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Evidence for the role of gonadotropin hormones in the development of Alzheimer disease

G. Casadesus[a], C. S. Atwood[b], X. Zhu[a], A. W. Hartzler[a], K. M. Webber[a], G. Perry[a], R. L. Bowen[c] and M. A. Smith[a,*]

[a] Institute of Pathology, Case Western Reserve University, 2085 Adelbert Road, Rm 501, Cleveland Ohio 44106 (USA), Fax: + 216 368 8964, e-mail: mark.smith@case.edu
[b] School of Medicine, University of Wisconsin and William S. Middleton Memorial Veterans Administration, Madison, Wisconsin 53705 (USA)
[c] Voyager Pharmaceutical Corporation, Raleigh, North Carolina 27615 (USA)

Abstract. Differences in the prevalence and age of onset of Alzheimer disease (AD) in men and women, and observations that hormone replacement therapy (HRT) may prevent the development of AD, caused many to hypothesize that estrogen deficiency contributes to AD. However, recent trials using estrogen failed to show any benefit in preventing or alleviating the disease. To address this and other inconsistencies in the estrogen hypothesis, we suspect that another hormone of the hypothalamic-pituitary-gonadal axis, luteinizing hormone (LH), as a major factor in AD pathogenesis. Individuals with AD have elevated levels of LH when compared with controls, and both LH and its receptor are present in increased quantities in brain regions susceptible to degeneration in AD. LH is also known to be mitogenic, and could therefore initiate the cell cycle abnormalities known to be present in AD-affected neurons. In cell culture, LH increases amyloidogenic processing of amyloid-β protein precursor, and in animal models of AD, pharmacologic suppression of LH and FSH reduces plaque formation. Given the evidence supporting a pathogenic role for LH in AD, a trial of leuprolide acetate, which suppresses LH release, has been initiated in patients.

Key words. Hormone replacement therapy (HRT); luteinizing hormone (LH); leuprolide acetate.

Etiology of Alzheimer disease (AD)

Despite being the leading cause of dementia, the etiologic events that lead to the pathology of Alzheimer disease (AD), namely neuronal loss, neurofibrillary tangles, amyloid-β (Aβ) and senile plaques, remain unknown [1]. Current mechanistic hypotheses that attempt to explain AD are based on the pathological hallmarks. For example, the Aβ hypothesis [2], the most well accepted to date, is supported by the fact that familial forms of AD, resulting from mutations in either the Aβ protein precursor or presenilins-1/2, all affect the processing of Aβ [3]. However, while genetic evidence supports this theory, the fact that experimental manipulation of Aβ in cell or animal models does not yield the multitude of biochemical and cellular changes found in the human disease [4–6] hints at other factors being involved. In fact evidence now suggests that Aβ may be a consequence rather than causative factor in disease pathogenesis [7–9]; thus, alternative theories such as tau phosphorylation [10–12], oxidative stress [13], metal ion deregulation [14] and inflammation [15] are now emerging. However, it is likely that not a single theory but rather a combination of these may be necessary to be able to explain the spectrum of abnormalities found in this disorder. Unfortunately, the incomplete understanding of pathogenic mechanisms associated with AD has resulted in a lack of efficient therapeutic strategies such that, at present, the disease is primarily targeted by palliative treatment of symptoms rather than by attempting to forestall the progression of the disease. Therefore, urgent attention is needed to resolve the mechanistic underpinnings of AD. The role of sex steroids, and particularly estrogen, in AD has been given a prominent role because of its rather unique multi-encompassing role in the development of disease, impinging on factors such as age, genetics and environment. However, recent contradictory reports, based on a randomized clinical trial, regarding the effects

* Corresponding author.

of HRT on AD incidence and progression have created much confusion and significantly lessened interest in the role of estrogen in AD [16–18]. In this review we propose that the hypothalamic-pituitary-gonadal (HPG) axis remains a major player in this disease, but suggest that it is not sex steroids, such as estrogen, but rather gonadotropins, which are key elements in the development of AD. Of prime importance, we provide a potential mechanism of action as well as a new therapeutic avenue to treat this insidious disease.

Sex steroids in the etiology of AD

The decrease in the serum concentrations of these neurotrophic steroids following menopause (and andropause) is associated with disease progression and has been based on epidemiological studies and findings that estrogen deficiency in vitro and in vivo promotes biochemical and neuropathological changes consistent with those observed in the aging and AD brains (see previous reviews).

