Rebecca E. Ruggiero, B.S., Djurdjica Coss, PhD.
University of California, Riverside, Riverside, CA, USA.

Gonadotropin releasing hormone (GnRH) from the hypothalamus regulates the synthesis and secretion of gonadotropin hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH regulates steroidogenesis in both sexes and ovulation in females, while FSH stimulates folliculogenesis in females and spermatogenesis in males. LH and FSH are heterodimers of a common alpha subunit and a unique beta subunit, which provides biological specificity and is the rate limiting factor in hormone synthesis. Immediate early genes, early growth response 1 (Egr1) and fos proto-oncogene (cFos) are critical in the induction of LH-beta and FSH-beta subunits by GnRH, respectfully. However, gaps exist in our understanding of developmental initiation and hormonal regulation of gonadotropin gene expression. Specifically, epigenetic mechanisms that may play a role in beta subunit transcriptional regulation are unknown. The aim of this work was to identify transcriptional cofactors that are recruited to gonadotropin beta-subunit promoters with or without GnRH. Transcription factors interact with cofactors that recruit chromatin remodeling enzymes in order to regulate transcription. Identification of cofactors may explain tight regulation of gonadotropin hormone levels in reproductive physiology. Previous studies identified regions on the beta-subunit promoters that are necessary for GnRH responsiveness. These regions were used to pull down interacting proteins that bind to these response elements using nuclear extracts from the immortalized mature gonadotrope cell line, LβT2. Using a discovery proteomics approach, we identified different transcriptional cofactors that are recruited to beta subunit promoters with or without GnRH. Approximately 1500-2000 proteins were identified per pulldown. As expected, proteins known to interact with beta subunit promoters, such as Egr1, cFos and cJun were identified in the DNA pulldown experiments as positive controls. We identified 63 proteins unique for LH-beta promoter under control conditions and 60 unique for FSH-beta promoter, of which 7 proteins for LH-beta and 8 proteins for FSH-beta may play a role as corepressors. We further identified 97 proteins that were pulled down with the LH-beta promoter following GnRH treatment, of which 9 proteins were also pulled down with Egr1 as potential coactivator candidates. We also identified 72 proteins that were pulled down with the FSH-beta promoter following GnRH treatment, of which 6 proteins were pulled down with cFos as potential coactivator candidates. Functional studies to identify roles of these cofactors in gonadotropin hormone expression are in progress. The identification of epigenetic regulators will allow for better understanding of the transcriptional regulation of gonadotropin beta-subunit gene expression, which is critical for reproductive function.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

***Estrogen Differentially Regulates Protein Abundance in Exosomes Released From Immortalized Kisspeptin Neurons** in Vitro*

Teagan James, BS, Patrick Everett Chappell, PhD.
Oregon State University, Corvallis, OR, USA.

