 doi:10.1111/j.1471-4159.2006.04307.x

Luteinizing hormone receptor mediates neuronal pregnenolone production via up-regulation of steroidogenic acute regulatory protein expression

Tianbing Liu,* Jay Wimalasena,† Richard L. Bowen‡,[1] and Craig S. Atwood*,§,¶

*Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, Wisconsin, USA
†Department of Obstetrics and Gynecology, Graduate School of Medicine, University of Tennessee, Knoxville, Tennessee, USA
‡Voyager Pharmaceutical Corporation, Raleigh, North Carolina, USA
§Case Western Reserve University, Cleveland, Ohio, USA
¶Department of Medicine, University of Wisconsin and the Geriatrics Research, Education and Clinical Center, VA Hospital, Madison, Wisconsin, USA

Abstract

The functional consequences of luteinizing hormone/human chorionic gonadotropin signaling via neuronal luteinizing hormone/human chorionic gonadotropin receptors expressed throughout the brain remain unclear. A primary function of luteinizing hormone (LH) in the gonads is the stimulation of sex steroid production. As LH can cross the blood–brain barrier, present in cerebrospinal fluid and is expressed by neuronal cells, we tested whether LH might also modulate steroid synthesis in the brain. Treatment of differentiated rat primary hippocampal neurons and human M17 neuroblastoma cells with LH (100 mIU/mL) resulted in a twofold increase in pregnenolone secretion in both cell types, suggesting an increase in P450scc-mediated cleavage of cholesterol to pregnenolone and its secretion from neurons. To explore how LH might regulate the synthesis of pregnenolone, the precursor for steroid synthesis, we treated rat primary hippocampal neurons with LH (0, 10 and 100 mIU/mL) and measured changes in the expression of LH receptor and steroidogenic acute regulatory protein (StAR). LH induced a rapid (within 30 min) increase in the expression of StAR, but induced a dose-dependent decrease in LH receptor expression. Consistent with these results, the suppression of serum LH in young rats treated with leuprolide acetate for 4 months down-regulated StAR expression, but increased LH receptor expression in the brain. Taken together, these results indicate that LH induces neuronal pregnenolone production by modulating the expression of the LH receptor, increasing mitochondrial cholesterol transport and increasing P450scc-mediated cleavage of cholesterol for pregnenolone synthesis and secretion.

Keywords: brain, estrogen, human, leuprolide acetate, luteinizing hormone, luteinizing hormone receptor, neuron, pregnenolone, rat, steroidogenic acute regulatory protein.
J. Neurochem. (2007) 10.1111/j.1471-4159.2006.04307.x

Neurosteroids are synthesized by both neurons and glia, and a number of different functions have been attributed to specific neurosteroids. These include the modulation of memory and behavior, neuroprotection, and neurotrophic activities, such as the regulation of myelinization, microtubule assembly, and the growth of axons and dendrites (e.g. Schumacher *et al.* 2004). The importance of neurosteroids in maintaining normal cognitive function (Simpkins *et al.* 2005) is clearly demonstrated by the decrement in cognitive performance associated with the sudden suppression of serum sex steroid to castrate levels in pre-menopausal women following chemical castration [using gonadotropin-

Received June 9, 2006; revised manuscript received August 10, 2006; accepted August 25, 2006.

Address correspondence and reprint requests to Craig S. Atwood, Department of Medicine, University of Wisconsin-Madison, Wm. S. Middleton Memorial VA Hospital (GRECC 11G), 2500 Overlook Terrace, Madison, WI 53705, USA. E-mail: csa@medicine.wisc.edu
[1]The present address of Richard L. Bowen is OTB Research, Raleigh, North Carolina, USA.

Abbreviations used: CSF, cerebrospinal fluid; EIA, enzyme immunoassay; FBS, fetal bovine serum; LH/hCG, luteinizing hormone/human chorionic gonadotropin; LRBP, LH receptor mRNA binding protein; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; StAR, steroidogenic acute regulatory protein.

releasing hormone (GnRH) superagonists], and by the amelioration of these effects with administration of estrogen (Varney *et al.* 1993; Newton *et al.* 1996; Sherwin and Tulandi 1996). The neuroprotective effects of neurosteroids also are demonstrated by recent findings that sex steroid levels are decreased in the brains of individuals with mild neurological changes or Alzheimer's disease (Bernardi *et al.* 2000; Weill-Engerer *et al.* 2002; Rosario *et al.* 2004; Yue *et al.* 2005). Similar correlations between peripheral sex steroid concentrations and cognition have previously been reported in numerous epidemiological studies (Jorm *et al.* 1987; McGonigal *et al.* 1993; Bowen *et al.* 2000; Manly *et al.* 2000; Hogervorst *et al.* 2001, 2003, 2004). In this regard, hormone replacement therapies have been found to decrease the incidence, and delay the onset, of cognitive decline among women and men following menopause/andropause (reviewed in Gleason *et al.* 2005). Indeed, estrogen and androgen replacement therapies in post-menopausal women and andropausal men have been associated with improved cognitive function (Jacobs *et al.* 1998; Tan and Pu 2003).

In the gonads and adrenal glands, luteinizing hormone (LH) stimulates steroidogenesis by up-regulating the rate-limiting transport of cholesterol from the outer to the inner mitochondrial membrane by increasing the expression of the mitochondrial cholesterol transport protein, steroidogenic acute regulatory protein (StAR; reviewed in Strauss *et al.* 1999; Stocco 2001). Additionally, LH increases the expression of the P450 cholesterol side-chain cleavage enzyme (P450scc) located on the inner membrane of mitochondria, which catalyzes the conversion of cholesterol to pregnenolone, the first product in steroid hormone biosynthesis (reviewed in Stocco 1998, 2001; Strauss *et al.* 1999; Apaja *et al.* 2004).

Luteinizing hormone receptor has been shown by a number of workers to be expressed by neuronal cells throughout the brain (reviewed in Lei and Rao 2001; Vadakkadath Meethal and Atwood 2005). The findings that LH/human chorionic gonadotropin (LH/hCG) can cross the blood–brain barrier (Lukacs *et al.* 1995); is present in cerebrospinal fluid (CSF) (Ehrhardt 1931; Zondek 1937, 1942; Mathieu 1939; Vesell and Goldman 1944; McCormick 1954, Tashima *et al.* 1965; Bagshawe *et al.* 1968); is present in neuronal cells (Petrusz 1975; Pacold *et al.* 1978; Emanuele *et al.* 1981a,b, 1983, 1985; Hostetter *et al.* 1981; Strauss *et al.* 1994; Bowen *et al.* 2002); and can be produced by extra-hypothalamic neurons (Wilson *et al.* 2006) raise the intriguing question as to whether serum, CSF or *de novo* synthesized LH might signal via neuronal LH receptors to modulate neurosteroid synthesis. Supporting this idea that LH might induce neuronal steroidogenesis are findings that the expression of StAR is induced in both neurons and glia by cAMP (King *et al.* 2002), and that LacZ under the control of the LH/hCG receptor promoter is expressed in neurons and colocalizes with P450scc (Apaja *et al.* 2004).

In this study, we demonstrate that LH increases pregnenolone secretion from differentiated rat embryonic hippocampal neurons and from human neuroblastoma cells. As in the ovary, LH appears to regulate pregnenolone synthesis and secretion by modulating the expression of the LH receptor and the cholesterol transport protein StAR.