Experimental support for sex steroids in promoting neurodegenerative-like changes has come primarily from studies demonstrating an increase in Aβ deposition following suppression of serum sex steroid concentrations (following menopause and ovariectomy/castration), and by the modulation of AßPP processing and Aβ generation by sex steroids in vitro. At high concentrations of sex steroids, 17β-Estradiol and testosterone have been shown to alter neuronal Aβ protein precursor (AβPP) processing towards the non-amyloidogenic pathway both in vitro and in vivo. 17β-Estradiol at concentrations between 2 and 2000 nM 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 [19–22]. Likewise, testosterone treatment (200–2000 nM) of mouse neuroblastoma cells and rat primary cerebrocortical neurons increases secretion of sAβPPα and decreases the secretion of Aβ [23]. Ovariectomy, which suppresses serum estrogen levels, also has been shown to increase total Aβ concentrations in guinea pigs [22] and AβPP transgenic mice [24]. Conversely, 17β-estradiol treatment was shown to partially and totally reverse the effects of ovariectomy in guinea pigs [25] and AβPP transgenic mice (Tg2576 and Tg2576 × mutant PS1; [24]), respectively. A more recent study using AβPPSWE transgenic mice did not see a significant increase in brain Aß following ovariectomy [26]. One difference that might explain this discrepancy is that the APPSWE mice were ovariectomized prior to puberty (4 weeks), while the Tg2576 mice were ovariectomized after puberty, indi cating that establishment of an intact HPG axis is important with regards to AβPP processing and pathologic deposition of Aβ. In humans, one study has shown a higher incidence of amyloid plaques in the brains of women, together with a greater age-related increase in the incidence rate of plaques [27]. Interpretation of these results must be carefully considered, since changes in AβPP processing and Aβ generation in all the above studies could also be explained by the changes in serum concentrations of other hormones induced by the loss of the sex steroids. Likewise, the high concentrations of sex steroids used in vitro have been shown to alter LH receptor number [19], and also may be exerting their effects indirectly. The results indicate the importance of maintaining serum sex steroid concentrations, but may fall short of a complete mechanistic explanation of how the axis modulates AβPP processing and Aβ generation.

The role of gonadotropins in AD

Despite the large body of evidence supporting the protective role of estrogen in AD, controversy regarding the benefits of HRT in the disease clearly reveals that falling levels of steroid hormones associated with menopause/andropause do not successfully account for patterns of AD susceptibility; only when one takes into account the role of the other hormones of the HPG axis, during i.e. peri-menopause and beyond, that the events leading to AD pathogenesis can be accurately characterized.

Hormones of the HPG axis include gonadotropin-releasing hormone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, progesterone, testosterone, activin, inhibin and follistatin. Each of these hormones, by participating in a complex feedback loop initiated by the hypothalamic secretion of gonadotropin-releasing hormone (reviewed in [28]) and, via the stimulation of anterior pituitary hormones such as LH, lead to stimulation of oogenesis/spermatogenesis, and production of the sex steroids ultimately decreases gonadotropin secretion from the hypothalamus and pituitary gland. Importantly, menopause and andropause can shift the balance of this feedback loop, and this shift results in an increase in the production of gonadotropins along with the bioavailability of activin and a decrease in gonadal inhibin production in both women [29, 30] and men [31].

Surprisingly, despite the tight relationship between sex steroids and gonadotropins, the effects of increased circulating LH or FSH due to the loss of negative feedback on the aging brain remain unexplored. In fact if these secondary hormonal changes are taken into account, it can be speculated that benefits of HRT may be dependent on the integrity of the HPG axis feedback loop. To this end, the levels of gonadotropins, including LH, are highest during perimenopause and early menopause [32],

when HRT has been observed to be most successful in preventing dementia [33]. Therefore it is possible that during perimenopause and early menopause, when the HPG axis is still functional, estrogen therapy is beneficial. Once this loop is shut down after chronic low levels of estrogen and high levels of gonadotropins such as LH, as later in menopause, HRT loses therapeutic value or may even become detrimental [34].

