Extracellular Expression of Luteinizing Hormone by Human Neurons Possessing GnRH Receptor I

Andrea C. Wilson1, M. Shahriar Salamati1, Ryan J. Haasl1, Sivan Vaddakadath Meethal1, Mark A. Smith2, El Terasawa3, Richard L. Bowen4 and Craig S. Atwood1,2

1Department of Medicine, University of Wisconsin and Geriatric Research, Education and Clinical Center, Veterans Administration Hospital, Madison, WI, 53705. 2Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106. 3Department of Pediatrics, University of Wisconsin, Madison, WI 53705. 4Voyager Pharmaceutical Corporation, Raleigh, NC, 27615.

ABSTRACT

Luteinizing hormone (LH) has recently been localized to extrapituitary regions of the brain including pyramidal neurons of the aged human and rat. Although the source of extrapituitary LH is unclear, we have previously shown that the levels of neutral LH are elevated in Alzheimer’s disease (AD). Given the expression of gonadotropin-releasing hormone receptor 1 (GnRHR I) on extrapituitary cells, and the marked increase in secretion of hypothalamic GnRH following menopause/endropause during aging, it is possible that human neurons are capable of LH synthesis like endocrine glands. To test this hypothesis, human M17 and SH-SY5Y neuroblastoma cells were cultured in serum free conditions with 10-15 µM GnRH for 6 h. Immunohistochemistry indicated the presence of LH variants (~30, 46 and 60 kDa) that were upregulated at higher GnRH concentrations. LH expression was also found to increase in differentiating embryonic rat primary cortical neurons. To determine the potential for GnRH I signaling in the brain, human hippocampal tissue was immunostained for GnRHR I. Robust GnRHR I immunoreactivity was detected in the cell body as well as along the apical dendrites of pyramidal neurons in the CA1, CA3, and end plate, but was clearly lower in the subiculum of the hippocampus. Immunoblotting also was evident in cortical neurons, including those located in the entorhinal cortex and septal-lateral gyrus but was not observed within the granular layer of the dentate gyrus. No differences in immunohistochemical staining were observed among control and AD tissues, although immunohistochemical analysis of brain homogenates indicated decreased expression of the ~30 and 116 kDa GnRHR I variants, but not the mature 64 kDa variant. Decreased expression of the 116 kDa receptor complex in the AD cortex, and in neuroblastoma cells following GnRH I treatment, suggests that GnRHR I may mediate the coupling of the mature 64 kDa GnRHR I to the G-protein coupled receptor complex. Our results demonstrate that extrapituitary neurons expressing GnRHR I are functional, responding to GnRH I by upregulating LH production.

INTRODUCTION

- Nearly 4.5 million Americans have Alzheimer’s disease (AD), a neurodegenerative disease of the elderly, which causes memory loss, language and behavior impairments, and eventually leads to death.
- With menopause/endropause, the loss of sex steroids and inhibitory production by the gonads leads to the dysregulation of the serum concentrations of all hormones of the hypothalamic-pituitary-gonadal (HPG) axis (Bowen and Atwood, 2004).
- Some of these hormones and their receptors may be important in explaining certain age-related diseases since:
  - GnRH receptor I has been localized to pyramidal neurons of the rat brain, although their presence in extrapituitary regions of the human brain is not yet reported.
  - LH expression has been detected in extrapituitary neurons and its expression is increased in the AD brain (Bowen et al., 2002).
  - Leuprolide acetate, a GnRH agonist, inhibits LH suppression serum LH, has been shown to decrease amyloid in the brains of normal and aged AbetaPP-transgenic mice (Bowen et al., 2004; Casadeous et al., 2004).

METHODS AND RESULTS

- Previous studies have shown that activation of GnRH receptors by a GnRH agonist, specifically leuprolide acetate, induced a long-lasting enhancement of synaptic transmission mediated by ionotropic glutamate receptors in CA1 pyramidal neurons of rat hippocampal slices (Yang et al., 1999).
- Binding of GnRH to the aged hypothalamic GnRH receptors may also explain a potential source for the extrapituitary, intracellular LH which has been localized to the cytoplasm of pyramidal neurons of the cerebral cortex and hippocampus of the rat and human brains (Bowen et al., 2002).
- Extracellular LH may contribute to the aging process, specifically extrapituitary GnRH receptors, promoting LH production and in turn activating LH receptors in the brain. These receptors induce a long-term enhancement of excitatory postsynaptic currents mediated by ionotropic glutamate receptors (B). GnRH treatment differentially induces the expression of LH in human neuroblastoma cells. No significant differences in expression were observed in control and AD tissues (both hippocampus and cortex).
- GnRH receptor I also was localized to human M17 neuroblastoma cells and rat primary cortical neurons. GnRH receptor I expression differentially induces the expression of LH, yet this expression is independent of GnRH expression.
- Both GnRHR I and LH mRNA were detected in the M17 cells in order to confirm their presence, and the use of these cells as a model system.

CONCLUSIONS

- We have identified GnRHR I on extrapituitary cells of the human brain.
- GnRHR I was immunolocalized to the cell bodies and apical dendrites of pyramidal neurons in the hippocampus.
- GnRHR expression also was found to increase in differentiating embryonic rat primary cortical neurons. GnRH receptor I also was localized to human M17 neuroblastoma cells and rat primary cortical neurons.
- GnRH receptor I expression differentially induces the expression of LH, yet this expression is independent of GnRH expression.

DISCUSSION

- GnRHR I expression increases with differentiation in primary rat cortical neurons.

REFERENCES