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Novel therapeutic strategies for Alzheimer's disease based on the forgotten reproductive hormones

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Abstract. The relationship between hormones and Alzheimer's disease (AD) has been intensely researched. While the majority of this work has focused on the sex steroids, estrogens, and more recently androgens, a serendipitous patient encounter led one of us (R.L.B.) to question whether other hormones of the hypothalamic-pituitary-gonadal axis might play a role in the pathogenesis of AD. The age-related decline in reproductive function results in a dramatic decrease in serum estrogen and testosterone concentrations and an equally dramatic compensatory increase in serum gonadotropin concentrations. Indeed, there is growing evidence that the gonadotropin luteinizing hormone, which regulates serum estrogen and testosterone concentrations, is an important causative factor in the development of AD. This review provides information supporting the 'gonadotropin hypothesis'. We put forth a novel mechanism of how changes in serum luteinizing hormone concentrations could contribute to the pathogenesis of AD and discusses potential therapeutic anti-gonadotropin compounds.

Key words. Alzheimer's disease; FSH; LH; GnRH analogue.

Introduction

It has long been postulated that hormonal manipulation may be of benefit in AD. Early epidemiological studies comparing women who took estrogen replacement therapy (ERT) suggested estrogen confers protection against the disease [1–3]. However, a subsequent large epidemiological study indicated that estrogen only exerts its protective effect if taken during a 'critical period' that exists during the onset of menopause and the first few years thereafter [4, 5]. Indeed, there was no benefit seen in prospective AD prevention studies of women who were given conjugated equine estrogen replacement later in life [6, 7]. We propose that one explanation for this is that estrogen is only one member of the complex hypothalamic-pituitary-gonadal (HPG) hormone feedback loop. In addition to estrogen and the other sex steroids, progesterone and testosterone, the HPG axis (fig. 1) comprises inhibins, activins, follistatin, gonadotropin-releasing hormone (GnRH), and the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Given this complex interactive system and the fact that these hormones have been shown to have multiple non-reproductive functions (see Atwood, this series), it is difficult to determine which of these hormones is the most important in the pathogenesis of AD.

The impetus for the gonadotropin hypothesis

The gonadotropin hypothesis was stimulated by a serendipitous patient encounter one of us (R.L.B.) had during the late 1990s. The patient, Mrs Louis Newton (permission obtained), was a post-menopausal female being counseled as to the risks and benefits of ERT. When informed that there was evidence that estrogen may provide protection from AD, she provided the following vignette. 'That's interesting. My husband has Alzheimer's disease. When his memory loss started to affect his daily activities, I took him to the doctor to be evaluated. He underwent a complete history and physical exam, and the doctor said that he probably had Alzheimer's disease. The doctor also found that he had prostate cancer, and in light of the Alzheimer's disease, he recommended conservative treatment with a hormone. Soon after receiving the hormone there was a slight improvement in my husband's memory, and it has stayed that way ever since. And that was more than five years ago.'

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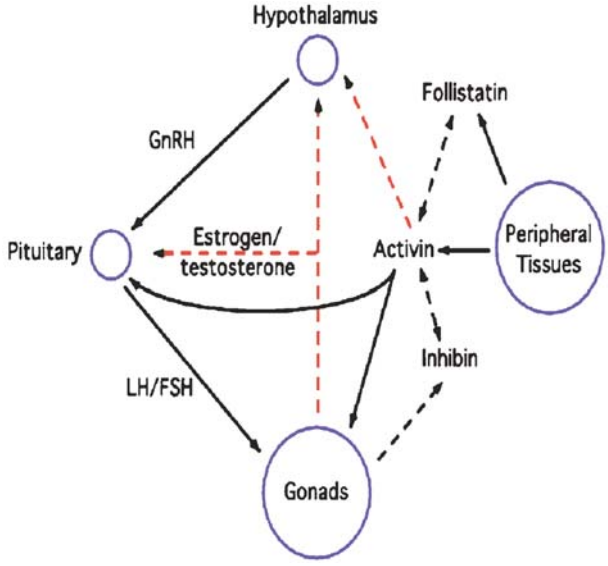

Figure 1. A schematic of the hypothalamic-pituitary-gonadal axis.