Material and methods

Reagents and preparation

Human LH was purchased from the National Hormone and Peptide Program, Harbor-UCLA, Torrance, CA, USA. LH stock solution was made by dissolving 1.0 mg of LH in 0.5% BSA in sterile phosphate-buffered saline (PBS) (Invitrogen, Carlsbad, CA, USA) and filtered through a 0.22-μm filter (Millipore, Bedford, MA, USA) and kept at −80°C. 17β-Estradiol was purchased from Sigma (St Louis, MO, USA) and was made fresh each time by dissolving in 100% ethanol. Neurobasal medium without phenol red, B27 supplement, and glutamine were purchased from Invitrogen. Anti-rat/human LH receptor monoclonal antibody 3B5 was provided by Dr Wimalasena (University of Tennessee, Knoxville, TN, USA). DURIN™-leuprolide and DURIN placebo implants (Lot No. 03523) were from Durect Corporation, Birmingham, AL, USA. Rabbit anti-rat/human StAR serum was a kind gift from Dr Jerome Strauss at University of Pennsylvania.

GnRH agonist treatment of rats and collection of brains and serum

All animal experimentation met the criteria for animal care as outlined in the Guide for the Care and Use of Laboratory Animals (National Research Council 1996). Sprague–Dawley rats (18 weeks old; Harlan Sprague–Dawley Inc., Indianapolis, IN, USA) kept at 18–26°C, 30–70% humidity, on a 12 h light–dark cycle and fed *ad libitum* were treated with DURIN-leuprolide or DURIN placebo implants for 4 months via two consecutive subcutaneous implantations 2 months apart (i.e., at 0 and 2 months) at the dose of 12.4 mg/kg. Subcutaneous implantation was via a single small skin incision closed using a surgical adhesive. The dose of leuprolide administered was ∼80-fold greater than that used clinically. No known toxic level has been observed for leuprolide acetate. Blood samples were collected after an overnight fast from the abdominal vena cava prior to killing by CO_2 followed by exsanguinations, and the serum stored at −80°C until analysis. After weighing, the right cerebral cortex was removed from the brain of each rat, frozen in liquid nitrogen, and stored at ≤ −70°C until analysis. The remaining brain was fixed in 10% neutral buffered formalin for subsequent processing and light microscopic examination for histopathological findings.

Radioimmunoassay

Serum was analyzed for LH and FSH by radioimmunoassay at The National Hormone and Peptide Program, National Institute of Diabetes, Digestive and Kidney Disease (Dr Albert Parlow, Harbor-UCLA Medical Center, Torrance, CA, USA).

Primary rat hippocampal neuron culture

Embryonic day 18 Sprague–Dawley rat hippocampi (BrainBits, Springfield, IL, USA) were dissociated by trituration in Hibernate E

media (Brainbits, Southern Illinois University School of Medicine, Springfield, IL, USA). Undispersed tissue was allowed to settle for 1 min prior to transferring the supernatant to a new tube and centrifugation at 200 *g* for 1 min. The supernatant was removed and the cells resuspended in serum-free neurobasal medium (without phenol red) containing B-27 supplement (Invitrogen), 25 μmol/L L-glutamate, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.5 mmol/L L-glutamine, counted using a hemocytometer and then equal volumes of mixed cells were plated into six-well poly-L-lysine-coated plates (0.5 or 1×10^5 cells/well; Falcon; Fisher Scientific, Pittsburgh, PA, USA). Hippocampal cultures were grown in 5% CO_2, 85% humidity in the above media. Half of the medium was replaced with the same media without L-glutamate every 3 days.

Immunofluorescent staining of primary neurons

Differentiated primary rat E18 neurons were either untreated or treated with digitonin (40 μg/mL in PBS on ice for 20 min) and then washed with PBS, fixed in 4% paraformaldehyde in PBS for 15 min, washed 3 × 5 min each with PBS containing 100 mmol/L glycine and permeabilized with 0.1% Triton X-100 in PBS for 4 min. The permeabilized cells were then washed in PBS for 3 × 5 min, incubated in 1% bovine serum albumin in PBS with 3% goat serum for 1 h, followed by incubation with primary antibody for 2 h, washing with PBS for 3 × 10 min, and incubation with secondary antibodies for 2 h. The slides were then mounted with mounting solution (Vector Laboratories, Burlingame, CA, USA) and viewed under a Zeiss Axiophot microscope (Carl Zeiss Micro Imaging, Inc., Thornwood, NY, USA).

Neuroblastoma cell culture

The M17 human neuroblastoma cell line was maintained in OPTI-MEM (Invitrogen) media supplemented with 0.5% (v/v) fetal bovine serum (FBS), 50 IU/mL penicillin, and 50 μg/mL streptomycin. All cells were cultured in a humidified atmosphere containing 5% CO_2 in air at 37°C. Cells were plated at a density of $\sim 1.0 \times 10^6$ cells/mL in OPTI-MEM media containing 0.5% FBS and grown to ~50% confluence. Cells were switched to fresh media for 24 h prior to treatment with human LH (0–100 mIU/mL).

Immunoblot analyses

The frontal cortex and mid-region were cut coronally from each brain, homogenized in lysis buffer (20 mmol/L Tris–HCl, pH 7.6, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% sodium dodecyl sulfate (SDS), and protease inhibitors (10 μg/mL aprotinin, 10 μg/mL leupeptin, 1 μg/mL pepstatin A, 1 mmol/L phenylmethylsulfonyl fluoride; Roche Diagnostics, Indianapolis, IN, USA), and then centrifuged for 10 min at 10 000 *g*. Cells were collected into lysis buffer and then sonicated for 30 s. Following protein assay (bicinchoninic acid protein assay kit; Pierce, Rockford, IL, USA), equal amounts of protein were loaded onto 10–20% tricine gels (Invitrogen) for SDS–polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA, USA), fixed with glutaraldehyde (1%, v/v), blocked with milk (5%, w/v) and then probed with primary antibody overnight at 4°C. The blot was then incubated with the corresponding horseradish peroxidase conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 h at 22°C, and developed with enhanced chemiluminescence reagent (Santa Cruz Biotechnology) as per the manufacturer's instructions. The chemiluminescent signal was captured on autoradiographs (Eastman Kodak, Rochester, NY, USA), which were scanned and the intensity of the autoradiagraph signals (including a blank region) was determined using the NIH ImageJ Sotware. Briefly, images were captured and the intensity of the autoradiograph signals (including a blank region) was determined. Control and treatment values were corrected for blank values, normalized to their respective glyceraldehyde-3-phosphate dehydrogenase (GADPH) band intensity and the results then expressed as a fold change or percentage over control levels. Molecular size markers were from Bio-Rad Laboratories.

Pregnenolone assay

The M17 neuroblastoma cells cultured in OPTI-MEM medium with 0.5% of FBS without phenol red were switched to Dulbecco's modified Eagle's medium without phenol red with 0.5% charcoal stripped calf serum ± LH for 24 h. For primary cells, E18 primary hippocampal neurons were differentiated *in vitro* in neurobasal medium with B27 supplement for 7 days and then treated ± LH for 24 h. Media was then collected and the concentration of total pregnenolone was determined using the ALPCO enzyme immunoassay (EIA) kit (Salem, New Hampshire, UK) as per instructions. Briefly, the competition assay was performed by adding 50 μL of pregnenolone standards (0, 0.1, 0.4, 1.6, 6.4, and 25.6 ng/mL; $n = 3$ wells) or media from treated cells ($n = 6$ wells) into plates pre-coated with antipregnenolone antibody. To each well was added pregnenolone–horseradish peroxidase conjugate working solution (100 μL), the plate incubated for 2 h at 20 °C while shaking, the wells were then washed five times with wash buffer (300 μL), and tetramethylbenzidine (TMB) substrate (150 μL) added to each well. After incubation for 15 min at 20 °C with shaking the reaction was stopped and the plate read at 450 nm using a Spectramax 190 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). Cross-reactivity with other steroids is typically below 1%; cross-reactivity with progesterone is 6%. The sensitivity of our assays was 0.05 ng/mL. Intra-assay coefficients of variation were typically <10%. Inter-assay coefficients of variation were ~15%.