Estrogen (E2) is essential for multiple physiological effects in females, ensuring maximum reproductive fitness and maintaining skeletal homeostasis. E2 has been shown to stimulate cancellous bone formation via activation of estrogen receptor alpha (ERα), an effect widely accepted to be mediated directly at bone. A recent landmark study (Herber et al., *Nat Commun* 2019) demonstrated bone density increases in female mice harboring ERα-deletions specifically in arcuate Kiss-1 neurons. In this study, bone from transgenic females showed higher osteoblast functioning and increases in the expression of *sp7* and *runx2,* positing a direct neural-bone regulatory axis altered by circulating E2 acting in brain. Our laboratory has used two immortalized Kisspeptin (Kiss1)-expressing and -secreting cell lines, KTaR-1 (representative of female arcuate Kiss-1 neurons) and KTaV-3 cells (representative of female AVPV Kiss-1 neurons) as models to explore the role of Kiss-1 in multiple physiological regulatory contexts. We recently determined that factors in the media of female ARC-derived KTaR-1 cells can affect parameters of osteoblast function *in vitro*, including increases in *sp7* and *runx2* expression, and formation of bone matrix (evaluated by Alizarin Red assay). Exposure of canine osteosarcoma cells to conditioned media from KTaR-1 cells led to increases in *sp7* expression in an E2-dependent manner, and 24h E2-deprivation of these neurons stimulated secretion of osteogenic factors. In this current study, we have used LCMS-MS proteomic analysis to determine the contents of exosomes isolated from Kisspeptin neurons under varying E2 exposure conditions *in vitro*. Preliminary results reveal ~150-170 proteins up-regulated by E2 exposure and ~200-220 proteins downregulated by E2 exposure in exosomes of both KTaR-1 and KTaV-3 Kisspeptin neurons. Estrogen-regulated Kiss-1 exosomal proteins include several candidates involved in bone remodeling (pentraxin, osteonectin, osteoclast-stimulating factor-1) and neuronal synaptic plasticity and signaling (annexins, semaphorins, connexins). Current work is exploring the effects of exposure of purified exosomes on morphology and gene expression in immortalized GnRH neurons and osteoblasts. While further study is required, initial results suggest that exosomes may represent additional cellular communication pathways utilized by Kisspeptin neurons to elicit changes in brain and bone.

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NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Expression of IGF-1, IGF-1 Receptor and Growth Hormone Receptor in Hepatic Tissue in Adults Across the Spectrum of Nonalcoholic Fatty Liver Disease (NAFLD)

Laura E. Dichtel, MD[1], Sonu Subudhi, PhD, MB, BS[2], Hannah Drescher, PhD[2], Lea Bartsch, MD[2], Stephanie Osganian, BA[3], Mark Chicote, BS[4], Ricard Masia, MD[5], Miriam Bredella, MD[6], Sangeeta Bhatia, MD, PhD[7], Georg Lauer, MD, PhD[2], Karen Klahr Miller, MD[1], Kathleen Corey, MD[2].

[1]Neuroendocrine Unit, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA, [2]Division of Gastroenterology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA, [3]Division of Gastroenterology, Massachusetts General Hospital, Boston, MA, USA, [4]Neuroendocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [5]Department of Pathology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA, [6]Department of Radiology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA, [7]Massachusetts Institute of Technology, Cambridge, MA, USA.

Background: Obesity is a state of relative growth hormone (GH) and insulin-like growth factor-1 (IGF-1) deficiency, and the GH/IGF-1 axis has been implicated in the pathophysiology of nonalcoholic fatty liver disease (NAFLD) and the progression to steatohepatitis (NASH) in preclinical models and human studies. GH has both lipolytic and anti-inflammatory properties while IGF-1 has been implicated in reducing hepatic fibrosis and promoting hepatic regeneration. The GH/IGF-1 axis may be a therapeutic target in NAFLD/NASH, however, IGF-1, IGF-1 receptor (IGF-1R) and GH receptor (GHR) expression in adult human hepatic tissue has not been studied across the spectrum of disease severity.
Methods: We quantified IGF-1, IGF-1R, and GHR gene expression in hepatic tissue from 318 adults with obesity using the Nanostring nCounter assay. Subjects were classified into four categories of disease severity based on histopathology: normal liver histology (NLH) (n=76, 24%), steatosis only (Steatosis) (n=88, 28%), NASH without fibrosis (NASH F0) (n=72, 23%), and NASH with fibrosis (NASH F1-F4) (n=82, 26%). Gene expression analysis is presented as normalized gene counts by group with p-value of the generalized linear model controlled for age, sex and BMI.
Results: Mean (±SD) age (whole cohort 44.0±12 years) and BMI (whole cohort 46.8±7.2 kg/m^2) did not differ across groups (p=0.2 for both). ALT was higher with increasing disease severity (NLH 30.1±26.7, Steatosis 31.9±15.7, NASH F0 35.7±16.5, NASH F1-4 48.4±34.9, p<0.001). IGF-1 gene expression was lower in all NAFLD/NASH groups compared to the NLH reference group (NLH 485.4±292.7; Steatosis 396.3±238.0, p=0.04; NASH F0 349.8±220.1, p=0.01 and NASH F1-4 341.2±268.6, p=0.03, all p-values vs NLH). There was no difference in IGF-1R or GHR gene expression across disease severity groups (IGF-1R NLH 43.3±10.2, Steatosis 41.4±11.6, NASH F0 38.8±8.8 and NASH F1-4 39.1±8.1, p>0.05 between any disease state; GHR NLH 6382±2366, Steatosis 6544±2699, NASH F0 7220±2542 and NASH F1-4 5997±2352, p>0.05 between any disease state).
Conclusion: We demonstrated that IGF-1 gene expression was lower in liver tissue from patients with NAFLD and NASH than healthy controls. This is consistent with our prior finding that histologic NASH and fibrosis are associated with lower serum IGF-1 levels. Moreover, we demonstrated that hepatic IGF-1R and GHR gene expression is not lower in liver tissue from patients with NAFLD and does not decline across disease severity. This reinforces our prior finding that GHR staining intensity and zonality by immunohistochemistry does not change with increasing disease severity in NAFLD/NASH. These data demonstrate that the GH axis is relatively suppressed but that expression of GHR and IGF-1R receptors is stable with worsening disease severity in NAFLD/NASH, suggesting that GH augmentation may be a viable therapeutic target in NAFLD.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Expression of Kisspeptin, Neurokinin B, and Dynorphin During Pubertal Development in Female Sheep

