2002; 288: 2170-2
36. Sherwin BB. Estrogen and memory in women: how can we reconcile the findings? Horm Behav 2005; 47: 371-5
37. Daniel JM, Hulst JL, Berbling JL. Estradiol replacement enhances working memory in middle-aged rats when initiated immediately after ovariectomy but not after a long-term period of ovarian hormone deprivation. Endocrinology 2006; 147: 607-14
38. Genazzani AR, Gastaldi M, Bidzinska B, et al. The brain as a target organ of gonadal steroids. Psychoneuroendocrinology 1992; 17: 385-90
39. Webber KM, Casadesus G, Marlatt MW, et al. Estrogen bows to a new master: the role of gonadotropins in Alzheimer pathogenesis. Ann N Y Acad Sci 2005; 1052: 201-9
40. Chakravarti S, Collins WP, Forecast JD, et al. Hormonal profiles after the menopause. BMJ 1976; 2: 784-7
41. Neaves WB, Johnson L, Porter JC, et al. Leydig cell numbers, daily sperm production, and serum gonadotropin levels in aging men. J Clin Endocrinol Metab 1984; 59: 756-63
42. Short RA, Bowen RL, O'Brien PC, et al. Elevated gonadotropin levels in patients with Alzheimer disease. Mayo Clin Proc 2001; 76: 906-9
43. Schupf N, Kapell D, Nightingale B, et al. Earlier onset of Alzheimer's disease in men with Down syndrome. Neurology 1998; 50: 991-5
44. Lukacs H, Hiatt ES, Lei ZM, et al. Peripheral and intracerebroventricular administration of human chorionic gonadotropin alters several hippocampus-associated behaviors in cycling female rats. Horm Behav 1995; 29: 42-58
45. Lei ZM, Rao CV. Neural actions of luteinizing hormone and human chorionic gonadotropin. Semin Reprod Med 2001; 19: 103-9
46. Temeli E, Oprescu M, Coculescu M, et al. LH and FSH levels in serum and cerebrospinal fluid (CSF) of human fetus. Endocrinologie 1985; 23: 55-9
47. Al-Hader AA, Lei ZM, Rao CV. Neurons from fetal rat brains contain functional luteinizing hormone/chorionic gonadotropin receptors. Biol Reprod 1997; 56: 1071-6
48. Al-Hader AA, Lei ZM, Rao CV. Novel expression of functional luteinizing hormone/chorionic gonadotropin receptors in cultured glial cells from neonatal rat brains. Biol Reprod 1997; 56: 501-7
49. Lei ZM, Rao CV, Kornyei JL, et al. Novel expression of human chorionic gonadotropin/luteinizing hormone receptor gene in brain. Endocrinology 1993; 132: 2262-70
50. Bowen RL, Smith MA, Harris PL, et al. Elevated luteinizing hormone expression colocalizes with neurons vulnerable to Alzheimer's disease pathology. J Neurosci Res 2002; 70: 514-8
51. Bowser R, Smith MA. Cell cycle proteins in Alzheimer's disease: plenty of wheels but no cycle. J Alzheimers Dis 2002; 4: 249-54
52. Raina AK, Monteiro MJ, McShea A, et al. The role of cell cycle-mediated events in Alzheimer's disease. Int J Exp Pathol 1999; 80: 71-6
53. Zhu X, Raina AK, Smith MA. Cell cycle events in neurons: proliferation or death? Am J Pathol 1999; 155: 327-9
54. Raina AK, Takeda A, Smith MA. Mitotic neurons: a dogma succumbs. Exp Neurol 1999; 159: 248-9
55. Zhu X, Raina AK, Perry G, et al. Alzheimer's disease: the two-hit hypothesis. Lancet Neurol 2004; 3: 219-26
56. McShea A, Harris PL, Webster KR, et al. Abnormal expression of the cell cycle regulators P16 and CDK4 in Alzheimer's disease. Am J Pathol 1997; 150: 1933-9
57. Zhu X, McShea A, Harris PL, et al. Elevated expression of a regulator of the G2/M phase of the cell cycle, neuronal CIP-1-associated regulator of cyclin B, in Alzheimer's disease. J Neurosci Res 2004; 75: 698-703
58. Ogawa O, Zhu X, Lee HG, et al. Ectopic localization of phosphorylated histone H3 in Alzheimer's disease: a mitotic catastrophe? Acta Neuropathol (Berl) 2003; 105: 524-8
59. Ogawa O, Lee HG, Zhu X, et al. Increased p27, an essential component of cell cycle control, in Alzheimer's disease. Aging Cell 2003; 2: 105-10
60. Zhu X, Rottkamp CA, Boux H, et al. Activation of p38 kinase links tau phosphorylation, oxidative stress, and cell cycle-related events in Alzheimer disease. J Neuropathol Exp Neurol 2000; 59: 880-8
61. Zhu X, Raina AK, Boux H, et al. Activation of oncogenic pathways in degenerating neurons in Alzheimer disease. Int J Dev Neurosci 2000; 18: 433-7