Statistical analyses

Changes between treatments were assessed using the Student's *t*-test, or where applicable, the one-way analysis of variance followed by Tukey's HSD *post hoc* test to determine significant changes between treatment groups. The type I error rate is set at 0.05 for all analyses.

Results

Hormonal modulation of neuronal LH receptor expression

Although the presence of LH receptor has been demonstrated in the brain, the functionality of the receptor and the functional consequences of LH signaling via this neuronal receptor with respect to neurosteroid synthesis have not been determined. We initially hypothesized that if the LH receptor

was functional, its expression might be regulated by related hormones. Therefore, to determine if changes in the concentrations of serum LH and sex steroids modulate the expression of the LH receptor *in vivo*, we treated rats with DURIN-leuprolide or DURIN placebo implants for 4 months as described in the Methods section. Leuprolide acetate is a GnRH receptor superagonist that suppresses serum LH and FSH and is used for the treatment of prostate cancer, precocious puberty, and other reproductive disorders (Schally *et al.* 1980). Measurement of the serum concentrations of LH and follicle-stimulating hormone (FSH) by radioimmunoassay (RIA) confirmed that leuprolide acetate treatment suppressed the concentrations of serum LH (−80%, $p = 0.017$, $n = 3–5$; Fig. 1a) and FSH (−46%, $p = 0.023$, $n = 3–5$; Fig. 1b). We next determined whether these changes altered the expression of LH receptor in the rat brain. As reported by another group (Apaja *et al.* 2004), we also detected two major LH receptor variants, i.e., the mature glycosylated 92-kDa variant and a 73-kDa variant (Fig. 2a). Treatment with leuprolide acetate increased the expression of the 73-kDa and 92-kDa LH receptor variants by 74% ($p = 0.039$) and 67% ($p = 0.07$), respectively, in the brain after treatment with leuprolide acetate (Fig. 2b). Analyses of the frontal cortex showed an almost identical pattern of LH receptor expression in leuprolide-treated and -untreated animals (data not shown). As leuprolide acetate treatment induces a decrease in the concentration of both serum gonadotropins and sex steroids, the increased expression of brain LH receptor may be due to decreases in sex steroids or gonadotropins, or both.

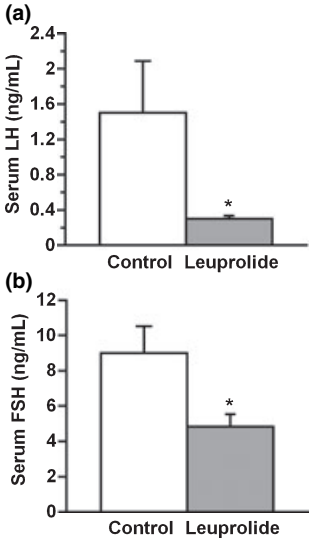

Fig. 1 Leuprolide acetate suppresses the serum concentrations of LH and FSH in rats. Serum collected from 8-month-old rats treated with ($n = 5$) and without ($n = 3$) leuprolide acetate (12.4 mg/kg) for 4 months was measured for LH/FSH, as described in the Methods section. Serum LH (a) and FSH (b) are presented as mean ± SEM, $^*p < 0.05$).

To differentiate between whether gonadotropins and/or sex steroids were responsible for modulating neuronal LH receptor expression, we first confirmed the presence of LH receptor on *in vitro* differentiated primary rat hippocampal neurons by immunofluorescence, using the well-characterized monoclonal anti-LH receptor antibody 3B5 (Indrapichate *et al.* 1992). Staining was clearly detected on the cell body and neurites, but did not appear to be associated with the nucleus of the neuron (Fig. 3). No staining was detected with the same concentration of mouse IgG_1 (negative control). To determine whether this staining was associated with the plasma membrane or was intracellular, we pre-treated cells with digitonin before fixation. The loss of staining indicated that the LH receptor was primarily located on the cell membrane. We next treated primary rat E18 hippocampal neurons differentiated for 5 days with increasing concentrations of either LH or 17β-estradiol for 48 h and measured the expression of LH receptor by immunoblot analyses. LH induced a significant dose-dependent decrease in the expression of the 73-kDa LH receptor variant such that at 100 mIU/mL, receptor expression was 46% that of untreated control neurons (Fig. 4a). In contrast, no change in expression was detected with increasing concentrations of 17β-estradiol treatment (Fig. 4b). Interestingly, we did not detect the 92-kDa variant of the receptor in primary neurons under these conditions, despite detecting both variants in the rat brain (Fig. 2). This disparity may be explained by the use of fetal primary neurons that are either not sufficiently differentiated or lack sufficient trophic support to allow for post-translational modification (glycosylation; Apaja *et al.* 2004) of the receptor to its mature form. These results also suggest that while primary neurons respond to LH by down-regulating its receptor, further neuron differentiation may be required in order for LH receptor expression to be modulated by 17β-estradiol, as reported earlier for neuroblastoma cells maintained in serum media (Bowen *et al.* 2004). The modulation of neuronal LH receptor expression by LH demonstrates the functionality of neuronal LH receptors.

LH rapidly up-regulates StAR expression in neurons

Luteinizing hormone receptors mediate mitochondrial cholesterol transport by up-regulating the expression of the cholesterol transport protein StAR in granulosa cells of the ovary (Strauss *et al.* 1999; Stocco 2001). Given that neuronal LH receptors are functional, we decided to determine whether LH also modulated StAR expression by incubating M17 neuroblastoma cells and primary neurons with typical concentrations of serum LH found during reproduction (10 mIU/mL) and post-menopause (100 mIU/mL). Both the 37-kDa pre-protein and the 30-kDa mature form of the protein were detected in both cell types (Figs 5a and b). We also detected bands intermediate

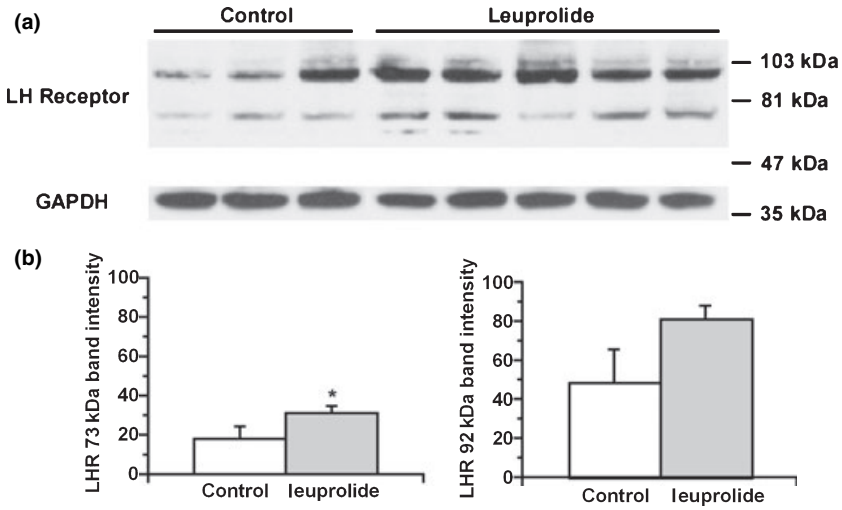

Fig. 2 Leuprolide acetate treatment up-regulates the expression of LH receptor in the rat brain. Brain tissue (mid-cortical region) collected from rats treated with (n = 5) and without (n = 3) leuprolide acetate for 4 months were homogenized and the supernatant underwent immunoblot analysis as described in the Methods section. (a) Immunoblot of brain tissue using the monoclonal anti-LH receptor antibody 3B5. (b) Quantification of the immunoreactive bands in (a) (mean ± SEM, *$p < 0.05$). Molecular weight markers are shown on the right and are representative for both autoradiographs.

