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Luteinizing Hormone, a Reproductive Regulator That Modulates the Processing of Amyloid-β Precursor Protein and Amyloid-β Deposition*

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Hormonal changes associated with the dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis following menopause/andropause have been implicated in the pathogenesis of Alzheimer's disease (AD). Experimental support for this has come from studies demonstrating an increase in amyloid-β (Aβ) deposition following ovariectomy/castration. Because sex steroids and gonadotropins are both part of the HPG feedback loop, any loss in sex steroids results in a proportionate increase in gonadotropins. To assess whether Aβ generation was due to the loss of serum 17β-estradiol or to the up-regulation of serum gonadotropins, we treated C57Bl/6J mice with the anti-gonadotropin leuprolide acetate, which suppresses both sex steroids and gonadotropins. Leuprolide acetate treatment resulted in a 3.5-fold ($p < 0.0001$) and a 1.5-fold ($p < 0.024$) reduction in total brain Aβ1–42 and Aβ1–40 concentrations, respectively, after 8 weeks of treatment. To further explore the role of gonadotropins in promoting amyloidogenesis, M17 neuroblastoma cells were treated with the gonadotropin luteinizing hormone (LH) at concentrations equivalent to early adulthood (10 mIU/ml) or post-menopause/andropause (30 mIU/ml). LH did not alter amyloid-β precursor protein (AβPP) expression but did alter AβPP processing toward the amyloidogenic pathway as evidenced by increased secretion and insolubility of Aβ, decreased αAβPP secretion, and increased AβPP-C99 levels. These results suggest the marked increases in serum LH following menopause/andropause as a physiologically relevant signal that could promote Aβ secretion and deposition in the aging brain. Suppression of the age-related increase in serum gonadotropins using anti-gonadotropin agents may represent a novel therapeutic strategy for AD.

Alzheimer's disease (AD)[1] is a neurodegenerative disorder of the elderly that leads to progressive memory loss, impairments in behavior, language, visuo-spatial skills, and ultimately death. The one or more underlying biochemical mechanisms leading to AD are unknown. Genetic studies have shown that mutations in AβPP and the presenilin genes lead to early onset (≤65 years) AD, which accounts for ~5% of all AD cases. The vast majority of these mutations promote the overproduction and deposition of amyloid-β (Aβ) (1–7), the major component of the extracellular amyloid plaques, in the hippocampus and frontal cortex (8, 9). Amyloid deposition also is a hallmark of the late-onset or "sporadic" form of AD, which accounts for ~95% of AD cases. The primary factors responsible for Aβ deposition and disease progression in late onset AD remain to be elucidated.

Aging, the strongest risk factor for late-onset AD, is associated with major changes in serum concentrations of all hormones that comprise the hypothalamic-pituitary-gonadal (HPG) axis, including declines in the serum concentrations of the sex steroids, 17β-estradiol and testosterone. Such changes have been correlated with the prevalence of the disease (*e.g.* Refs. 10–14), and it has been shown that there is a decreased incidence (15) and delay in the onset (16) of AD among women on hormone replacement therapies following menopause (17). There also is biochemical evidence suggesting that sex steroids modulate Aβ deposition in animal models (18, 19). Taken together, these studies have driven the idea that the loss of sex steroids following menopause/andropause is primarily responsible for the cognitive and neuropathological changes observed in the disease. Sex steroid production is, however, under the control of complex feedback loops within the HPG axis, such that during menopause the loss of negative feedback by estrogen on gonadotropin production (20) results in 3–4-fold and 4–18-fold increases in the concentrations of serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH), respectively (21). Likewise, during andropause, men experience increases of more than 2- and 3-fold in LH and FSH, respectively (22). Thus, the neurological and biochemical changes previ-

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[1] The abbreviations used are: AD, Alzheimer's disease; Aβ, amyloid-β; HPG, hypothalamic-pituitary-gonadal; LH, luteinizing hormone; FSH, follicle-stimulating hormone; AβPP, amyloid-β precursor protein; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; PBS, phosphate-buffered saline; GnRH, gonadotropin-releasing hormone; PS1, presenilin-1; α-AβPPs, α-AβPP soluble.

TABLE I
Concentration of pituitary hormones in fetal calf serum

	Concentration
	ng/ml
Luteinizing hormone	0.3–0.7
Follicle-stimulating hormone	0.25–0.35
Prolactin	9–21

ously ascribed to sex steroids could just as easily be explained by the large increases in the circulating concentrations of LH and/or FSH or other hormones of the HPG axis that are dysregulated by menopause/andropause. Evidence for a role of gonadotropins in the disease process is indicated by the 2-fold increase in the concentration of circulating gonadotropins in individuals with AD, above that of the already elevated concentrations of serum gonadotropins in age-matched control individuals (13, 23). Further support for a role of LH in AD is given by the findings that the highest density of LH receptors in the brain is found within the hippocampus (24–26), that LH crosses the blood-brain barrier (27), and that LH accumulates intracellularly in the pyramidal neurons of AD compared with age-matched control brains (28). These findings have led us to investigate whether the expression and processing of AβPP, a molecular pathway relevant to AD neuropathology, also is regulated by gonadotropins.