Elevations in LH are associated with AD pathology

Epidemiological data demonstrate that patients with AD have higher levels of LH than do individuals who do not develop the disease [35, 36]. Moreover, in addition to explaining the therapeutic controversies associated with HRT and dementia, increasing LH levels can also explain the higher predominance for developing AD in females when compared to males [37–41]. In this regard, LH levels are significantly higher in females as compared to males [29, 30]. Further evidence supporting gnadotropins, and in particular LH, as a central mediator of disease stems from examining individuals with Down syndrome who develop precocious AD-type pathology and cognitive alterations. To this end, in Down syndrome, there is a gender-reversal in the incidence of AD-type changes such that males with this disorder, and not females, are at significantly higher risk for developing AD-type changes. Interestingly, in this population while sex steroid levels are comparable to those found in non-Down syndrome individuals, contrary to what we see in a the normal population where females have higher LH levels than males [30, 31] after menopause/andropause, LH levels in Down syndrome males are much higher than in females [42]. Therefore, LH levels account for the gender reversal seen in this syndrome, and with regard to AD, represents and affords the only explanation thus far for the gender selectivity in AD and Down syndrome.

More recently, studies have begun to support these epidemiological data with mechanistic evidence. In this regard, several findings now indicate an experimental link between elevations in LH and the development of AD (reviewed in [43]). For example, LH can cross the blood-brain barrier [44] and thus may play a direct driving pathogenic force in this disease [45]. In support of this view, neurons of individuals with AD show significant elevations of LH when compared to aged-matched control cases [45]. More important, these elevations parallel the distribution of LH receptors [46], which show the highest density within the hippocampus [46–48], the most vulnerable and affected area in AD [1]. Because such increases in LH appear to be a very early event in disease history and are predictive of neuronal populations at risk of degeneration and death, these findings suggest that LH is likely contribute to other pathological events seen in the disease indicating a cause-effect relationship [49–51]. Further support for the role of LH in AD stems from its capacity to modulate neurodegenerative-like changes such as Aβ deposition, the most prominent pathological entity. In this regard, addition of LH to neuroblastoma cells has been shown to alter AβPP processing toward the amyloidogenic pathway, as evidenced by increased generation and secretion of Aβ, decreased AβPP secretion, and increased AβPPCT100 levels [19]. In this regard, the expression of presenilin-1 and -2 genes, 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 [52]. Confirming these results, suppression of LH with the anti-gonadotropin drug leuprolide acetate in mice leads to AD-like changes in AβPP processing [45]. Notably, alterations in AβPP processing were due to the suppression of LH [19], since decreased brain Aβ followed treatment with leuprolide acetate (i.e. ablation of LH) but not with the loss of sex steroids [20, 23, 53]. More important, suppression of gonadotropins with leuprolide acetate improves cognitive performance and decreases Aβ deposition in transgenic mice carrying the AβPP with the Swedish mutation [54].

Based on the aforementioned notion that LH is a driving pathogenic force in AD, leuprolide acetate, a gonadotropin-releasing hormone agonist which has been shown to suppress luteinizing hormone to undetectable levels by downregulating pituitary gonadotropin-releasing hormone receptors, might be an effective method of treatment for patients with AD. As stated above, administration of leuprolide acetate to C57BL/6 mice produced significant decreases of brain Aβ [19] and improved cognitive function in a transgenic mouse model of AD, improvements that correlated to decreases in Aβ-burden [54]. Importantly, the latter study used mice at a very advanced stage of cognitive decline, indicating therapeutic efficacy at later stages of disease. Therefore, targeting LH release may be a successful strategy to combat the incidence as well as the progression of AD [43, 55].

Mechanistic link between gonadotropins and AD

The above evidence demonstrates that gonadotropins such as LH may indeed play an important role in the development of AD. However, successful treatment strategies can only stem from careful examination of the mechanisms involved in such actions. Previously, we have discussed that elevations of LH levels in vulnerable neuronal populations of patients with AD compared to aged-matched control cases [45] appear to be a very early change in disease history serving to predict neuronal populations at risk of degeneration and death. One early change that temporally

matches these elevations in LH and that is also observed in these vulnerable neuronal populations is the expression of cell-cycle markers that are not associated with post-mitotic cellular populations. Neuronal changes supporting the involvement of cell cycle-related events in the etiology of the disease include the ectopic expression of markers of the cell cycle [56], organelle kinesis [57] and cytoskeletal alterations, including tau phosphorylation [58]; in fact, these neurons seem to attempt an unscheduled initiation of a mitotic division cycle which in a mature, normally post-mitotic neuron leads to an abortive reactivation of a variety of cell cycle components and ultimately the demise of the cell [8, 49, 59]. With regard to LH, this gonadotropin hormone has powerful mitogenic properties [60, 61]. For example, restoration of LH levels after testosterone and estrogen-induced LH deprivation leads to increased proliferation of leydig cells in the testis of adult rats [62]. Likewise, LH levels are associated with increased T-cell proliferation and activation [63, 64] and peripheral blood lymphocyte proliferation [65]. Finally, LH secretion has been associated with increased proliferation of porcine granulosa cells and activation of mitogen activated protein kinases such as extracellular signal-regulated kinase [66], and other signal transduction and transcription activators [67], all of which play a major role in cell cycle events [68] and, more important, are associated with AD pathology [12, 58, 69–71].