Initially, this observation seemed counterintuitive, since estrogen is supposed to be beneficial and the drug she was referring to, leuprolide acetate, suppresses estrogen in women and testosterone in men to castration levels. However, the sex steroid suppression is mediated by leuprolide acetate's suppression of pituitary LH secretion. Interestingly, the age-related decline in ovarian estrogen production results in an equally dramatic compensatory increase in serum LH concentrations, which is partially countered by estrogen replacement [8]. Therefore, we hypothesize that the detrimental effects attributed to a decrease in estrogen could be attributed to an increase in LH, and hence the gonadotropin hypothesis.

Evidence supporting a role for gonadotropins in AD

The gonadotropin hypothesis is consistent with numerous other findings regarding AD. In addition to the studies mentioned earlier, there are epidemiological studies revealing an approximate 2:1 ratio of female predominance of AD compared to males [9, 10] which is consistent with the earlier loss of reproductive function and an earlier increase in serum gonadotropin concentrations in women. LH is known to cross the blood-brain barrier [11], and LH receptors are expressed in the brain [12] with higher expression levels in brain regions susceptible to AD neuropathology [13, 14]. In addition, pyramidal neurons contain intracytoplasmic LH, and AD brains contain significantly greater amounts than age-matched controls [15]. Further evidence that gonadotropins may play a role in AD is found in studies from individuals with Down syndrome who have elevated serum concentrations of gonadotropins throughout life and develop AD-like neuropathology if they live into the fourth decade [16, 17]. Interestingly, males with Down syndrome develop these neuropathological changes earlier and more often than their female counterparts. The reversal in gender predilection in Down syndrome individuals from that seen in the general population cannot be explained by differences in sex steroid concentrations, since these levels are similar. However, the increase in gonadotropin concentrations is more pronounced and occurs at an earlier age in Down syndrome males than in Down syndrome females [18].

To test the gonadotropin hypothesis, two studies were performed to evaluate serum gonadotropin concentrations in patients with dementia. The first study revealed that male AD patients had significantly higher serum gonadotropin concentrations than age and sex-matched control patients: FSH (30.2 ± 3.8 vs. 15.8 ± 2.0 mIU/ml) ($p = 0.003$) and LH (8.1 ± 1.0 vs. 4.9 ± 0.8 mIU/ml) ($p = 0.01$) (fig. 2) [19]. A second study revealed that LH and FSH were significantly elevated in female AD patients who had never received estrogen replacement compared to female AD patients taking estrogen: (26.3 IU/L vs. 20.1 IU/L) ($p = 0.046$) (62.0 IU/L vs. 47.7 IU/L) ($p = 0.007$), respectively [20]. LH levels also were significantly higher in estrogen-free female AD patients compared to female frontotemporal patients: (26.3 IU/L vs. 20.7 IU/L) ($p = 0.0286$). In the same study, LH and FSH levels were elevated in male AD patients compared to normal controls in the univariate analysis: (6.2 IU/L vs. 4.7 IU/L) ($p = 0.037$) and (9.6 IU/ L vs. 7.6 IU/L) ($p = 0.060$). However, this did not hold for the multivariate analysis due to age differences between the AD and control group. To further assess differences among males, a paired analysis was performed, matching AD patients within 3 years of

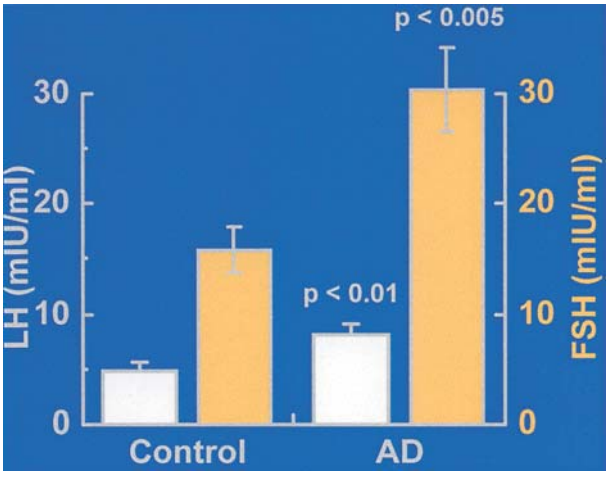

Figure 2. Serum LH and FSH levels were determined by radioimmunoassay in (AD) and control subjects. LH and FSH levels were higher in AD subjects compared to control ($p < 0.01$) and ($p < 0.005$), respectively. Control subjects ($n = 29$), AD subjects ($n = 40$).