Eliana G. Aerts, B.S.[1], KaLynn E. Harlow, M.S.[2], Max J. Griesgraber, B.S.[1], Elizabeth C. Bowdridge, Ph.D.[1], Steven L. Hardy, Ph.D.[1], Casey C. Nestor, Ph.D.[2], Stanley M. Hileman, Ph.D.[1].
[1]West Virginia University, Morgantown, WV, USA, [2]North Carolina State University, Raleigh, NC, USA.

Puberty onset depends upon an increase in pulsatile GnRH/LH secretion, which in sheep is the result of reduced sensitivity to estrogen negative feedback. Neurons within the arcuate nucleus of the hypothalamus (ARC) expressing kisspeptin, neurokinin B (NKB), and dynorphin (i.e. KNDy neurons) express estrogen receptors and are believed to play a key role in mediating the effects of estrogen on GnRH/LH secretion. Therefore, the purpose of this study was to assess changes in kisspeptin, NKB, and dynorphin within the ARC across pubertal development in female sheep. Blood samples were collected at 12-minute intervals for 4 hours and assessed for LH secretion in five age groups of ewes: 5 months (n=6), 6 months (n=6), 7 months (n=5), 8 months (n=5), and 10 months (n=6) of age. Following each bleed, ewes were sacrificed, hypothalamic tissue containing the ARC was collected, and then processed for use in dual immunofluorescence and RNAscope. Mean LH and LH pulse frequencies followed the expected patterns: concentrations and frequencies were low during the prepubertal ages (5-7 months of age), intermediate during the peripubertal age (8 months of age), and elevated in the postpubertal age group (10 months of age). Using immunofluorescence, kisspeptin and NKB immuno-positive cell numbers did not change significantly (P > 0.50) over time with cell numbers averaging 235.3±16.8 and 231.3±16.8, respectively. Colocalization of kisspeptin and NKB was greater than 90% for all age groups. Using RNAscope, the total number of cells expressing mRNA for kisspeptin and dynorphin did not change significantly (P > 0.05) over time with cell numbers averaging 46.7±12.0 and 28.3±10.0 cells/hemisection, respectively. Taken together, our data suggest that the increase in LH secretion that drives puberty onset is not limited by changes in kisspeptin, NKB, or dynorphin expression, but may instead depend on other factors such as changes in receptor expression or changes in KNDy neuron activity via a reduction in inhibitory and/or an increase in stimulatory afferent inputs.

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