Based on the above evidence and considering that elevation of LH parallels the ectopic expression of cell cycle and oxidative markers that represent one of the initiating pathological changes preceding neuronal degeneration by decades [50, 51], it is likely, given this temporal and spatial overlap with mitotic changes in AD [unpublished observations], that elevations in LH are responsible for inappropriate cell cycle reentry in neurons [45, 49]. Obviously, such a notion does not preclude the involvement of other hormones of the HPG axis, such as activin or inhibin, that also exhibit significant changes in serum concentrations during aging, however, these findings provide the first evidence for a potential mechanism by which elevations in LH may take part in the development and progression of AD.

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1 Smith M. A. (1998) Alzheimer disease. Int. Rev. Neurobiol. **42:** 1–54
2 Selkoe D. J. (2003) Aging, amyloid and Alzheimer's disease: a perspective in honor of Carl Cotman. Neurochem. Res. **28:** 1705–1713
3 Selkoe D. J. (1997) Alzheimer's disease: genotypes, phenotypes and treatments. Science **275:** 630–631
4 Savonenko A. V., Xu G. M., Price D. L., Borchelt D. R. and Markowska A. L. (2003) Normal cognitive behavior in two distinct congenic lines of transgenic mice hyperexpressing mutant APP SWE. Neurobiol. Dis. **12:** 194–211
5 Takeuchi A., Irizarry M. C., Duff K., Saido T. C., Hsiao Ashe K., Hasegawa M. et al. (2000) Age-related amyloid beta deposition in transgenic mice overexpressing both Alzheimer mutant presenilin 1 and amyloid beta precursor protein Swedish mutant is not associated with global neuronal loss. Am. J. Pathol. **157:** 331–339
6 Irizarry M. C., Soriano F., McNamara M., Page K. J., Schenk D., Games D. et al. (1997) 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. **17:** 7053–7059
7 Perry G., Nunomura A., Raina A. K. and Smith M. A. (2000) Amyloid–beta junkies. Lancet **355:** 757
8 Obrenovich M. E., Joseph, J.A., Atwood, C.S., Perry, G., Smith, M.A. (2003) Alzheimer Disease – A New Beginning, or a Final Exit? Landes Bioscience, TX
9 Rottkamp C. A., Atwood C. S., Joseph J. A., Nunomura A., Perry G. and Smith M. A. (2002) The state versus amyloid-beta: the trial of the most wanted criminal in Alzheimer disease. Peptides **23:** 1333–1341
10 Trojanowski J. Q., Clark C. M., Arai H. and Lee V. M. (1999) Elevated levels of tau in cerebrospinal fluid: implications for the antemortem diagnosis of alzheimer's disease. J. Alzheimers Dis. **1:** 297–305
11 Avila J. (2000) Tau aggregation into fibrillar polymers: taupathies. FEBS Lett. **476:** 89–92
12 Zhu X., Lee H. G., Raina A. K., Perry G. and Smith M. A. (2002) The role of mitogen-activated protein kinase pathways in Alzheimer's disease. Neurosignals **11:** 270–281
13 Perry G., Castellani R. J., Hirai K. and Smith M. A. (1998) Reactive oxygen species mediate cellular damage in Alzheimer disease. J. Alzheimers Dis. **1:** 45–55
14 Perry G., Sayre L. M., Atwood C. S., Castellani R. J., Cash A. D., Rottkamp C. A. et al. (2002) The role of iron and copper in the aetiology of neurodegenerative disorders: therapeutic implications. CNS Drugs **16:** 339–352
15 Attwood C. S., Huang X., Moir R. D., Smith M. A., Tanzi R. E., Roher A. E. et al. (2001) Neuroinflammatory Responses in the Alzheimer's Disease Brain Promote the Oxidative Post-Translational Modification of Amyloid Deposits, John Wiley, UK
16 Shumaker S. A., Legault C., Rapp S. R., Thal L., Wallace R. B., Ockene J. K. et al. (2003) 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 **289:** 2651–2662
17 Mulnard R. A. (2000) Estrogen as a treatment for Alzheimer disease. JAMA **284:** 307–308
18 Mulnard R. A., Cotman C. W., Kawas C., van Dyck C. H., Sano M., Doody R. et al. (2000) Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: a randomized controlled trial. Alzheimer's Disease Cooperative Study. JAMA **283:** 1007–1015
19 Bowen R. L., Verdile G., Liu T., Parlow A. F., Perry G., Smith M. A. et al. (2004) Luteinizing hormone, a reproductive regulator that modulates the processing of amyloid-beta precursor protein and amyloid-beta deposition. J. Biol. Chem. **279:** 20539–20545
20 Jaffe A. B., Toran-Allerand C. D., Greengard P. and Gandy S. E. (1994) Estrogen regulates metabolism of Alzheimer amyloid beta precursor protein. J. Biol. Chem. **269**: 13065–13068
21 Manthey D., Heck S., Engert S. and Behl C. (2001) Estrogen induces a rapid secretion of amyloid beta precursor protein via the mitogen-activated protein kinase pathway. Eur. J. Biochem. **268:** 4285–4291
22 Chang D., Kwan J. and Timiras P. S. (1997) Estrogens influence growth, maturation and amyloid beta-peptide production in