age with normal subjects. Signed rank tests on the resulting 38 pairs were statistically significant for LH ($p = 0.048$), but not for FSH ($p = 0.342$) [20]. In contrast, Mayeux and colleagues saw no significant difference in serum gonadotropin concentrations between control and AD females [21]. This discrepancy may be explained by the fact that the mean age of the control group was 5.86 years younger than the AD group and that serum gonadotropin concentrations continuously decrease after the menopausal peak in women [22]. In a study by Hogervorst et al. that was limited to males, there was no difference in age between AD and control groups, and the AD group did have higher serum concentrations of gonadotropins [23]. The study also found that older male AD patients had higher gonadotropin concentrations than younger AD patients, but no such correlation was found for the control group. While this would seem to support the gonadotropin hypothesis, when the authors 'controlled' for the gonadotropin/age correlation in the AD group, this led to their contention that there was no difference in gonadotropin concentrations.

Lack of estrogen: not the whole story

Much of the research on estrogen's role in AD points to the likelihood that while there is an association of AD with estrogen, it does not appear that estrogen is playing the primary role. One large epidemiological study indicated that estrogen only exerts its protective effect if taken during a critical period that exists during the onset of menopause and the first few years thereafter [4]. Interestingly, the critical period for estrogen effectiveness correlates to the time when serum LH concentrations are at their peak and would be expected to cause the most damage. Suppressing the LH peak with estrogen might well prevent this damage and delay the onset of AD. One large prospective study in older postmenopausal women not only failed to show a protective effect of ERT, but there was even a slight increase in the risk for developing AD [6]. Likewise, studies of ERT in females who had already developed AD have produced mixed results, but the majority of females showed no benefit [24, 25]. These studies utilized conjugated equine estrogens and/or medroxyprogesterone, neither of which are endogenous human steroids [7]. The findings suggest that equine estrogens are similar to other estrogen receptor modulators such as tamoxifen, and thereby affect receptor activation differently than endogenous estrogens. Indeed, a small prospective study utilizing 17β-estradiol rather than conjugated equine estrogens has shown beneficial effects on cognition [26]. Although estrogen receptor modulators such as tamoxifen bind to the estrogen receptor, they are used as a treatment for breast cancer. Women who took equine estrogen experienced a decrease in the incidence of breast cancer. Similarly, the conjugated equine estrogens may not suppress serum gonadotropins as effectively as endogenous estrogens. This is supported by studies in women who underwent oophorectomy and received ERT [27] showing that gonadotropins remained elevated. Irrespective of this, the question remains whether estrogen replacement alone or the suppression of serum gonadotropins is responsible for the cognitive benefits. If the loss of sex steroids is responsible for neurodegeneration, then one might expect to see AD neuropathology during childhood, a time of life when circulating sex steroid concentrations are almost undetectable. Since this is not the case, it is becoming clear that the absence of estrogen alone is not enough to explain the pathogenesis of AD.

Gonadotropins and the cell cycle hypothesis

The cell cycle hypothesis of AD states that AD is a result of aberrant reentry of neurons into the cell cycle. This hypothesis is consistent with all known abnormalities found in the disease. Aberrant cell cycle reentry has been suggested by many to result from an age-related upregulation of an unknown mitogen. We suggest that LH is this mitogen. Human chorionic gonadotropin (hCG) and LH have been shown to be mitogenic in certain reproductive tissues [28–30] and are frequently expressed by tumor cells [31–33]. In addition, LH has been shown to activate extracellular signal-regulated protein kinase (ERK) and mitogen activated protein kinase (MAP) [34, 35]. Serum concentrations also correlate with periods of rapid growth: fetal life, the first year of life and puberty (fig. 3). Once reproductive maturity is reached, we believe the mitogenicity of LH is countered by the newly produced sex steroids and inhibins. However, protection against the

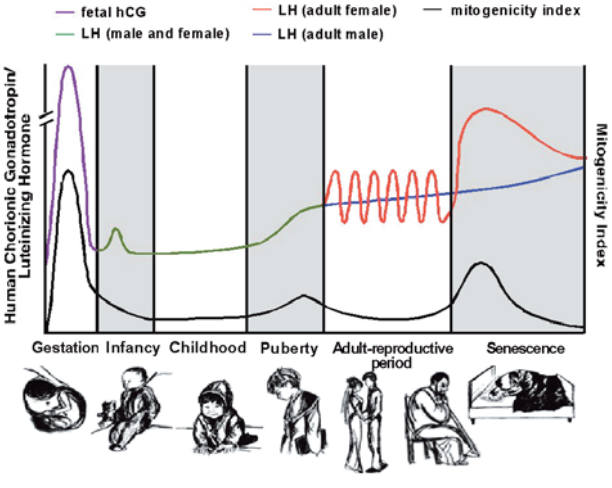

Figure 3. Gonadotropin secretion throughout life.