EXPERIMENTAL PROCEDURES

Antibodies—The human-specific PS1 monoclonal antibody, NT1, directed against residues 41–49 of PS1 and the AβPP monoclonal antibody C1/1.6, directed against the last 20 residues of AβPP, have been described previously (29, 30). Antibody WO2, raised against amino acid residues 5–8 (31) of the Aβ domain of AβPP, was kindly provided by Prof. C. L. Masters (University of Melbourne, Victoria, Australia). Antibody 6E10 (amino acid residues 1–17 of Aβ) was from Senetek (Maryland Heights, MO). Rabbit antisera R208 (specific for Aβ40) was kindly provided by Prof. P. D. Mehta (Institute for Basic Research in Developmental Disabilities, New York, NY). Rabbit anti-rat LH receptor polyclonal antibodies (raised against the N-terminal peptide sequence (15–38) of the rat LH/CG receptor) were kindly provided by Dr. P. C. Roche (Mayo Clinic, Rochester, MN). Anti-human LH receptor monoclonal antibody 3B5 was kindly provided by Dr. J. Wimalasena (The University of Tennessee, Knoxville, TN). Horseradish peroxidase linked goat anti-mouse and goat anti-rabbit IgG were from Amersham Biosciences (Buckinghamshire, UK).

Radioimmunoassay—Pooled fetal calf serum (FCS) (from HyClone, Logan, UT) was analyzed for LH, FSH, and prolactin by radioimmunoassay using reagents from The National Hormone and Peptide Program, NIDDK, National Institutes of Health. Table I summarizes the concentration ranges of pituitary hormones in FCS. The concentration of bovine LH in media containing 0.5% FCS was ~80-fold lower than that of the lowest concentration (1 mIU/ml or 0.25 ng/ml) of human LH added to media.

Cell Culture—The M17 human neuroblastoma cell line was maintained in Opti-MEM media supplemented with 5% (v/v) FCS, 2 mM L-glutamine, 10 mM D-glucose, 50 IU/ml penicillin, 50 μg/ml streptomycin, and 0.7 mM β-mercaptoethanol. All cells were cultured in a humidified atmosphere containing 5% CO_2 in air, at 37 °C. Cells were plated at a density of 1.0×10^6 cells/ml 1 day prior to experiments in Opti-MEM media containing dialyzed 0.5% FCS (to remove endogenous sex steroids and to minimize the effects of endogenous gonadotropins). The next day, M17 cells were treated with human LH (0–30 mIU/ml; National Hormone and Peptide Program, Harbor-UCLA, Torrance, CA) or 17β-estradiol (0–100 nM) at 37 °C for various time periods as indicated in the results section. Medium was changed with fresh medium containing the appropriate hormone concentration every 2 days.

Cell Lysis and Western Immunoblotting—Following incubation with LH the M17 cells were scraped in ice-cold PBS using a rubber policeman and centrifuged at 200 × *g* for 10 min to obtain a cell pellet. The pellet was washed with ice-cold PBS prior to lysing the cells with lysis buffer (50 mM Tris-HCl, pH 7.6, containing 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, 5 μg/ml leupeptin, 5 μg/ml aprotinin, and 0.1 mM phenylmethylsulfonyl fluoride). Total protein (25 μg) was resuspended in sample buffer (70 mM Tris-HCl, pH 6.8, containing 3.2% (w/v) SDS, 0.4 mM glycine, 6 M urea, 0.1 M dithiothreitol, and 0.01% (w/v) phenol red) and separated using polyacrylamide gel electrophoresis. Following electrophoresis and electrophoretic transfer, the membranes were immunoblotted using the antibodies against full-length AβPP (WO2), PS1 (NT1), AβPP-C99 (C1/1.6), and/or LH receptor (rabbit polyclonal and 3B5) using standard techniques as previously described (29, 32).

Sub-cellular Fractionation and Quantitation of Aβ—Following incubation with LH, the cells were washed twice with ice-cold PBS. After washing, the cells were lysed with 50 mM Tris-HCl (pH 7.6), containing 1% Triton X-100 and 150 mM NaCl. The cell lysate was centrifuged at 60,000 × *g* for 20 min to obtain a supernatant and cell pellet. The supernatant (Triton-soluble fraction) was analyzed using ELISA. The cell pellet was washed once with 50 mM Tris-HCl (pH 7.6), containing 1% Triton X-100 and 150 mM NaCl, to remove remaining Triton-soluble proteins. The pellet was sonicated in 50 mM Tris-HCl (pH 7.6) containing 6 M guanidine hydrochloride (33–35). Following centrifugation at 250,000 × *g* for 20 min, the supernatant was diluted 1:12 to give a final concentration of guanidine hydrochloride of 0.5 M (which has been shown not to affect protein-antibody binding (34)). The samples were then subjected to a double sandwich ELISA as previously described (36, 37). Briefly, ELISA plates were coated with the antibody 6E10, and the Aβ was captured with the rabbit polyclonal R208 antibody conjugated with biotin. The optical density was measured at 450 nm using a Bio-Rad Model 3550 microplate reader. All samples were treated similarly to allow comparisons between treatments. The levels of Aβ are expressed as picograms per milligram of total protein.