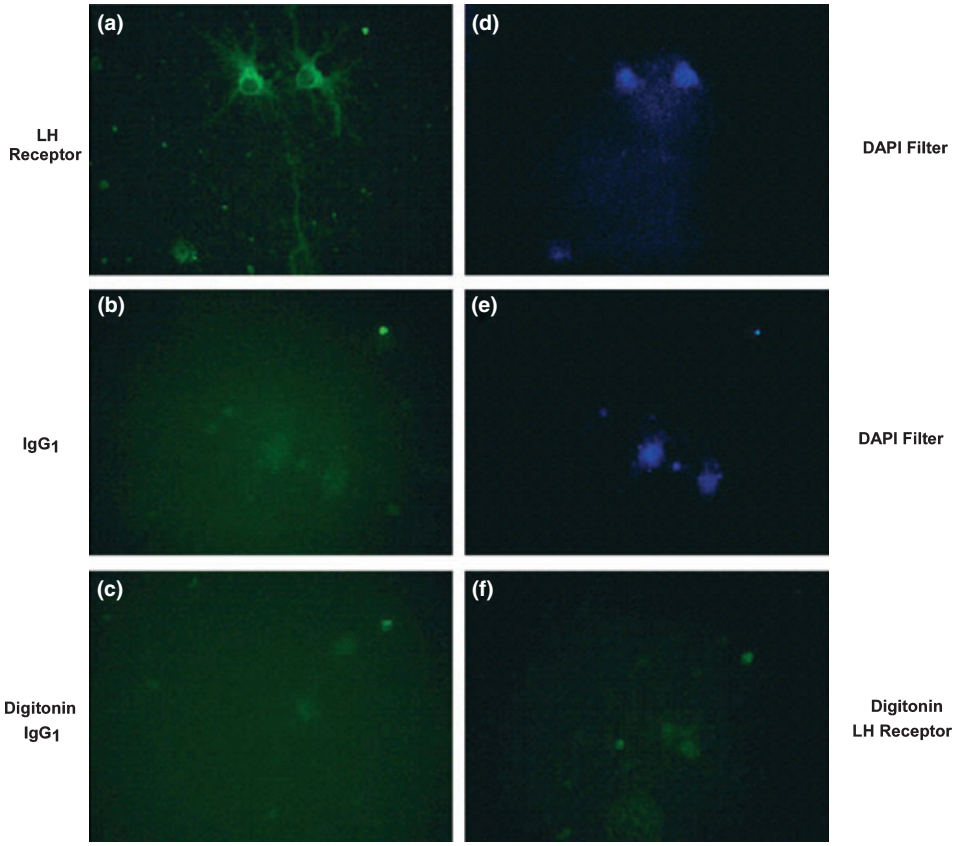

Fig. 3 Expression of LH receptor in primary hippocampal neurons. Neurons from rat embryonic day 18 hippocampi were differentiated in culture for 7 days and probed with the monoclonal anti-LH receptor antibody 3B5, which is of IgG_1 subtype (a), or the same concentration of mouse IgG_1 (b), followed by FITC conjugated anti-mouse secondary antibody; the same fields were observed under DAPI filter (d and e) and pseudocolored images are shown for the localization of nuclei of the corresponding cells in (a) and (b), respectively; neurons also were pre-treated with digitonin before fixation, and then stained with mouse IgG_1 (c) or 3B5 (f).

between the pre-protein and the 30-kDa mature protein, suggesting sequential processing of the StAR pre-protein, as has been proposed by Clark *et al.* (1994), as well as two higher molecular weight bands at ~59 and ~64-kDa.

A rapid increase in the expression of the pre-protein was observed after 28 min of LH treatment of 7-day differentiated primary rat neurons (Fig. 5a) and after 3 h of LH treatment in neuroblastoma cells (Fig. 5b). The processing

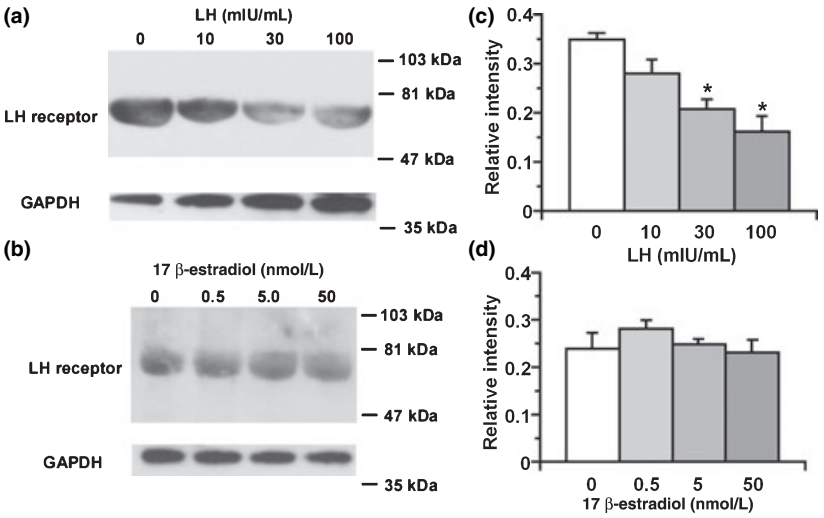

Fig. 4 Modulation of the expression of LH receptor in rat primary hippocampal neurons by LH and 17β-estradiol. Neurons from rat embryonic day 18 hippocampi were cultured for 5 days, and then treated with (a) LH (0–100 mIU/mL) or (b) 17β-estradiol (0–50 nmol/L) for 48 h and the expression of LH receptor determined by immunoblot. Quantitation of the immunoreactive bands in (a) and (b) are shown in (c) and (d), respectively (mean ± SEM, $^*p < 0.05$). Molecular weight markers are shown on the right. Experiments are representative of at least three experiments.

of the pre-protein to the mature 30 kDa form of the protein increased with LH concentration (Fig. 5). To determine whether the suppression of serum LH, FSH (Fig. 1), and 17β-estradiol in rats following leuprolide treatment down-regulated StAR expression, the same samples used for LH receptor analysis (Fig. 2) were used to study the expression of StAR. As expected, the expression of both forms of StAR were significantly down-regulated (~70%) in leuprolide-acetate-treated animals (Figs 5c–e). Together, these results suggest that LH regulates neuronal StAR expression *in vitro* and *in vivo*, and may therefore be involved in neurosteroid production.

LH stimulates neuronal steroid synthesis and secretion

To test whether LH might modulate neurosteroid production, human M17 neuroblastoma cells and rat primary hippocampal neurons were treated with LH at doses of 10 and 100 mIU/mL for 24 h, and the concentration of total pregnenolone in the media was assayed by EIA. Treatment of M17 neuroblastoma cells (at a density of $\sim 10^6$ cells/well) with LH induced a dose-dependent statistically significant increase in the concentration of pregnenolone in the media (Fig. 6a). This equated to 131 ± 4, 172 ± 4, and 237 ± 10 ng (mean ± SEM) of pregnenolone synthesized per mg of cellular protein for 0, 10, and 100 mIU/mL LH, respectively. Treatment of rat primary hippocampal neurons ($\sim 10^4$ cells/well) differentiated *in vitro* for 8 days with LH for 24 h only significantly increased the media concentration of pregnenolone at the higher dose (Fig. 6b). The concentration of pregnenolone synthesized per mg of cellular protein for 0, 10, and 100 mIU/mL LH was 6.2 ± 0.6, 6.4 ± 0.7, and 10.0 ± 0.6 ng (mean ± SEM), respectively, an ~20-fold lower level of pregnenolone production than that detected for the human neuroblastoma cells. A significant increase in the concentration of pregnenolone was not observed at the lower concentration of LH (10 mIU/mL),

