pulses over three hours were similar in ovariectomized WT and KI mice; however, KI mice had significantly reduced LH secretion, as measured by area under the curve. Similarly, GnRH treatment induced a diminished LH response in intact KI compared to WT males. *In vitro* cultures of hemi-pituitaries from gonadectomized WT and KI males were exposed to 0.01 nM GnRH and LH secretion into culture media was measured by ELISA at 0, 0.5, 1, 2, and 4 hours. There was no difference in basal LH secretion between WT and KI pituitaries but GnRH induction of LH was significantly lower in cultures from AP-1 mutant mice, indicating a direct impairment of GnRH action at the level of the pituitary. Taken together, these data indicate that the gonadotroph *Gnrhr* AP-1 promoter motif is critical for normal reproductive function. Prevention of AP-1 binding to the *Gnrhr* proximal promoter element decreases GnRH-induced *Gnrhr, Lhb, and Fshb* levels, impairs GnRH-stimulated LH secretion, and disrupts pubertal development and reproductive cyclicity in female mice.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Neuroendocrine Basis for Disrupted Ovarian Cyclicity in Females During Chronic Undernutrition: A Mouse Model

Kirollos S. Tadrousse, UC San Diego Undergraduate, Richard B. McCosh, PhD, Michael J. Kreisman, MS, Kellie M. Breen, PhD.
University of California, San Diego, La Jolla, CA, USA.

Chronic undernutrition is a type of metabolic stress that impairs reproduction across species and, in women, is implicated in the development of functional hypothalamic amenorrhea. Although the tight coupling of energy balance to reproductive capacity is recognized *in principle*, the neuroendocrine loci and molecular mechanisms that mediate ovarian cycle dysfunction during undernutrition remain poorly understood. Ovarian cyclicity is dependent on a population of kisspeptin (Kiss1) neurons in arcuate nucleus (ARC^{Kiss1}) for luteinizing hormone (LH) pulses and in the anteroventral periventricular nucleus ($AVPV^{Kiss1}$) for LH surge secretion. Here, we present a series of studies in which we tested the hypothesis that inhibition of both Kiss1 cell populations underlies the impairment of the cycle by undernutrition. During a baseline period, body weight, feed intake, and ovarian cycle stage (via vaginal cytology) were evaluated in female c57bl6 mice. Then, animals were randomly assigned into one of two groups (n=6-8/grp): 1) ad libitum fed controls or 2) feed restricted (70% of feed consumed during the baseline period). Control animals displayed clear and regular cycles throughout the 4-week treatment period. In contrast, feed restriction caused a significant and rapid cessation of ovarian cyclicity (4.8±0.3 vs. 1.5±0.5 estrus cycles/4 weeks; control vs. restricted, $p<0.05$), causing all females to enter and remain mostly in diestrus. Based on these results, we conducted two experiments to directly test the hypothesis that undernutrition inhibits both modes of LH secretion (and both Kiss1 cell populations) using two well-defined estradiol (E) replacement paradigms. We first evaluated LH pulses in mice that were ovariectomized and implanted subcutaneously with a pellet containing a diestrus level of E (100 ng, OVX+LowE). Following 3 days of feed restriction or control diet (n=3/grp), serial blood samples were collected every 8 min for 88 min. Undernutrition prevented LH pulses and significantly reduced mean LH (5.2±0.6 vs. 0.6±0.2 ng/mL; control vs. restricted, $p<0.05$). Fixed neural tissue was evaluated by immunohistochemistry to determine whether undernutrition impairs ARC^{Kiss1} neuronal activation, using c-Fos as a marker. The percent of ARC^{Kiss1} neurons expressing cFos was reduced by 90% ($p<0.05$). We next evaluated the LH surge. After 3 days, control or feed restricted mice were OVX and implanted subcutaneously with a surge-inducing estradiol implant (OVX+HighE, 1 μg, n=3-4/grp). Undernutrition completely blocked the E-induced LH surge (1.9±0.3 vs. 0.2±0.02 ng/mL; control vs. restricted, $p<0.05$) and diminished *Kiss1* mRNA abundance in micropunches of the AVPV (42%, $p<0.05$). Collectively, these studies clearly show that undernutrition impairs both ARC^{Kiss1} control of LH pulses and $AVPV^{Kiss1}$ induction of the LH surge, via mechanisms that remain to be identified.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Neuromedin B Receptor Antagonist Suppresses Pomc Expression in AtT-20 Cells and Human Corticotroph Adenoma Cells

Tomonori Sekizaki, MD, Hiraku Kameda, MD, PhD, Akinobu Nakamura, PhD, Hiroshi Nomoto, PhD, Kyu Yong Cho, PhD, Yukitomo Ishi, MD, Hiroaki Motegi, PhD, Hideaki Miyoshi, PhD, Tatsuya Atsumi, PhD.
HOKKAIDO University GRAD SCHL OF Medical, Sapporo, Japan.