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mitogenic effects of LH is lost with the age-related decline in reproductive function, which results in a decrease in sex steroids and inhibins and an increase in LH. While this hormonal profile may be advantageous in the developing brain of the fetus, terminally differentiated adult neurons are likely unable to respond appropriately to the mitogenic stimulus, resulting in the neuronal dysfunction and death characteristic of AD.

LH as a diagnostic marker for AD?

In the early stages, AD diagnosis is far from precise, and it is thought that significant brain damage is already present at the time of diagnosis. One study of 650 autopsy cases with a clinical diagnosis of AD found that 22% had some other form of dementia [36]. Therefore, much research has focused on finding a quantifiable marker that could be used to make the diagnosis of AD. While progress is being made, especially with various neuroimaging techniques, a reliable serum marker of AD has remained elusive. If the age-associated increase in LH is playing a major role in the pathogenesis of AD, it begs the question, 'Can serum LH concentrations be used as a diagnostic marker?' Unfortunately, this is not likely the case. As mentioned previously, LH is a pulsatile hormone with a frequency of 20.6 ± 3.6 pulses/24 h [37], and peak serum concentrations are frequently 5–10-fold higher than trough concentrations. For an assay of serum LH to provide an accurate indication of circulating LH concentrations, it would require collection of serial specimens at least every 45 min for 24 h and calculation of the mean value [38]. However, even this would not provide a true picture of LH signaling due to polymorphisms of LH and its receptor, both of which affect ligand binding affinity. Since LH is part of a feedback loop, these polymorphisms would result in differences in LH serum concentrations but not necessarily a difference in receptor activation [39]. While serum LH concentrations would not be useful as a diagnostic tool for AD in a single individual, LH levels are useful in clinical studies with an appropriate number of subjects since the variations should even out and be randomized equally between groups.

Therapeutic strategies for AD based on the gonadotropin hypothesis

Currently, there are two classes of drugs approved in the United States for the treatment of AD. Acetylcholinesterase inhibitors are approved for the treatment of mild to moderate AD, and an N-methyl-D-aspartate inhibitor is marketed for severe AD. These compounds primarily offer symptomatic improvement with no significant effects on disease progression.

According to the gonadotropin hypothesis, drugs that inhibit gonadotropin their synthesis and secretion should result in a halting or significant slowing of the disease process. Indeed, we have shown in vitro and in vivo that gonadotropins modulate amyloid-β precursor protein (AβPP) processing and Aβ generation [40]. In addition, human granulosa cells stimulated with gonadotropins are characterized by upregulation of presenilin-1 and -2 gene expression [41], which code for proteins involved in AβPP processing.

There are a number of Food and Drug Administration (FDA)-approved drugs that effectively suppress gonadotropins. These drugs fall into two classes: GnRH agonists and GnRH antagonists. GnRH agonists were developed as a method of suppressing sex steroid production as an alternative to surgical castration in the treatment of advanced prostate cancer. However, GnRH analogues have since been used in a number of other hormone-related conditions, including endometriosis, uterine fibroids and infertility, and are even approved for use in children suffering from precocious puberty [42–45].

For chronic use, the GnRH agonists are more effective than the antagonists at suppressing gonadotropins. The GnRH antagonists were developed to inhibit gonadotropin and sex steroid synthesis without the initial spike in gonadotropins and sex steroids associated with the agonists. While antagonists do prevent this initial burst, there is more 'breakthrough' in LH and testosterone secretion than with the agonists [46]. This phenomenon may be due to a compensatory increase in hypothalamic GnRH secretion, which alters the ratio of the competing ligands, resulting in activation of the receptor. In contrast, with GnRH agonists, a compensatory increase in hypothalamic GnRH would only serve to potentiate receptor downregulation. In addition to this efficacy issue, the antagonists are associated with occasional anaphylactic reactions due to their high histamine-releasing properties [47].