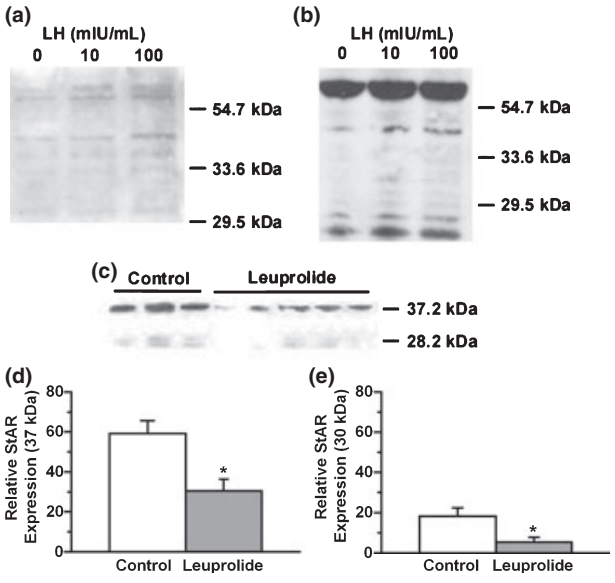

Fig. 5 LH up-regulates the expression of StAR in human neuroblastoma cells and rat primary hippocampal neurons *in vitro* and *in vivo*. (a) Neurons from rat embryonic day 18 hippocampi were cultured for 7 days, and then treated with LH (0, 10, and 100 mIU/mL) for 28 min prior to collection for immunoblot analysis using the anti-rat/human StAR rabbit serum. (b) Human M17 neuroblastoma cells cultured in 0.5% fetal bovine serum were incubated with LH (0, 10, and 100 mIU/mL) for 3 h prior to collection for immunoblot analysis using anti-rat/human StAR rabbit serum. (c) Brain tissue samples treated with and without leuprolide acetate, as described in Fig. 2, underwent immunoblot analysis using anti-rat/human StAR rabbit serum. Molecular weight markers are shown on the right hand side. (d) Quantification of the 37 kDa immunoreactive band in (c) (mean ± SEM, $^*p < 0.05$). (e) Quantification of the 30 kDa immunoreactive band in (c) (mean ± SEM, $^*p < 0.05$).

likely because of the low level of pregnenolone secretion and the limited number of primary hippocampal cells available for these experiments. Taken together, these results indicate

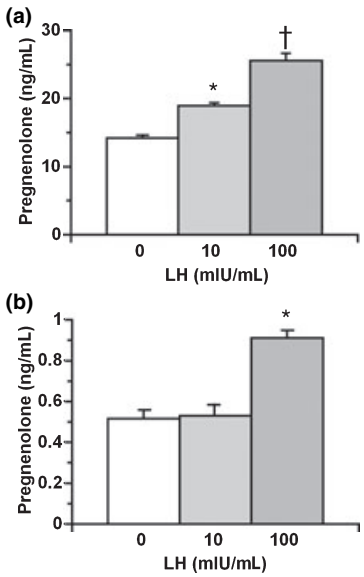

Fig. 6 LH increases the secretion of pregnenolone by rat primary hippocampal neurons and human neuroblastoma cells. (a) Human M17 neuroblastoma cells were treated with LH (0, 10, and 100 mIU/mL) for 24 h, and the media collected for pregnenolone assay. Results are normalized for differences in total protein content for each well and expressed as the mean ± SEM (*$p < 0.01$ vs. 0 and 100 mIU/mL LH; †$p < 0.01$ vs. 0 and 10 mIU/mL LH). Experiments are representative of three separate experiments. (b) Neurons from rat embryonic day 18 hippocampi cultured for 8 days were treated with LH (0, 10, and 100 mIU/mL) for 24 h and the media collected for pregnenolone assay. Results are normalized for differences in total protein content for each well and expressed as the mean ± SEM (*$p < 0.01$ vs. 0 and 10 mIU/mL LH). Pregnenolone was not detectable in media alone. Experiments are representative of three separate experiments.

that LH promotes the increased secretion of pregnenolone from neurons.

Discussion

We demonstrate for the first time that LH signaling in neurons promotes the synthesis and secretion of steroids (Fig. 6). This LH-induced production of neurosteroids has features that resemble LH-mediated sex steroid production by the gonads: LH modulation of LH receptor expression (Figs 2 and 4), LH up-regulation of StAR protein expression (Fig. 5) and the increased P450scc cleavage of cholesterol to pregnenolone and its secretion from the cell (Fig. 6). The amount of pregnenolone secreted by the M17 human neuroblastoma cell line (~130–240 ng/mg cellular protein/24 h) is consistent with previous findings that SH-SY5Y cells contain 1–20 ng/mg pregnenolone (Guarneri *et al.* 2000). The high level of basal pregnenolone production in neuroblastoma cells maintained in serum-containing media when compared with primary neurons (~6–10 ng/mg cellular protein/24 h) maintained in defined media indicates that other serum factors also may be required for optimal pregnenolone synthesis and secretion. Alternatively, 8-day cultured embryonic hippocampal neurons may be insufficiently differentiated to allow considerable side chain cleavage, despite their apparent ability to transport cholesterol to the mitochondria (Fig. 5a).

The source of LH (and hCG in humans) to signal for neurosteroid production is unclear, but is most likely from pituitary LH secreted into the blood stream, which crosses the blood–brain barrier (Lukacs *et al.* 1995; Toth *et al.* 1994), or from the *de novo* synthesis of LH by neuronal cells (Wilson *et al.* 2006). The finding that LH/hCG is present in CSF (reviewed in Bagshawe *et al.* 1968) and neurons (Petrusz 1975; Pacold *et al.* 1978; Emanuele *et al.* 1981a,b, 1983, 1985; Hostetter *et al.* 1981; Strauss *et al.* 1994; Bowen *et al.* 2002) supports neuronal LH signaling via neuronal LH/hCG receptors.

The presence of LH receptor and its mRNA has previously been reported in the central and peripheral nervous system of a number of animals (Lei *et al.* 1993; Lei and Rao 1994; al-Hader *et al.* 1997a,b; You *et al.* 2000; Bhatnagar *et al.* 2002; Bukovsky *et al.* 2003; Apaja *et al.* 2004; Figs 2–4). LH receptor is present in the fetal and adult rat brain, with the highest density of receptors being found within the hippocampus followed by the hypothalamus, cerebellum, choroid plexus, ependymal tanycytes of third, fourth, and lateral ventricles, cortex, brain stem, and anterior pituitary (Lei *et al.* 1993; al-Hader *et al.* 1997a,b). All human neuronal brain cell types studied to date possess LH/hCG receptors (Bukovsky *et al.* 2003; Bowen *et al.* 2004), as do immortalized mouse hypothalamic and hippocampal GT1-7 and HN33p cells and human M17 neuroblastoma cells (Zhang *et al.* 1999; Bowen *et al.* 2004). In this study, we confirm that LH receptor is present on primary neurons and in addition demonstrate that its expression is regulated by its cognate ligand (Fig. 4), as has been reported in the ovary (Hoffman *et al*. 1991). A significant difference in the regulation of neuronal LH receptor expression, however, is the lack of FSH input into LH receptor expression, as FSH receptors have not been reported on neurons. FSH is well known to modulate LH receptor expression in the ovary (for a review, see Menon *et al.* 2004).