Leuprolide Acetate Treatment of Mice and Quantitation of Brain Aβ—Female C57Bl/6J mice (3 months of age) received either leuprolide acetate (1.5 mg/kg, slow release (depot) formulation) or vehicle for 0, 4, or 8 weeks and were then sacrificed. Brains were removed and dissected in half, and one hemisphere was frozen (−80 °C) for biochemical analysis. This hemisphere (to maximize yield and minimize inter-animal variability) was homogenized and used to determine the concentrations of Aβ1–40 and Aβ1–42 using an ELISA assay as described above or previously (38) with a modification for rodent brain tissues (Techno-Synapse Inc., Quebec, Canada) (39). Each sample was run in duplicate or triplicate and normalized with α-tubulin content for each sample. As in other species, leuprolide acetate has been shown to lower serum gonadotropin and sex steroid levels in previous studies (40, 41) and in this study.[2]

Data Analysis—Exposed films from Western blots were scanned (transmission scanning) using a visual light scanner (UMAX) at a resolution of 600 dpi. Scanned images were quantified using the public domain program Image (National Institutes of Health; version 1.61) to measure the density of the protein bands. Data collation and analysis were performed using Microsoft® Excel 2000. This program was also used for statistical analysis of data via the Students *t* test (independent, two-tailed, assuming unequal variances).

RESULTS

Gonadotropin Lowering Hormone Decreases the Concentration of Brain Aβ in Mice—To determine whether gonadotropins alter AβPP processing and Aβ generation, we administered a GnRH analogue (leuprolide acetate, 1.5 mg/kg) to depress serum LH concentrations in 3-month-old C57Bl/6J female mice ($n = 6$) and then analyzed Aβ1–40 and Aβ1–42 levels. Leuprolide is a GnRH agonist that down-regulates GnRH receptors and acts as a potent antagonist, suppressing LH and FSH secretion (reviewed in Ref. 42). The actions of GnRH and its analogues are mediated by high affinity receptors for GnRH found on the membranes of the pituitary gonadotrophs (43). An acute administration of agonists of GnRH induces a marked initial release of LH and FSH. However, continuous stimulation of the pituitary by chronic administration of GnRH agonists produces an inhibition of the hypophyseal-gonadal axis through the process of "down-regulation" of pituitary receptors for GnRH, desensitization of the pituitary gonadotrophs, and a suppression of circulating levels of LH and sex steroids (42). This down-regulation of GnRH receptors, produced by sustained administration of GnRH agonists, prevents GnRH sig-

[2] LH: control mice (8 months old = 0.5 ng/ml *versus* ovariectomized mice (8 months old) = 2.1 ng/ml; FSH: control mice (8 months old) = 10.2 ng/ml *versus* ovariectomized mice (8 months old) = 74.5 ng/ml (65).

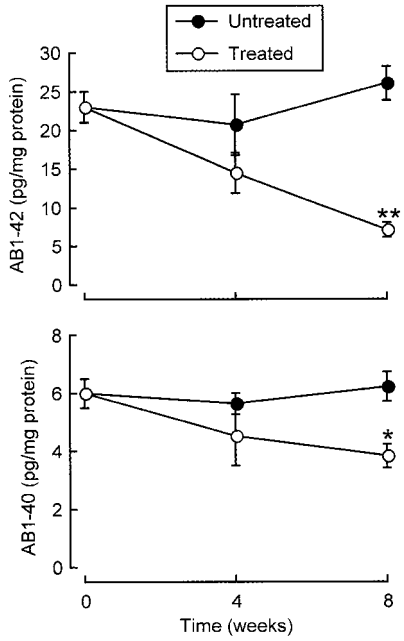

FIG. 1. **Leuprolide, a gonadotropin-lowering drug, decreases brain Aβ levels in mice.** C57Bl/6J mice (3 months old) were administered either vehicle or a slow release leuprolide acetate (1.5 mg/kg; intraperitoneal monthly) mixture at 0 and 4 weeks. Mice were euthanized at 0, 4, and 8 weeks, the brains were dissected, the frontal cortex tissues were homogenized and centrifuged, and the supernatant analyzed for Aβ1–40 and Aβ1–42 levels via an Aβ ELISA assay. Results are expressed as picograms/mg of total protein (mean ± S.D., $n = 6$ mice at each time point). *, $p < 0.05$; **, $p < 0.0001$ for differences between vehicle and treated animals at the same time point.

naling for the secretion of LH and FSH and provides the basis for clinical applications in numerous reproductive hormone-related disorders (42).

Leuprolide treatment dramatically reduced the concentration (picograms/mg of protein) of brain Aβ1–42, by 40% after 4 weeks (24.1 ± 2.0 *versus* 14.5 ± 2.6, $p < 0.015$) and further decreased the concentration to 71% (24.1 ± 2.0 *versus* 7.1 ± 1.0, $p < 0.0001$; mean ± S.E.) that of controls after 8 weeks (Fig. 1). Leuprolide treatment also reduced brain Aβ1–40 levels by 23% after 4 weeks (5.8 ± 0.3 *versus* 4.5 ± 1.1, ns) and further reduced the concentration to 35% (5.8 ± 0.3 *versus* 3.8 ± 0.4, $p < 0.024$) after 8 weeks compared with control mice. Given that leuprolide acetate suppresses serum gonadotropin concentrations, these results suggest that gonadotropin hormones may modulate Aβ generation (44).