GnRH agonists are analogues of the endogenous GnRH decapeptide, with specific amino acid substitutions (fig. 4). Replacement of the GnRH carboxyl-terminal glycinamide residue with an ethylamide group greatly increases the affinity these analogues possess for the GnRH receptor compared to the endogenous peptide. Many of these analogues also have a longer half-life than endogenous GnRH [47]. Administration of GnRH analogues results in an initial increase in serum gonadotropin concentrations that persists for several days (there is also a corresponding increase in testosterone in men and estrogen in premenopausal women), which is followed by a precipitous decrease in gonadotropins. This suppression is secondary to the loss of GnRH signaling due to downregulation of pituitary GnRH receptors [48], and is a consequence of the increased concentration of ligand, the increased affinity of the ligand for the receptor and the con-

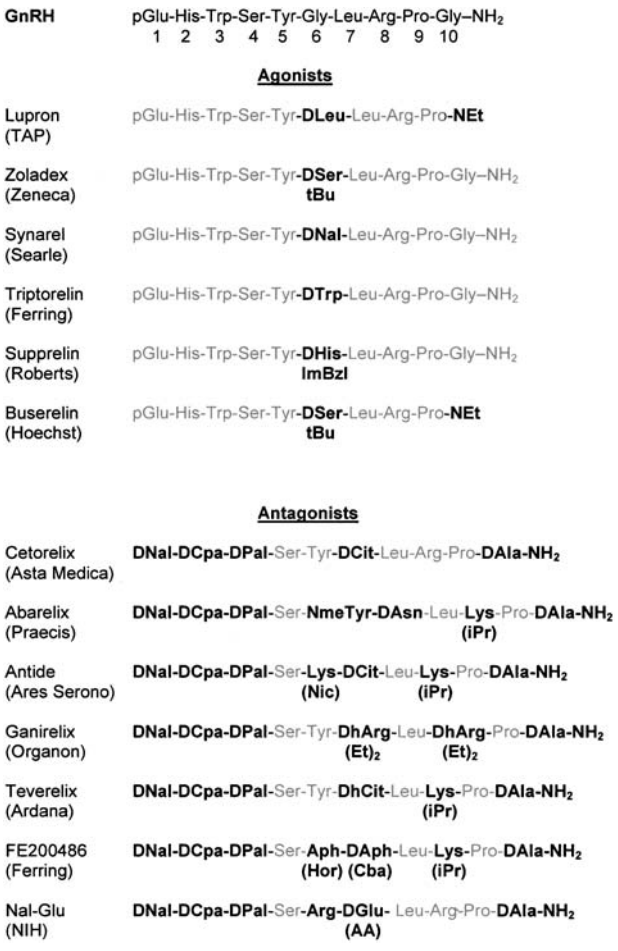

Figure 4. GnRH agonists and antagonists.

tinuous receptor exposure to ligand. This is in opposition to the intermittent exposure that occurs with physiological pulsatile secretion.

Since these GnRH agonists are small peptides, they are not amenable to oral administration. Therefore, they are administered subcutaneously, intramuscularly or via nasal spray. The agonists are extremely potent, with serum concentrations of less than 1 ng/ml of leuprolide acetate required for testosterone suppression [49]. Due to their small size and extreme potency, these peptides are ideal molecules for use in long-acting depot delivery systems. Four such products are currently marketed in the United States and their duration of action ranges from 1 month to 1 year. Three of these products contain the GnRH agonist leuprolide acetate, the other goserelin (fig. 4). Leuprolide acetate has been on the market for close to 2 decades and continues to demonstrate a favorable side-effect profile. Most of the side effects, such as hot flashes and osteoporosis, can be attributed to the loss of sex steroid production [50]. For treatment of female Alzheimer's patients, sex steroid suppression should not be a major issue since they are postmenopausal, and their estrogen production is already significantly decreased. However, since males in this age group continue to produce appreciable amounts of testosterone, add-back testosterone supplementation should counter symptoms associated with its suppression. The safety of GnRH agonists is supported further by the fact that it is estimated that well over 100 million doses have been administered to date (based on sales figures), with no serious consistent adverse effects. In addition, the low toxicity of GnRH agonists was demonstrated in a clinical trial in which men with prostate cancer received daily injections for up to 2 years that were 20-fold higher (20 mg per day) than the currently approved 1 mg per day dose. The 20-mg dose did not result in any adverse effects different from what was seen with the 1-mg dose [51]. Impressive safety profiles along with delivery systems that insure compliance for long periods make these compounds well suited for the AD population. Double-blind, placebo-controlled, clinical trials are currently under way and will help determine whether treatment with an antigonadotropin is a viable therapeutic strategy for AD.