Different mechanisms underlying the down-regulation of LH receptor expression by LH have been proposed in non-neuronal tissues or cells, including endocytosis of the hormone–receptor complex and subsequent degradation in the lysosomes (Ascoli *et al.* 2002; Amsterdam *et al*. 2002), and a novel mechanism including increased degradation of LH/CG receptor mRNA after hCG treatment (Lu *et al.* 1993). It was later found that a cytoplasmic LH receptor mRNA binding protein (LRBP) was a trans-acting factor in regulating LH receptor mRNA levels (Wang and Menon 2005), and that LRBP was further identified as mevalonate kinase (MVK), which is an enzyme involved in *de novo*

synthesis of cholesterol. The levels of MVK mRNA and protein were shown to be induced in response to hCG treatment prior to the down-regulation of LH receptor mRNA expression (Nair and Menon 2005; Menon *et al.* 2006). Whether similar mechanisms of LH/CG receptor regulation exist in neurons is unknown.

Luteinizing hormone has been shown to influence a number of physiological parameters, including the electrical activity of the brain, respiration, excitability, behavior, and learning (Faure and Loiseau 1954; Faure 1956; Kawakami and Sawyer 1959; Telegdy *et al.* 1971; Inselman-Temkin and Flynn 1973; Emanuele *et al.* 1991; Toth *et al.* 1994; Lukacs *et al.* 1995; Lei and Rao 2001; Rao 2001). At the cellular and molecular levels, LH has been shown to affect sexual differentiation (Sheridan *et al.* 1973), morphology (Ifft 1964; Parada and Rodriguez-Echandia 1973), and the biochemistry and metabolism (Moguilevsky *et al.* 1970; MacKinnon and ter Haar 1971) of different regions of the brain. In this regard, hCG has been shown to induce a dose-dependent increase in the outgrowth of neuritic processes and total cellular protein while decreasing apoptosis in rat primary neurons (al-Hader *et al.* 1997a,b). Recently, we demonstrated that LH modulates the neuronal processing of amyloid-β precursor protein and the generation of amyloid-β (Bowen *et al.* 2004), the major component of amyloid plaques that deposit in the brain during Alzheimer's disease. While some of these LH-induced responses appear to be a direct effect of LH (Bowen *et al.* 2004), changes in other parameters may be an indirect result of LH/hCG-induced neurosteroid synthesis (Murphy and Segal 2000, Sakamoto *et al*. 2001, 2002, 2003; Segal and Murphy 2001).

Like the ovary (Stocco and Clark 1996), LH treatment increases the expression of StAR and the production of pregnenolone by rat primary neurons and human neuroblastoma cells (Figs 5 and 6). The contribution of steroids produced in the brain versus those produced in the periphery to normal neuronal function is unknown. Given the importance of neurosteroids to brain function, it is possible that neurosteroid production may be a mechanism to fine-tune the level of sex steroids in the brain. This neuronal production of sex steroids may be more crucial to brain function when gonadal sex steroid production decreases with menopause and andropause. This is supported by a recent study showing that despite the decrease in serum sex steroids following ovariectomy, brain estrogen levels remain as high as in control mice (Yue *et al.* 2005), possibly because of increased neurosteroid production as a result of ovariectomy-induced increases in serum LH (Parlow 1964). In men however, brain neurosteroid levels decrease when serum LH levels are increasing with age (Rosario *et al.* 2004). Neurosteroid concentrations also are decreased in individuals with AD versus age-matched controls, although serum 17β-estradiol levels are unchanged (Yue *et al.* 2005) and serum LH levels are elevated in AD (Short *et al.* 2001; Hogervorst *et al.* 2004).

In summary, our results demonstrate that neuronal LH receptors are functional and that LH-mediated sex steroid production in the brain is similar to that in the ovary. Future studies are required to determine whether physiological conditions (i.e., pregnancy, menstrual cycle, menopause, etc.) or drugs that modulate LH/hCG production impact neurosteroid production.

Acknowledgements

This material is based on work supported in part by the office of Research and Development, Department of Veteran Affairs. This work is also supported by funds from the National Institute of Health (RO1 AG19356), Alzheimer's Association and Voyager Pharmaceutical Corporation to CSA.

References

al-Hader A. A., Tao Y. X., Lei Z. M. and Rao C. V. (1997a) Fetal rat brains contain luteinizing hormone/human chorionic gonadotropin receptors. *Early Pregnancy* **3**, 323–329.

al-Hader A. A., Lei Z. M. and Rao C. V. (1997b) Neurons from fetal rat brains contain functional luteinizing hormone/chorionic gonadotropin receptors. *Biol. Reprod.* **56**, 1071–1076.

Amsterdam A., Hanoch T., Dantes A., Tajima K., Strauss J. F. and Seger R. (2002) Mechanisms of gonadotropin desensitization. *Mol. Cell. Endocrinol.* **187**, 69–74.

Apaja P. M., Harju K. T., Aatsinki J. T., Petaja-Repo U. E. and Rajaniemi H. J. (2004) Identification and structural characterization of the neuronal luteinizing hormone receptor associated with sensory systems. *J. Biol. Chem.* **279**, 1899–1906.

Ascoli M., Fanelli F. and Segaloff D. L. (2002) The lutropin/choriogonadotropin receptor: a 2002 perspective. *Endocr. Rev.* **23**, 141–174.

Bagshawe K. D., Orr A. H. and Rushworth A. G. (1968) Relationship between concentrations of human chorionic gonadotrophin in plasma and cerebrospinal fluid. *Nature* **217**, 950–951.

Bernardi F., Lanzone A., Cento R. M., Spada R. S., Pezzani I., Genazzani A. D., Luisi S., Luisi M., Petraglia F., Genazzani A. R. (2000) Allopregnanolone and dehyroepiandrosterone response to corticotropin-releasing factor in patients suffering from Alzheimer's disease and vascular dementia. *Eur. J. Endocrinol.* **142**, 466–471.

Bhatnagar K. P., Li X., Lei Z. M. and Rao C. V. (2002) Human pineal luteinizing hormone receptors. *Biotech. Histochem.* **77**, 223–228.

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.

Bowen R. L., Smith M. A., Harris P. L., Kubat Z., Martins R. N., Castellani R. J., Perry G. and Atwood C. S. (2002) Elevated luteinizing hormone expression colocalizes with neurons vulnerable to Alzheimer's disease pathology. *J. Neurosci. Res.* **70**, 514–518.

Bowen R. L., Verdile G., Liu T., Parlow A. F., Perry G., Smith M. A., Martins R. N. and Atwood C. S. (2004) Luteinizing hormone, a reproductive regulator that modulates the processing of amyloid-beta precursor protein and amyloid-beta deposition. *J. Biol. Chem.* **279**, 20 539–20 545.

Bukovsky A., Indrapichate K., Fujiwara H. *et al.* (2003) Multiple luteinizing hormone receptor (LHR) protein variants, interspecies reactivity of anti-LHR mAb clone 3B5, subcellular localization of LHR in human placenta, pelvic floor and brain, and possible role

for LHR in the development of abnormal pregnancy, pelvic floor disorders and Alzheimer's disease. *Reprod. Biol. Endocrinol.* **1**, 46.

Clark B. J., Wells J., King S. R. and Stocco D. M. (1994) The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). *J. Biol. Chem.* **269**, 28 314–28 322.

Ehrhardt K. (1931) Blazenmole nmd Schwaugerschaffts Reaktion. *Med. Klin.* **27**, 426–427.

Emanuele N., Oslapas R., Connick E., Kirsteins L. and Lawrence A. M. (1981a) Hypothalamic LH may play a role in control of pituitary LH release. *Neuroendocrinology* **33**, 12–17.

Emanuele N., Connick E., Howell T., Anderson J., Hojvat S., Baker G., Souchek J., Kirsteins L. and Lawrence A. M. (1981b) Hypothalamic luteinizing hormone (LH): characteristics and response to hypophysectomy. *Biol. Reprod.* **25**, 321–326.