Secreted, Triton-soluble, and Triton-insoluble Amyloid-β Levels in Cells Treated with LH—To determine how gonadotropins may be acting to modulate the concentration of Aβ in the brain, we next assessed the effects of gonadotropins on the expression and processing of AβPP in a neuroblastoma cell line. Because FSH receptors are primarily located in reproductive tissues, and others and we have not detected FSH receptors in neuronal cell types, we focused our efforts on biochemical changes modulated by LH. To test whether LH impacts Aβ generation we used the M17 neuroblastoma cell line (widely used for studying AβPP processing (1, 3, 45, 46)) to examine whether LH altered AβPP expression and/or processing. Immunoblot analysis using polyclonal (Fig. 2*D*) and monoclonal (Fig. 2*E*) antibodies against LH receptor indicated that M17 neuroblastoma cells contain a number of LH receptor variants, including the mature glycosylated and phosphorylated LH receptor protein (~92 kDa), the immature full-length LH receptor (59 kDa isoform (47, 48)) and a number of minor variants migrating at 36, 40, 48, 68, and 110 kDa (Fig. 2, *D* and *E*).

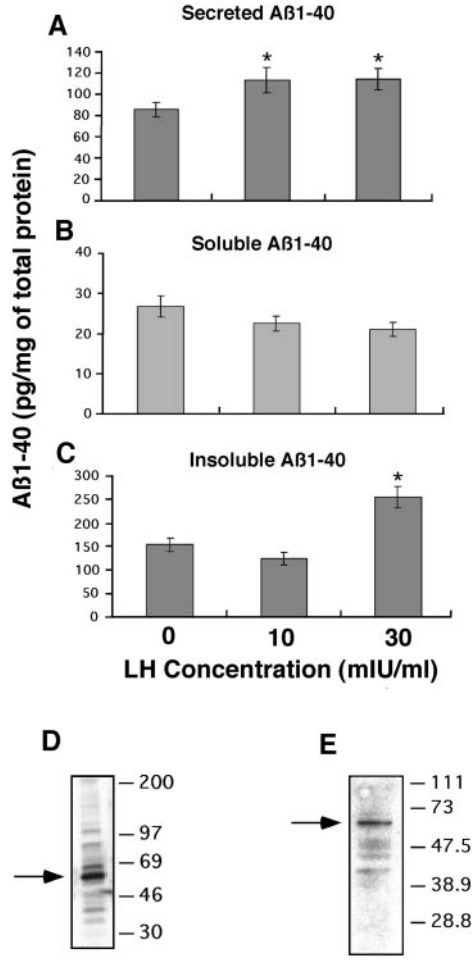

FIG. 2. **LH induces Aβ secretion and insolubility in neuroblastoma cells.** Human M17 neuroblastoma cells were cultured in Opti-MEM medium with 0.5% of dialyzed donor calf serum and 1% of penicillin/streptomycin and treated with 0, 10, and 30 mIU/ml of LH for 5 days. Media with corresponding LH concentrations were replaced every 2 days. The medium from each experiment was used to measure secreted Aβ1-40 (*A*). Cell pellets were solubilized in Triton X-100 and centrifuged to generate soluble (*B*) and insoluble fractions (*C*). Aβ concentration is expressed as picograms/mg of total protein (mean ± S.D.). Experiments were performed three times in duplicate (*i.e.* $n = 6$, $p < 0.01$). LH receptor expression pattern in human M17 neuroblastoma cells was determined by immunoblot analysis with (*D*) a rabbit polyclonal antibody against residues 15–38 and (*E*) a mouse monoclonal antibody (3B5). *Arrows* indicate the immature (~59 kDa) full-length LH receptor.

These results indicate the presence of the immature, mature, and truncated forms of the LH receptor on M17 neuroblastoma cells by which LH might mediate signaling.

We first treated M17 neuroblastoma cells with concentrations of LH representative of serum concentrations during early adulthood (1–10 mIU/ml) and during menopause/andropause (15–30 mIU/ml) for 5 d. We then measured secreted, Triton X-100-soluble (cytoplasmic and membranous components) and Triton X-100-insoluble cell fractions for Aβ. LH treatment at both early adult and post-menopausal concentrations significantly increased the concentration of secreted Aβ (Fig. 2A), indicating that LH may promote secretion of Aβ from neurons. No change in the concentration of Triton X-100-soluble Aβ was detected with either LH concentration (Fig. 2*B*). Together these results indicate that LH is involved in either the processing of AβPP or the trafficking of AβPP/Aβ (see below). Interestingly, there was a doubling in the concentration of Triton X-100-insoluble Aβ with 30 mIU/ml but not with 10 mIU/ml LH (Fig. 2*C*), suggesting higher concentrations of LH