neuroblastoma cells and in a beta-APP transfected kidney 293 cell line. Adv. Exp. Med. Biol. **429:** 261–271
23 Gouras G. K., Xu H., Gross R. S., Greenfield J. P., Hai B., Wang R. et al. (2000) Testosterone reduces neuronal secretion of Alzheimer's beta-amyloid peptides. Proc. Natl. Acad. Sci. USA **97:** 1202–1205
24 Zheng H., Xu H., Uljon S. N., Gross R., Hardy K., Gaynor J. et al. (2002) Modulation of A(beta) peptides by estrogen in mouse models. J. Neurochem. **80:** 191–196
25 Petanceska S. S., Nagy V., Frail D. and Gandy S. (2000) Ovariectomy and 17beta-estradiol modulate the levels of Alzheimer's amyloid beta peptides in brain. Exp. Gerontol. **35:** 1317–1325
26 Levin-Allerhand J. A. and Smith J. D. (2002) Ovariectomy of young mutant amyloid precursor protein transgenic mice leads to increased mortality. J. Mol. Neurosci. **19:** 163–166
27 Stam F. C., Wigboldus J. M. and Smeulders A. W. (1986) Age incidence of senile brain amyloidosis. Pathol. Res. Pract. **181:** 558–562
28 Genazzani A. R., Gastaldi M., Bidzinska B., Mercuri N., Genazzani A. D., Nappi R. E. et al. (1992) The brain as a target organ of gonadal steroids. Psychoneuroendocrinology **17:** 385–390
29 Couzinet B. and Schaison G. (1993) The control of gonadotrophin secretion by ovarian steroids. Hum. Reprod. **8 Suppl. 2:** 97–101
30 Chakravarti S., Collins W. P., Forecast J. D., Newton J. R., Oram D. H. and Studd J. W. (1976) Hormonal profiles after the menopause. Br. Med. J. **2:** 784–787
31 Neaves W. B., Johnson L., Porter J. C., Parker C. R. Jr and Petty C. S. (1984) Leydig cell numbers, daily sperm production, and serum gonadotropin levels in aging men. J. Clin. Endocrinol. Metab. **59:** 756–763
32 Burger H. G. (1996) The endocrinology of the menopause. Maturitas **23:** 129–136
33 Henderson V. W. (2004) Hormone therapy and Alzheimer's disease: benefit or harm? Expert. Opin. Pharmacother. **5:** 389–406
34 Henderson V. W., Guthrie J. R., Dudley E. C., Burger H. G. and Dennerstein L. (2003) Estrogen exposures and memory at midlife: a population-based study of women. Neurology **60:** 1369–1371
35 Bowen R. L., Isley J. P. and Atkinson R. L. (2000) An association of elevated serum gonadotropin concentrations and Alzheimer disease? J. Neuroendocrinol. **12:** 351–354
36 Short R. A., Bowen R. L., O'Brien P. C. and Graff-Radford N. R. (2001) Elevated gonadotropin levels in patients with Alzheimer disease. Mayo Clin. Proc. **76:** 906–909
37 Breitner J. C., Silverman J. M., Mohs R. C. and Davis K. L. (1988) 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 **38:** 207–212
38 Jorm A. F. and Jolley D. (1998) The incidence of dementia: a meta-analysis. Neurology **51:** 728–733
39 McGonigal G., Thomas B., McQuade C., Starr J. M., MacLennan W. J. and Whalley L. J. (1993) Epidemiology of Alzheimer's presenile dementia in Scotland, 1974–88. BMJ. **306:** 680–683
40 Rocca W. A., Hofman A., Brayne C., Breteler M. M., Clarke M., Copeland J. R. et al. (1991) Frequency and distribution of Alzheimer's disease in Europe: a collaborative study of 1980–1990 prevalence findings. The EURODEM-Prevalence Research Group. Ann. Neurol. **30:** 381–390
41 Jorm A. F., Korten A. E. and Henderson A. S. (1987) The prevalence of dementia: a quantitative integration of the literature. Acta Psychiatr. Scand. **76:** 465–479
42 Schupf N., Kapell D., Nightingale B., Rodriguez A., Tycko B. and Mayeux R. (1998) Earlier onset of Alzheimer's disease in men with Down syndrome. Neurology **50:** 991–995