Emanuele N. V., Anderson J., Andersen E., Connick E., Baker G., Kirsteins L. and Lawrence A. M. (1983) Extrahypothalamic brain luteinizing hormone: characterization by radioimmunoassay, chromatography, radioligand assay and bioassay. *Neuroendocrinology* **36**, 254–260.

Emanuele N., Lipov S., Anderson J., Andersen E., Kirsteins L., Nishimura N. and Lawrence A. M. (1985) Subcellular localization of hypothalamic luteinizing hormone. *Neuroendocrinology* **40**, 339–344.

Emanuele M. A., Tentler J., Emanuele N. V., Kelley M. R. (1991) In vitro effects of acute E+OH in rat alpha and beta luteinizing hormone gene expression. *Alcohol*. **8**, 345–348.

Faure J. (1956) Changes in bioelectric activity of the rhinencephalon and in behavior after administration of pituitary gonadotropins in rabbits. *Rev. Neurol. (Paris)* **95**, 490–497.

Faure J. and Loiseau P. (1954) Effect of hormones on the electrical activity of the brain, respiration, excitability and behavior of the rabbit. *Rev. Neurol. (Paris)* **91**, 460–468.

Gleason C. E., Carlsson C. M., Johnson S., Atwood C. and Asthana S. (2005) Clinical pharmacology and differential cognitive efficacy of estrogen preparations. *Ann. N.Y. Acad. Sci.* **1052**, 93–115.

Guarneri P., Cascio C., Piccoli T., Piccoli F. and Guarneri R. (2000) Human neuroblastoma SH-SY5Y cell line: neurosteroid-producing cell line relying on cytoskeletal organization. *J. Neurosci. Res.* **60**, 656–665.

Hoffman Y. M., Peegal H., Sprock M. J., Zhang Q. Y. and Menon K. M. (1991) Evidence that human chorionic gonadotropin luteinizing hormone receptor down-regulation involves decreased levels of receptor messenger ribonucleic acid. *Endocrinology* **128**, 388–393.

Hogervorst E., Williams J., Budge M., Barnetson L., Combrinck M. and Smith A. D. (2001) Serum total testosterone is lower in men with Alzheimer's disease. *Neuro Endocrinol. Lett.* **22**, 163–168.

Hogervorst E., Combrinck M. and Smith A. D. (2003) Testosterone and gonadotropin levels in men with dementia. *Neuro Endocrinol. Lett.* **24**, 203–208.

Hogervorst E., Bandelow S., Combrinck M. and Smith A. D. (2004) Low free testosterone is an independent risk factor for Alzheimer's disease. *Exp. Gerontol.* **39**, 1633–1639.

Hostetter G., Gallo R. V. and Brownfield M. S. (1981) Presence of immunoreactive luteinizing hormone in the rat forebrain. *Neuroendocrinology* **33**, 241–245.

Ifft J. D. (1964) The effect of endocrine gland extirpations on the size of nucleoli in rat hypothalamic neurons. *Anat. Rec.* **148**, 599–603.

Indrapichate K., Meehan D., Lane T. A., Chu S. Y., Rao C. V., Johnson D., Chen T. T. and Wimalasena J. (1992) Biological actions of monoclonal luteinizing hormone/human chorionic gonadotropin receptor antibodies. *Biol. Reprod.* **46**, 265–278.

Inselman-Temkin B. R. and Flynn J. P. (1973) Sex-dependent effects of gonadal and gonadotropic hormones on centrally-elicited attack in cats. *Brain Res.* **60**, 393–410.

Jacobs D. M., Tang M. X., Stern Y., Sano M., Marder K., Bell K. L., Schofield P., Dooneief G., Gurland B. and Mayeux R. (1998) Cognitive function in nondemented older women who took estrogen after menopause. *Neurology* **50**, 368–373.

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.

Kawakami M. and Sawyer C. H. (1959) Induction of behavioral and electroencephalographic changes in the rabbit by hormone administration or brain stimulation. *Endocrinology* **65**, 631–643.

King S. R., Manna P. R., Ishii T. *et al.* (2002) An essential component in steroid synthesis, the steroidogenic acute regulatory protein, is expressed in discrete regions of the brain. *J. Neurosci.* **22**, 10 613–10 620.

Lei Z. M. and Rao C. V. (1994) Novel presence of luteinizing hormone/human chorionic gonadotropin (hCG) receptors and the down-regulating action of hCG on gonadotropin-releasing hormone gene expression in immortalized hypothalamic GT1-7 neurons. *Mol. Endocrinol.* **8**, 1111–1121.

Lei Z. M. and Rao C. V. (2001) Neural actions of luteinizing hormone and human chorionic gonadotropin. *Semin. Reprod. Med.* **19**, 103–109.

Lei Z. M., Rao C. V. and Kornyei J. L. (1993) Novel expression of human chorionic gonadotropin/luteinizing hormone receptor gene in brain. *Endocrinology* **132**, 2262–2270.

Lu D. L., Peegel H., Mosier S. M. and Menon K. M. (1993) Loss of lutropin/human choriogonadotropin receptor messenger ribonucleic acid during ligand-induced down-regulation occurs post transcriptionally. *Endocrinology* **132**, 235–240.

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.

MacKinnon P. C. and ter Haar M. B. (1971) Plasma LH levels and changes in protein synthesis in the median eminence and anterior pituitary during the oestrous cycle. *J. Physiol.* **218**, 21–22.

Manly J. J., Merchant C. A., Jacobs D. M., Small S. A., Bell K., Ferin M. and Mayeux R. (2000) Endogenous estrogen levels and Alzheimer's disease among postmenopausal women. *Neurology* **54**, 833–837.

Mathieu A. (1939) Recent developments in diagnosis and treatment of hydatidiform mole and chorioepthelioma. *American Journal of Obstetrics and Gynecology* **37**, 654–662.

McCormick J. B. (1954) Gonadotrophin in urine and spinal fluid; quantitative studies for chorionic moles and choriocarcinomas. *Obstet. Gynecol.* **3**, 58–66.

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.

Menon K. M., Munshi U. M., Clouser C. L. and Nair A. K. (2004) Regulation of luteinizing hormone/human chorionic gonadotropin receptor expression: a perspective. *Biol. Reprod.* **70**, 861–866.

Menon K. M., Nair A. K. and Wang L. (2006) A novel post-transcriptional mechanism of regulation of luteinizing hormone receptor expression by an RNA binding protein from the ovary. *Mol. Cell. Endocrinol.* **246**, 135–141.

Moguilevsky J. A., Libertun C. and Foglia V. G. (1970) Metabolic sensitivity of different hypothalamic areas to luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone. *Neuroendocrinology* **6**, 153–159.

Murphy D. D. and Segal M. (2000) Progesterone prevents estradiol-induced dendritic spine formation in cultured hippocampal neurons. *Neuroendocrinology* **72**, 133–143.

Nair A. K. and Menon K. M. (2005) Regulation of luteinizing hormone receptor expression: evidence of translational suppression in vitro by a hormonally regulated mRNA-binding protein and its endogenous association with luteinizing hormone receptor mRNA in the ovary. *J. Biol. Chem.* **280**, 42809–42816.

National Research Council (1996) *Guide for the Care and Use of Laboratory Animals*. Institute of Laboratory Animals Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C.

Newton C., Slota D., Yuzpe A. A. and Tummon I. S. (1996) Memory complaints associated with the use of gonadotropin-releasing hormone agonists: a preliminary study. *Fertil. Steril.* **65**, 1253–1255.

Pacold S. T., Kirsteins L., Hojvat S. and Lawrence A. M. (1978) Biologically active pituitary hormones in the rat brain amygdaloid nucleus. *Science* **199**, 804–806.