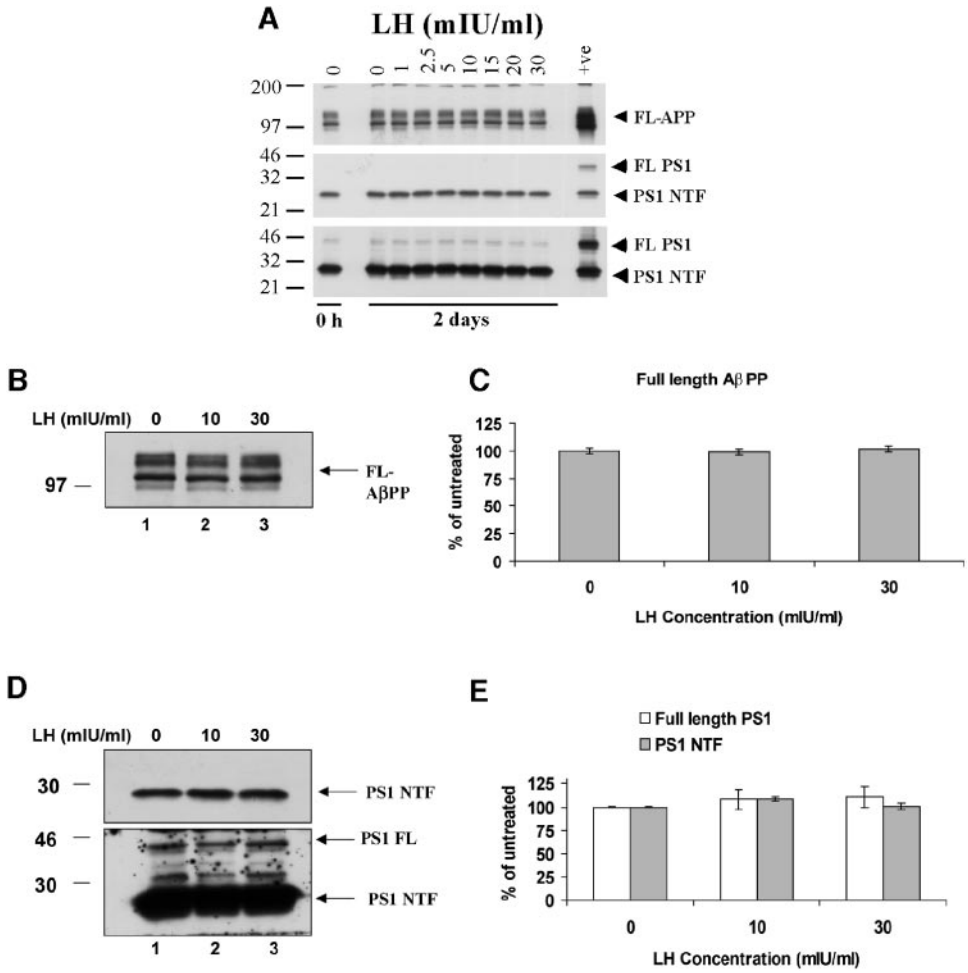

FIG. 3. **LH does not alter AβPP or PS1 expression.** Human M17 neuroblastoma cells were treated with 0–30 mIU/ml LH for 2 days (*A*) or 5 days (*B–E*) as described in Fig. 2. The cell lysates (*B–E* are same as used in Fig. 2) also underwent Western immunoblotting with antibodies against full-length AβPP (WO2), and full-length-PS1 and N-terminal PS1 (NT1). The full-length AβPP (*B*), PS1 holoprotein, and N-terminal fragment (*D*) signals were quantitated and were expressed as a percentage of untreated cells (*C* and *E*). *+ve* = HEK-293 cells overexpressing PS1wt and AβPP695. Experiments were performed three times in duplicate (*i.e.* $n = 6$, $p < 0.05$). Mol. weight markers are shown on the left hand-side.

might drive the aggregation of the protein. In summary, these results indicate that LH promotes the secretion of Aβ from neuroblastoma cells and that high concentrations of LH might promote Aβ insolubility, a cardinal feature of AD.

LH Does Not Modulate AβPP or PS1 Expression in Neuroblastoma Cells—To determine how LH was promoting the increased generation of Aβ, we examined whether LH was increasing the expression of AβPP. M17 neuroblastoma cells were treated with increasing concentrations of LH (0–30 mIU/ml) for 2 days, and changes in the expression of AβPP and PS1 were determined (Fig. 3*A*). Subsequent experiments were preformed at 0, 10, and 30 mIU/ml LH for quantitation. No significant change in the expression of AβPP was observed following quantitation at 5 days (or at 2 and 10 days, not shown), indicating that LH had no effect on modulating the expression of AβPP (Fig. 3, *B* and *C*).

Because evidence suggests PS1 is a component of the γ-secretase complex, we also tested whether LH altered the expression of cellular full-length PS1 and its N-terminal fragment. No change in PS1 expression was detected, indicating that LH does not promote γ-secretase cleavage by increasing PS1 expression (Fig. 3, *D* and *E*). LH also did not alter the processing of PS1 to its N-terminal fragment (Fig. 3, *D* and *E*). These results suggest that the mechanism by which LH increases Aβ generation is not via an increase in AβPP expression nor an increase in PS1 expression or processing.