43 Webber K. B. R., Casadesus G., Perry G., Atwood C. S. and Smith M. A. (2004) Gonadotropins and Alzheimer's disease: the link between estrogen replacement therapy and neuroprotection. Acta Neurobiol. Exp. **64:** 113–118
44 Lukacs H., Hiatt E. S., Lei Z. M. and Rao C. V. (1995) Peripheral and intracerebroventricular administration of human chorionic gonadotropin alters several hippocampus-associated behaviors in cycling female rats. Horm. Behav. **29:** 42–58
45 Bowen R. L., Smith M. A., Harris P. L., Kubat Z., Martins R. N., Castellani R. J. et al. (2002) Elevated luteinizing hormone expression colocalizes with neurons vulnerable to Alzheimer's disease pathology. J. Neurosci. Res. **70:** 514–518
46 Lei Z. M., Rao C. V., Kornyei J. L., Licht P. and Hiatt E. S. (1993) Novel expression of human chorionic gonadotropin/luteinizing hormone receptor gene in brain. Endocrinology **132:** 2262–2270
47 Al-Hader A. A., Lei Z. M. and Rao C. V. (1997) Novel expression of functional luteinizing hormone/chorionic gonadotropin receptors in cultured glial cells from neonatal rat brains. Biol. Reprod. **56:** 501–507
48 Al-Hader A. A., Lei Z. M. and Rao C. V. (1997) Neurons from fetal rat brains contain functional luteinizing hormone/chorionic gonadotropin receptors. Biol. Reprod. **56:** 1071–1076
49 Raina A. K., Zhu X., Rottkamp C. A., Monteiro M., Takeda A. and Smith M. A. (2000) Cyclin' toward dementia: cell cycle abnormalities and abortive oncogenesis in Alzheimer disease. J. Neurosci. Res. **61:** 128–133
50 Ogawa O., Lee H. G., Zhu X., Raina A., Harris P. L., Castellani R. J. et al. (2003) Increased p27, an essential component of cell cycle control, in Alzheimer's disease. Aging Cell **2:** 105–110
51 Nunomura A., Perry G., Aliev G., Hirai K., Takeda A., Balraj E. K. et al. (2001) Oxidative damage is the earliest event in Alzheimer disease. J. Neuropathol. Exp.Neurol. **60:** 759–767
52 Rimon E., Sasson R., Dantes A., Land-Bracha A. and Amsterdam A. (2004) Gonadotropin-induced gene regulation in human granulosa cells obtained from IVF patients: modulation of genes coding for growth factors and their receptors and genes involved in cancer and other diseases. Int. J. Oncol. **24:** 1325–1338
53 Xu H., Gouras G. K., Greenfield J. P., Vincent B., Naslund J., Mazzarelli L. et al. (1998) Estrogen reduces neuronal generation of Alzheimer beta-amyloid peptides. Nat. Med. **4:** 447–451
54 Casadesus G., Ogawa, O., Bowen, R.L., Atwood, C.S., Perry, G., Smith, M.A. (2003) Modulation of hippocampal plasticity and cognition in the swAPP transgenic mouse by the gonadotropin-releasing hormone agonist leuprolide acetate: involvement of luteinizing hormone in the pathogenesis and treatment of AD. Soc. Neuroci Abs., Program No. 945, 8, 2003
55 Smith M. A., Perry G., Atwood C. S. and Bowen R. L. (2003) Estrogen replacement and risk of Alzheimer disease. JAMA **289:** 1100; author reply 1101–1102
56 McShea A., Harris P. L., Webster K. R., Wahl A. F. and Smith M. A. (1997) Abnormal expression of the cell cycle regulators P16 and CDK4 in Alzheimer's disease. Am. J. Pathol. **150:** 1933–1939
57 Hirai K., Aliev G., Nunomura A., Fujioka H., Russell R. L., Atwood C. S. et al. (2001) Mitochondrial abnormalities in Alzheimer's disease. J. Neurosci. **21:** 3017–3023
58 Zhu X., Raina A. K., Boux H., Simmons Z. L., Takeda A. and Smith M. A. (2000) Activation of oncogenic pathways in degenerating neurons in Alzheimer disease. Int. J. Dev. Neurosci. **18:** 433–437