1 Kawas C., Resnick S., Morrision A., Brookmeyer R., Corrado, M., Zonderman, A. et al. (1997) A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging. Neurology **48:** 1517–1521
2 Tang M. X., Jacobs D., Stern Y., Marder K., Schofield P., Gurland B. et al. (1996) Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. Lancet **348:** 429–432
3 Mulnard R. A. (2000) Estrogen as a treatment for Alzheimer disease. JAMA **284:** 307–308
4 Zandi P. P., Carlson M. C., Plassman B. L., Welsh-Bohmer K. A., Mayer L. S., Steffens D.C. et al. (2002) Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. JAMA **288:** 2123–2129
5 Gibbs R. B. and Gabor R. (2003) Estrogen and cognition: applying preclinical findings to clinical perspectives. J. Neurosci. Res. **74:** 637–643
6 Resnick S. M. and Henderson V. W. (2002) Hormone therapy and risk of Alzheimer disease: a critical time. JAMA **288:** 2170–2172
7 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
8 Abdel-sayed W. S., Toppozada H. K., Said S. A. and El-sayed O. K. (1989) Some metabolic and hormonal changes in women using long acting injectable contraceptives. Alex J. Pharmaceut. Sci. **3:** 29–32
9 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
10 McGonigal G., Thamas B., McQuade C., Starr J. M., MacLennan L. W. and Whalley L. J. (1993) Epidemiology of Alzheimer's presenile dementia in Scotland. BMJ. **306:** 680–683
11 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 femal rats. Hormones Behav. **29:** 42–58
12 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
13 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
14 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
15 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
16 Oliver C. and Holland A. J. (1986) Down's syndrome and Alzheimer's disease: a review. Psychol. Med. **16:** 307–322
17 Mann D. M. (1988) Alzheimer's disease and Down's syndrome. Histopathol. **13:** 125–137
18 Schupf N., Kapell D., Nightingale B., Rodriguez A., Tycko B. and Mayeux R. (1998) Earlier onset of Alzheimer's disease in men with Down's syndrome. Neurology **50:** 991–995
19 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
20 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 Clinic Proc. **76:** 906–909
21 Hoskin E. K., Tang M. X., Manly J. J. and Mayeux R. (2004) Elevated sex-hormone binding globulin in elderly women with Alzheimer's disease. Neurobiol. Aging. **25:** 141–147
22 Wide L., Nillius S. J., Gemzell C. and Roos P. (1973) Radioimmunosorbent assay of follicle-stimulating hormone and luteinizing hormone in serum and urine from men and women. Acta Endocrinol. Suppl. **174:** 3–58
23 Hogervorst E., Combrinck M. and Smith A. D. (2003) Testosterone and gonadotropin levels in men with dementia. Neuroendocrinol. Lett. **24:** 203–208
24 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
25 Henderson V. W., Paganini-Hill A., Miller B. L., Elble R. J., Reyes P. F., Shoupe D. et al. (2000) Estrogen for Alzheimer's disease in women: randomized, double-blind, placebo-controlled trial. Neurology **54:** 295–301
26 Asthana S., Baker L. D., Craft S., Stanczyk F. Z., Veith R. C., Raskiind M. A. et al. (2001) High-dose estradiol improves cognition for women with AD: results of a randomized study. Neurol. **57:** 605–612
27 Utian W. H., Katz M., Davey D. A. and Carr P. J. (1978) Effect of premenopausal castration and incremental dosages of conjugated equine estrogens on plasma follicle-stimulating hormone luteinizing hormone and estradiol. Amer. J. Obstet. Gynecol. **132:** 297–302
28 Horiuchi A., Nikaido T., Yoshizawa T., Itoh K., Kobayashi Y., Toki T. et al. (2000) HCG promotes proliferation of uterine leiomyomal cells more strongly than that of myometrial smooth muscle cells in vitro. Molec. Human Reprod. **6:** 523–528
29 Davies B. R., Finnigan D. S., Smith S. K. and Ponder B. A. (1999) Administration of gonadotropins stimulates proliferation of normal mouse ovarian surface epithelium. Gynecol. Endocrinol. **13:** 75–81