LH Modulation of Secreted α-AβPPs Levels and AβPP-C99—Because LH did not affect the expression of AβPP or PS1 but did increase secreted Aβ levels, we next investigated if LH modulated AβPP processing through the amyloidogenic pathway (as reflected by a decrease in α-secretase cleavage of AβPP to soluble α-AβPP). The same media collected for Aβ analysis (Fig. 2) was used for immunoblotting of α-AβPPs using the mouse monoclonal antibody WO2 (recognizes α-AβPPs). Consistent with the increased concentration of soluble Aβ in the media of M17 neuroblastoma cells, there was a significant decrease in the concentration of α-AβPPs (Fig. 4). These results indicate that LH drives AβPP processing toward the amyloidogenic pathway.

Given that secreted Aβ levels increased and α-AβPPs levels decreased, we tested to confirm that there was a corresponding increase in the levels of AβPP-C99 (*i.e.* increased β-secretase activity) in the cell lysates treated with LH. The same cell lysate that was used for intracellular Aβ levels was therefore used for Western immunoblotting analysis of AβPP-C99 levels. Consistent with the decrease in α-AβPPs levels, there was an increase in AβPP-C99 levels at both 10 and 30 mIU/ml LH, supportive of the increased processing of AβPP to Aβ. These results further support the idea that LH modulates β-secretase cleavage of AβPP in the secretory pathway. These *in vitro* results indicate that LH drives AβPP processing toward the amyloidogenic pathway by decreasing α-secretase cleavage and

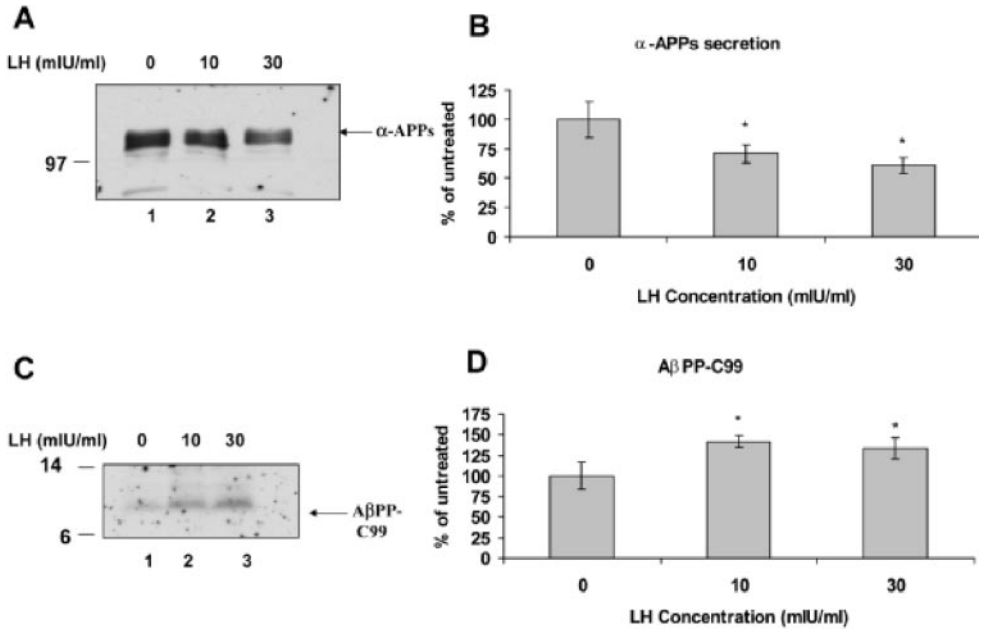

FIG. 4. **LH decreases α-AβPPs and promotes AβPP-C99 generation.** Human M17 neuroblastoma cells were treated as described in Fig. 2, and the media were analyzed for revised α-AβPPs (*A*) and cell lysates analyzed for AβPP-C99 generation (*C*) by immunoblot analysis. Immunoblotting with antibodies WO2 (residues 5–8 of the Aβ domain) or C1/1.6 revealed a band of ~110 kDa representing α-AβPPs (*A*) or a band of ~10 kDa representing AβPP-C99 (*C*), respectively. Signals were quantitated and are expressed as a percentage of untreated cells (*B* and *D*). Experiments were performed three times in duplicate (*i.e.* $n = 6$, $p < 0.05$).

promoting β-secretase (and γ-secretase) cleavage of AβPP to yield Aβ.

LH Receptor Expression and Hormonal Regulation—Because LH receptor expression is modulated in reproductive tissues by 17β-estradiol (49) and LH (50), we tested to determine whether 17β-estradiol (and LH) regulated LH receptor expression in neurons. Physiological concentrations of 17β-estradiol (0.1 nM) promoted an increase in the 59-kDa band representative of the immature form of the full-length LH receptor (Fig. 5) when compared with untreated M17 cells. However, treatment with increasing concentrations of 17β-estradiol, including physiologically relevant concentrations of 17β-estradiol (1 nM), decreased the expression of immature LH receptor in a dose-dependent manner (Fig. 5). Interestingly, levels of the mature forms of the LH receptor (~68, 92, and 110 kDa) were increased at high concentrations of 17β-estradiol (10 and 100 nM).