Parada M. I. and Rodriguez-Echandia E. L. (1973) A depolymerizing effect of the luteinizing hormone and follicle stimulating hormone on the neurotubules of the hypothalamus-hypophysial tract in the rat. *Experientia* **29**, 195–196.

Parlow A. F. (1964) Effect of ovariectomy on pituitary and serum gonadotrophins in the mouse. *Endocrinology* **74**, 102–107.

Petrusz P. (1975) Localization and sites of action of gonadotropins in brain, in *Anatomical Neuroendocrinology* (Stumpf W. E. and Grant L. D., eds) pp. 176–184. Karger, Basel.

Rao C. V. (2001) An overview of the past, present, and future of nongonadal LH/hCG actions in reproductive biology and medicine. *Semin. Reprod. Med.* **19**, 7–17.

Rosario E. R., Chang L., Stanczyk F. Z. and Pike C. J. (2004) Age-related testosterone depletion and the development of Alzheimer disease. *JAMA* **292**, 1431–1432.

Sakamoto H., Ukena K. and Tsutsui K. (2001) Effects of progesterone synthesized de novo in the developing Purkinje cell on its dendritic growth and synaptogenesis. *J. Neurosci.* **21**, 6221–6232.

Sakamoto H., Ukena K. and Tsutsui K. (2002) Dendritic spine formation in response to progesterone synthesized de novo in the developing Purkinje cell in rats. *Neurosci. Lett.* **322**, 111–115.

Sakamoto H., Mezaki Y., Shikimi H., Ukena K. and Tsutsui K. (2003) Dendritic growth and spine formation in response to estrogen in the developing Purkinje cell. *Endocrinology* **144**, 4466–4477.

Schally A. V., Coy D. H. and Arimura A. (1980) LH-RH agonists and antagonists. *Int. J. Gynaecol. Obstet.* **18**, 318–324.

Schumacher M., Guennoun R., Robert F. *et al.* (2004) Local synthesis and dual actions of progesterone in the nervous system: neuroprotection and myelination. *Growth Horm. IGF Res.* **14** (Suppl. A), S18–33.

Segal M. and Murphy D. (2001) Estradiol induces formation of dendritic spines in hippocampal neurons: functional correlates. *Horm. Behav.* **40**, 156–159.

Sheridan P. J., Zarrow M. X., Goldman B. D. and Denenberg V. H. (1973) The role of gonadotropins in the development of cyclicity in the rat. *Endocrinology* **92**, 500–508.

Sherwin B. B. and Tulandi T. 1(996) "Add-back" estrogen reverses cognitive deficits induced by a gonadotropin-releasing hormone agonist in women with leiomyomata uteri. *J. Clin. Endocrinol. Metab.* **81**, 2545–2549.

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.

Simpkins J. W., Yang S. H., Wen Y. and Singh M. (2005) Estrogens, progestins, menopause and neurodegeneration: basic and clinical studies. *Cell. Mol. Life Sci.* **62**, 271–280.

Stocco D. M. (1998) A review of the characteristics of the protein required for the acute regulation of steroid hormone biosynthesis: the case for the steroidogenic acute regulatory (StAR) protein. *Proc. Soc. Exp. Biol. Med.* **217**, 123–129.

Stocco D. M (2001) StAR protein and the regulation of steroid hormone biosynthesis. *Annu. Rev. Physiol.* **63**, 193–213.

Stocco D. M. and Clark B. J. (1996) Regulation of the acute production of steroids in steroidogenic cells. *Endocr. Rev.* **17**, 221–244.

Strauss B. L., Pittman R., Pixley M. R., Nilson J. H. and Boime I. (1994) Expression of the beta subunit of chorionic gonadotropin in transgenic mice. *J. Biol. Chem.* **269**, 4968–4973.

Strauss J. F. III, Kallen C. B., Christenson L. K., Watari H., Devoto L., Arakane F., Kiriakidou M. and Sugawara T. (1999) The steroidogenic acute regulatory protein (StAR): a window into the complexities of intracellular cholesterol trafficking. *Recent Prog. Horm. Res.* **54**, 369–394.

Tan R. S. and Pu S. J. (2003) A pilot study on the effects of testosterone in hypogonadal aging male patients with Alzheimer's disease. *Aging Male* **6**, 13–17.

Tashima C. K., Timberger R., Budick R., Leavy M. and Rawson R. W. (1965) Cerebrospinal fluid titer of chorionic gonadotropin in patients with intracranial metastatic choriocarcinoma. *J. Clin. Endocrinol. Metab.* **25**, 1493–1495.

Telegdy G., Rozsahegyi G. and Lissak K. (1971) Further data on the effect of human chorionic gonadotrophin on avoidance and exploratory behaviour in the rat. *Acta Physiol. Acad. Sci. Hung.* **40**, 215–220.

Toth P., Lukacs H., Hiatt E. S., Reid K. H., Iyer V. and Rao C. V. (1994) Administration of human chorionic gonadotropin affects sleep-wake phases and other associated behaviors in cycling female rats. *Brain Res.* **654**, 181–190.

Vadakkadath Meethal S. and Atwood C. S. (2005) The role of hypothalamic-pituitary-gonadal hormones in the normal structure and functioning of the brain. *Cell. Mol. Life Sci.* **62**, 257–270.

Varney N. R., Syrop C., Kubu C. S., Struchen M., Hahn S. and Franzen K. (1993) Neuropsychologic dysfunction in women following leuprolide acetate induction of hypoestrogenism. *J. Assist. Reprod. Genet.* **10**, 53–557.

Vesell M. and Goldman S. (1941) Friedman test on spinal fluid in cases of hydatidiform mole and pregnancy. *American Journal of Obstetrics and Gyneclogy* **42**, 272–275.

Weill-Engerer S., David J. P., Sazdovitch V., Liere P., Eychenne B., Pianos A., Schumacher M., Delacourte A., Baulieu E. E. and Akwa, Y. (2002) Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. *J. Clin. Endocrinol. Metab.* **87**, 5138–5143.

Wang L. and Menon K. M. (2005) Regulation of luteinizing hormone/chorionic gonadotropin receptor messenger ribonucleic acid expression in the rat ovary: relationship to cholesterol metabolism. *Endocrinology* **146**, 423–431.

Wilson A. C., Salamat S., Haasl R. J., Roche K. M., Karande A., Vadakkadath Meethal S., Terasawa E., Bowen R. L. and Atwood C. S. (2006) Human extra-pituitary neurons possess GnRH I receptors that respond to GnRH by expressing luteinizing hormone. *J. Endocrinol.* **191**, 651–665.

You S., Kim H., Hsu C. C., El Halawani M. E. and Foster D. N. (2000) Three different turkey luteinizing hormone receptor (tLH-R) isoforms I: characterization of alternatively spliced tLH-R isoforms and their regulated expression in diverse tissues. *Biol. Reprod.* **62**, 108–116.

Yue X., Lu M., Lancaster T., Cao P., Honda S., Staufenbiel M., Harada N., Zhong Z., Shen Y. and Li R. (2005) Brain estrogen deficiency accelerates Abeta plaque formation in an Alzheimer's

disease animal model. *Proc. Natl Acad. Sci. USA* **102**, 19 198–19 203.

Zhang W., Lei Z. M. and Rao C. V. (1999) Immortalized hippocampal cells contain functional luteinizing hormone/human chorionic gonadotropin receptors. *Life Sci.* **65**, 2083–2098.

Zondek B. (1937) Gonadotrophin hormone in diagnosis of chorioepithelioma. *JAMA* **108**, 607–611.

Zondek B. (1942) Importance of increased production and excretion of gonadotropic hormone for diagnosis of hydatidiform mole. *Journal of Obstetrics and Gynaecology of the British Empire* **49**, 397–411.