30 Webber R. J. and Sokoloff L. (1981) In vitro culture of rabbit growth plate chondrocytes. 1. Age-dependence of response to fibroblast growth factor and „chondrocyte growth factor". Growth. **45:** 252–268
31 Yokotani T., Koizumi T., Taniguchi R., Nakagawa T., Isobe T., Yoshimura M. et al. (1997) Expression of alpha and beta genes of human chorionic gonadotropin in lung cancer. Int. J. Cancer. **71:** 539–544
32 Krichevsky A., Campbell-Acevedo E. A., Tong J. Y. and Acevedo H. F. (1995) Immunological detection of membrane-associated human luteinizing hormone correlates with gene expression in cultured human cancer and fetal cells. Endocrinol. **136:** 1034–1039
33 Whitfield G. K. and Kourides I. A. (1985) Expression of chorionic gonadotropin alpha- and beta-genes in normal and neoplastic human tissues: relationship to deoxyribonucleic acid structure. Endocrinol. **117:** 231–236
34 Srisuparp S., Strakova Z., Brudney A., Mukherjee S., Reierstad S., Hunzicker-Dunn M. et al. (2003) Signal transduction pathways activated by chorionic gonadotropin in the primate endometrial epithelial cells. Biol. Reprod. **68:** 457–464
35 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
36 Mendez M. F., Mastri A. R., Sung J. H. and Frey W. H. (1992) Clinically diagnosed Alzheimer disease: neuropathologic findings in 650 cases. Alzheimer Dis. Assoc. Disord. **6:** 35–43
37 Veldhuis J. D., Evans W. S., Johnson M. L., Wills M. R. and Rogol A. D. (1986) Physiological properties of the luteinizing hormone pulse signal: impact of intensive and extended venous sampling paradigms on its characterization in healthy men and women. J. Clin. Endocrinol. Metabol. **62:** 881–891
38 Veldhuis J. D., Eveans W. S., Urban R. J., Rogol A. D. and Johnson M. L. (1988) Physiologic attributes of the luteinizing hormone pulse in the human. Cross-validation studies in men. J. Androl. **9:** 69–77
39 Themmen A. P. N. and Huhtaniemi I. T. (2000) Mutations of gonadotropins and gonadrotopin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocr. Rev. **21:** 551–583
40 Bowen R. L., Verdile G., Liu T., Parlow A. F., Perry G., Smith M. A. et al. (2004) Luteinizing hormone, a reproductive re gulator that modulates the processing of amyloid-b precursor protein and amyloid-b deposition. J. Biol. Chem. **279:** 20539–20545
41 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
42 Filicori M., Hall D. A., Loughlin J. S., Vale W. and Crowley W. F. Jr (1983) A conservative approach to the management of uterine leiomyoma: pituitary desensitization by a luteinizing hormone-releasing hormone analogue. Amer. J. Obstetr. Gynecol. **147:** 726–727
43 Laron Z., Kauli R., Zeev Z. B., Comaru-Schally A. M. and Schally A. V. (1981) D-TRP5-analogue of luteinising hormone releasing hormone in combination with cyproterone acetate to treat precocious puberty. Lancet **2:** 955–956
44 Meldrum D. R., Chang R. J., Lu J., Vale W., Rivier J. and Judd H. L. (1982) 'Medical oophorectomy' using a long-acting GNRH agonist – a possible new approach to the treatment of endometriosis. J. Clin. Endocrinol. Metabol. **54:** 1081–1083
45 Wildt L., Diedrich K., van der Ven H., al Hasani S., Hubner H. and Klasen R. (1986) Ovarian hyperstimulation for in-vitro

fertilization controlled by GnRH agonist administered in combination with human menopausal gonadotrophins. Human Reprod. **1:** 15–19
46 Praecis Pharmaceuticals Incorporated (2004)
47 Millar R. P., Lu Z. L., Pawson A. J., Flanagan C. A., Morgan K. and Maudsley S. R. (2004) Gonadotropin-releasing receptors. Endocr. Rev. **25:** 235–275
48 Belchetz P. E., Plant T. M., Nakai Y., Keogh E. J. and Knobil E. (1978) Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science **202:** 631–633
49 Fowler J. E., Flanagan M., Gleason D. M., Klimberg I. W., Gottesman J. E. and Sharifi R. (2000) Evaluation of an implant that delivers leuprolide for 1 year for the palliative treatment of prostate cancer. Urology **55:** 639–642
50 Stege R. (2000) Potential side-effects of endocrine treatment of long duration in prostate cancer. Prostate Suppl. **10:** 38–42
51 TAP Pharmaceuticals, Inc. (2003).