Treatment of M17 cells with LH (as little as 1 mIU/ml) resulted in a small dose-dependent increase in immature and mature LH receptor isoforms. Truncated forms of the LH receptor (<55 kDa) also increased in a dose-dependent manner with higher LH concentrations (10 and 30 mIU/ml). Together these results indicate that 17β-estradiol and LH can modulate LH receptor expression and maturation and may therefore regulate signaling mediated via the LH receptor.

DISCUSSION

Our results demonstrate that the gonadotropin LH modulates the processing of AβPP leading to increased Aβ generation. *In vivo*, the potent GnRH agonist, leuprolide acetate, which suppresses the concentrations of serum gonadotropin and sex steroids, decreased the concentration of total brain Aβ (Fig. 1). *In vitro*, LH directed AβPP processing toward the amyloidogenic pathway as evidenced by increased Aβ generation and secretion, a concurrent decrease in αAβPP secretion and increased AβPP-C99 generation (Figs. 2–4). Because LH induces biochemical changes consistent with those observed in the AD brain, our results implicate the normal age-related increase in serum LH following menopause/andropause as a potential mechanism responsible for the amyloidosis associated with aging and in AD.

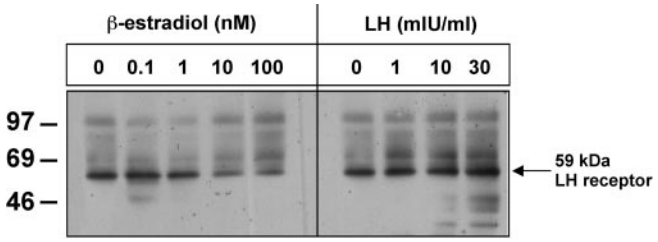

FIG. 5. **Human M17 neuroblastoma cells were treated with 17β-estradiol (0–100 nM) or LH (0–30 mIU/ml), and the cell lysates were analyzed by Western immunoblot for LH receptor isoforms using a rabbit polyclonal LH receptor antibody.**

Contribution of Sex Steroids and Gonadotropins to Amyloidosis—The importance of sex steroids in providing important trophic/protective support for normal brain function has been well described (51). That the decrease in the serum concentrations of these neurotrophic steroids following menopause (and andropause) is associated with disease progression 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. For example, 17β-estradiol and testosterone have been shown to alter neuronal AβPP processing toward the non-amyloidogenic pathway both *in vitro* and *in vivo*. 17β-estradiol at concentrations between 2 and 2000 nM increases α-AβPPs 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 (52–55). Likewise, testosterone treatment (200–2000 nM) of mouse neuroblastoma cells and rat primary cerebrocortical neurons increases secretion of α-AβPPs and decreases the secretion of Aβ (56). Ovariectomy, which suppresses serum estrogen levels, also has been shown to increase total Aβ concentrations in guinea pigs (18) and AβPP transgenic mice (19). Conversely, 17β-estradiol treatment was shown to partially and totally reverse the effects of ovariectomy in guinea pigs (18) and AβPP

transgenic mice (Tg2576 and Tg2576 × mutant PS1 (19)), respectively. A more recent study using AβPPSWE transgenic mice did not see a significant increase in brain Aβ following ovariectomy (57). One difference that might explain this discrepancy is that the AβPPSWE mice were ovariectomized prior to puberty (4 weeks), whereas the Tg2576 mice were ovariectomized after puberty, indicating that establishment of an intact HPG axis is important with regards to AβPP processing and pathological deposition of Aβ.

Although the above results indicate that a reduction in serum estrogen increases AβPP processing toward the amyloidogenic pathway *in vivo*, the fact that leuprolide acetate suppresses both gonadotropins and sex steroids suggests that the effect could well be mediated via LH. Physiological concentrations of serum estrogen are no greater than 1 nM in women, and serum testosterone concentrations are no greater than 35 nM in men (58). Given that physiologically relevant concentrations of 17β-estradiol (1 nM) decreased the expression of immature LH receptor in neuroblastoma cells (Fig. 5), it is likely that the high concentrations of sex steroids used in previous *in vitro* studies (~2–2000 nM 17β-estradiol and ~200–1000 nM testosterone) down-regulated neuronal gonadotropin receptors (and perhaps other HPG axis-relevant receptors). Thus, suppression of LH receptors by high concentrations of estrogen *in vitro* would have the same effect as elevated serum estrogen *in vivo*, suppressing gonadotropin signaling, diverting AβPP processing to the non-amyloidogenic pathway with a resultant decrease in Aβ generation. Although it is possible that high concentrations of LH suppress estrogen receptor expression, thereby modulating AβPP processing, the concentrations of LH used in this study would be unlikely to exert such an affect. In support of the affect of estrogen being dependent upon other hormones of the HPG axis such as LH, Manthey and colleagues have reported for mouse hippocampal HT22 and human neuroblastoma SK-N-MC cells that the enhancement of cellular α-AβPPs release was independent of estrogen receptor expression (55).