62. Hirai K, Aliev G, Nunomura A, et al. Mitochondrial abnormalities in Alzheimer's disease. J Neurosci 2001; 21: 3017-23

63. Perry G, Roder H, Nunomura A, et al. Activation of neuronal extracellular receptor kinase (ERK) in Alzheimer disease links oxidative stress to abnormal phosphorylation. Neuroreport 1999; 10: 2411-5

64. Vincent I, Zheng JH, Dickson DW, et al. Mitotic phosphoepitopes precede paired helical filaments in Alzheimer's disease. Neurobiol Aging 1998; 19: 287-96

65. Zhu X, Webber KM, Casadesus G, et al. Mitotic and gender parallels in Alzheimer disease: therapeutic opportunities. Curr Drug Targets 2004; 5: 559-63

66. Nunomura A, Perry G, Aliev G, et al. Oxidative damage is the earliest event in Alzheimer disease. J Neuropathol Exp Neurol 2001; 60: 759-67

67. German DC, Eisch AJ. Mouse models of Alzheimer's disease: insight into treatment. Rev Neurosci 2004; 15: 353-69

68. Raina AK, Zhu X, Rottkamp CA, et al. Cyclin' toward dementia: cell cycle abnormalities and abortive oncogenesis in Alzheimer disease. J Neurosci Res 2000; 61: 128-33

69. Harris D, Bonfil D, Chuderland D, et al. Activation of MAPK cascades by GnRH: ERK and Jun N-terminal kinase are involved in basal and GnRH-stimulated activity of the glycoprotein hormone LHbeta-subunit promoter. Endocrinology 2002; 143: 1018-25

70. Bowen RL, Verdile G, Liu T, et al. Luteinizing hormone, a reproductive regulator that modulates the processing of amyloid-beta precursor protein and amyloid-beta deposition. J Biol Chem 2004; 279: 20539-45

71. Casadesus G, Webber KM, Atwood CS, et al. Luteinizing hormone modulates cognition and amyloid-beta deposition in Alzheimer APP transgenic mice. Biochim Biophys Acta 2006; 1762: 447-52

72. Antigonadotropin-Leuprolide in Alzheimer's Disease Drug INvestigation (ALADDIN) VP 104 Study [online]. Available from URL: http://www.clinicaltrials.gov/ct/show/NCT00076440 [Accessed 2006 Mar 16]

73. Voyager Pharmaceutical Corp [online]. Available from URL: http://www.secinfo.com/d14D5a.z6483.htm, pp. 56-64 [Accessed 2006 Mar 16]

74. Hogervorst E, Combrinck M, Smith AD. Testosterone and gonadotropin levels in men with dementia. Neuro Endocrinol Lett 2003; 24: 203-8

75. Hogervorst E, Williams J, Combrinck M, et al. Measuring serum oestradiol in women with Alzheimer's disease: the importance of the sensitivity of the assay method. Eur J Endocrinol 2003; 148: 67-72

76. Casadesus G, Zhu X, Atwood CS, et al. Beyond estrogen: targeting gonadotropin hormones in the treatment of Alzheimer's disease. Curr Drug Targets CNS Neurol Disord 2004; 3: 281-5

77. Smith MA, Perry G, Atwood CS, et al. Estrogen replacement and risk of Alzheimer disease. JAMA 2003; 289: 1100

Correspondence and offprints: Dr *Mark A. Smith*, Department of Pathology, Case Western Reserve University, 2103 Cornell Road, Cleveland, OH 44106, USA.
E-mail: mark.smith@case.edu