59 Bowser R. and Smith M. A. (2002) Cell cycle proteins in Alzheimer's disease: plenty of wheels but no cycle. J. Alzheimers. Dis. **4:** 249–254
60 Sriraman V., Rao V. S., Rajesh N., Vasan S. S. and Rao A. J. (2001) A preliminary study on the possible role for luteinizing hormone in androgen independent growth of prostate. Reprod. Biomed. Online **3:** 6–13
61 Harris D., Bonfil D., Chuderland D., Kraus S., Seger R. and Naor Z. (2002) 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 **143:** 1018–1025
62 Keeney D. S., Sprando R. L., Robaire B., Zirkin B. R. and Ewing L. L. (1990) Reversal of long-term LH deprivation on testosterone secretion and Leydig cell volume, number and proliferation in adult rats. J. Endocrinol. **127:** 47–58
63 Athreya B. H., Pletcher J., Zulian F., Weiner D. B. and Williams W. V. (1993) Subset-specific effects of sex hormones and pituitary gonadotropins on human lymphocyte proliferation in vitro. Clin. Immunol. Immunopathol. **66:** 201–211
64 Sabharwal P., Varma S. and Malarkey W. B. (1992) Human thymocytes secrete luteinizing hormone: an autocrine regulator of T-cell proliferation. Biochem. Biophys. Res. Commun. **187:** 1187–1192
65 Costa O., Bouthet C., Sauvage P., Michel J. P. and Deschaux P. (1990) Age-dependent LH and FSH effect on the proliferation of women's peripheral blood lymphocytes in vitro. Int. J. Immunopharmacol. **12:** 821–829
66 Cameron M. R., Foster J. S., Bukovsky A. and Wimalasena J. (1996) Activation of mitogen-activated protein kinases by gonadotropins and cyclic adenosine 5′-monophosphates in porcine granulosa cells. Biol. Reprod. **55:** 111–119
67 Carvalho C. R., Carvalheira J. B., Lima M. H., Zimmerman S. F., Caperuto L. C., Amanso A. et al. (2003) Novel signal transduction pathway for luteinizing hormone and its interaction with insulin: activation of Janus kinase/signal transducer and activator of transcription and phosphoinositol 3-kinase/Akt pathways. Endocrinology **144:** 638–647
68 Rubinfeld H. and Seger R. (2004) The ERK cascade as a prototype of MAPK signaling pathways. Methods Mol. Biol. **250:** 1–28
69 Zhu X., Castellani R. J., Takeda A., Nunomura A., Atwood C. S., Perry G. et al. (2001) Differential activation of neuronal ERK, JNK/SAPK and p38 in Alzheimer disease: the 'two hit' hypothesis. Mech. Ageing. Dev. **123:** 39–46
70 Zhu X., Raina A. K., Perry G. and Smith M. A. (2004) Alzheimer's disease: the two-hit hypothesis. Lancet Neurol. **3:** 219–226
71 Perry G., Roder H., Nunomura A., Takeda A., Friedlich A. L., Zhu X. et al. (1999) Activation of neuronal extracellular receptor kinase (ERK) in Alzheimer disease links oxidative stress to abnormal phosphorylation. Neuroreport **10:** 2411–2415