Our results clearly indicate that 17β-estradiol modulates immature LH receptor expression. At physiological concentrations of 17β-estradiol (0.1 nM), expression of the 59-kDa LH receptor was maximal (greater than 0 nM 17β-estradiol) but subsequently declined in a dose-dependent manner to low levels by 10 nM. Thus, following menopause when 17β-estradiol levels are low (<0.1 nM), a moderate expression of tissue (brain) LH receptor would still be expected. Interestingly, the levels of higher molecular weight (mature) LH receptor isoforms increased at the higher concentrations of 17β-estradiol (10–100 nM). Although the physiological relevance of this finding is unclear, high concentrations of 17β-estradiol might drive LH receptor maturation at such times when pituitary LH secretion is decreased in order to mediate lowered LH signaling.

In rat ovaries (and testis), the preovulatory LH surge causes a marked down-regulation of cell surface LH receptors and its cognate mRNA, but both of these recover upon luteinization (reviewed in Ref. 50). At typical adult and post-reproductive concentrations of LH (10 and 30 mIU/ml, respectively) used in this study, we found that LH receptor expression increased slightly (Fig. 5). It will be interesting in future studies to determine the affects of LH concentrations normally detected during the LH surge (80 mIU/ml) on LH receptor numbers. Although down-regulation of receptor number by LH is associated with decreased responsiveness to subsequent LH administration, loss of receptor number alone may not completely explain the desensitization, because cAMP was shown to be ineffective in reversing desensitization, suggesting LH also inhibits some post-receptor signaling events (59).

Our results suggest that LH regulates Aβ generation via modulation of AβPP processing rather than via its clearance. It is unclear whether LH is mediating its affects via the LH receptor, although the modulation of LH receptor expression by both 17β-estradiol and LH (Fig. 5) supports this possibility. Full-length LH receptor consists of 335 residues containing six *N*-linked glycosylation sites (60, 61). We have identified mature, immature, and truncated forms of the LH receptor in M17 neuroblastoma cells (Fig. 2*D*). Similar findings have recently been reported in numerous mouse, rat, and human tissues, including brain (62). Post-translational changes in glycosylation and phosphorylation of the immature LH receptor (~59 kDa) result in several LHR variants that migrate between ~93 and 44 kDa (Ref. 62 and references therein). Lower molecular mass species, below 48 kDa, are thought to represent the glycosylated extracellular domain (truncated receptor) and retain hormone-binding specificity (63). Whether mature, immature, or other variants of LH receptor identified in neuroblastoma cells play a role in regulating amyloidogenesis remains to be determined. It also is becoming clear that LH can mediate its affects not only via LH receptors but via mechanisms independent of the LH receptor and traditional downstream signaling mechanisms (59, 64). These mechanisms by which LH exerts its LH receptor-independent affects are yet to be defined. Whether LH acts via one of these traditional or non-traditional pathways, or both, in regulating AβPP processing, remains to be determined. Given the complex feedback loops present within the HPG axis, it will be prudent in future studies to examine other HPG axis hormones and their receptors in mediating AD-related neurodegenerative changes.

Menopause/Andropause-induced Dysregulation of the HPG Axis—The decline in reproductive function late in life leads to the complete dysregulation in the concentrations of all hormones of the hypothalamic-pituitary-gonadal (HPG) axis. In particular, the loss of negative feedback of 17β-estradiol and testosterone on the hypothalamus/pituitary leads to a large increase in the circulating concentrations of the gonadotropins, LH and FSH. Ovariectomy also results in a well-documented increase in serum LH and FSH concentrations in the mouse and other species (66–69). Therefore, during menopause/andropause or following castration, our results suggest that elevated LH could drive AβPP processing toward the amyloidogenic pathway. It is interesting to speculate that the increased deposition of Aβ in female compared with male AβPP-transgenic mice (70, 71) might be due to the more rapid dysregulation of the HPG axis in females compared with males.

LH, FSH, and human chorionic gonadotropin have been shown to play a critical role in brain development and neuron differentiation (72). During fetal development human chorionic gonadotropin, which binds to LH receptors, is present at concentrations 5000 times higher than during young adulthood. With the onset of menopause/andropause, LH concentrations increase to consistently higher levels (73, 74). Interestingly, the increase in LH is higher and occurs earlier in women, who are more susceptible to AD than men. The increase in gonadotropins following menopause/andropause suggests that the neuron hormonal environment reverts back to one more akin to that of the fetal brain, which has been shown to display many similarities to AD brains, namely the presence of Aβ (75), hyperphosphorylated tau (76), and presenilin expression (77). Because the expression of these biochemical parameters is normally associated with neurogenesis, it is possible that the post-reproductive change in HPG hormones might promote abberant re-entry of neurons into the cell cycle.

Our results indicate that the role of HPG axis hormones, other than the sex steroids, in the etiology of AD warrants further investigation. Because leuprolide acetate suppressed

Aβ generation in our *in vivo* study, blockage of the age-related increase in serum gonadotropins using gonadotropin-releasing hormone agonists/antagonists may represent a novel treatment strategy for AD.

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