Objective: We previously reported that Neuromedin B (NMB) is expressed in murine pituitary corticotrophs under adrenal insufficiency (1). Because NMB is also expressed in several cancer cells and stimulates ACTH secretion, we hypothesized that NMB is related to corticotroph adenoma cell proliferation and hormone secretion. To examine this hypothesis, we investigated the expression of NMB and its receptor NMBR in human corticotroph adenoma and the effects of a NMBR antagonist on AtT-20 cells, a mouse corticotroph adenoma cell line, and patient-derived corticotroph adenoma cells. **Methods:** 1. NMB and NMBR expression in human pituitary adenoma: We performed real-time qPCR and immunostaining on human pathological specimens of corticotrophs, somatotrophs, and non-functioning pituitary adenoma to investigate NMB and NMBR expression. 2. Experiments in AtT-20 cells: We extracted mRNAs and proteins from AtT-20 cells after incubation with 100nM NMBR antagonist PD168368, and performed real-time qPCR and western blotting analyses to investigate Pomc expression. 3. Experiments in patient-derived corticotroph adenoma cells: We isolated surgically resected human corticotroph adenoma cells from patients who underwent trans-sphenoidal surgery and investigated POMC mRNA expression by real-time qPCR after incubation with PD168368. Statistical analysis: One-way

ANOVA was employed to compare values among multiple groups. If the ANOVA revealed significant differences, the Tukey-Kramer post-hoc test was employed to compare values between two specific groups. Dunnett's post-hoc test was employed to compare values with the control group. Statistical significance was defined as $p < 0.05$. **Results:** 1. NMB and NMBR expression levels were significantly higher in human corticotroph adenoma (13 and 33 times higher than non-functioning adenoma, respectively) than in somatotroph adenoma (2 and 3 times higher than non-functioning adenoma, respectively) and non-functioning adenoma in the qPCR analyses. Immunostaining confirmed higher expression of NMB and NMBR in corticotroph adenoma than in somatotroph and non-functioning adenoma. 2. Treatment with 100 nM PD168368 significantly suppressed Pomc mRNA and protein expression in AtT-20 cells by 22%±3% and 25%±10%, respectively. 3. Treatment with 1 μM PD168368 significantly suppressed POMC mRNA expression in human corticotroph adenoma cells by 18%±1%. **Conclusions:** NMB and NMBR were both expressed in human corticotroph adenoma, suggesting that NMB may stimulate adenoma cell proliferation and hormone secretion in autocrine or paracrine manners. Because the NMBR antagonist suppressed Pomc expression in both AtT-20 cells and human corticotroph adenoma cells, it may represent a potential treatment for Cushing disease. **Reference:** (1) Kameda H et al., Endocrinology 2014;155(7):2492-9.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Overactive Reproductive Axis Due to Fragile X Gene Mutation

Pedro Villa, B.S., Nancy Lainez, PhD, Iryna Ethell, PhD, Djurdjica Coss, PhD.
University of California, Riverside, Riverside, CA, USA.

Women carrying a pre-mutation or mutation of the Fragile X mental retardation gene (FMR1) comprise the largest portion of premature ovarian failure (POF) cases due to known genetic factors. FMR1 mutation causes Fragile X syndrome, the most common cause of inherited mental impairment. The mutation inhibits the expression of the fragile X mental retardation protein (FMRP), a ubiquitously expressed mRNA binding protein. The specific molecular mechanism(s) leading to premature ovarian failure in Fragile X carriers are not known. Here, we utilize the complete KO mouse model, to mimic the lack of FMRP in Fragile X mutations and analyze the hypothalamic-pituitary-gonadal axis to uncover causes of POF due to FMR1 mutation. Consistent with mutations in human population, KO females experience early cessation of reproductive function and stop having litters at 150 days of age, compared to controls that stop reproducing at 250 days of age. Since POF can be caused by either insufficient pool of primordial follicles or by increased recruitment in each cycle and early depletion, we analyzed ovaries at 3 weeks of age and determined that the FMR1 KO mice had the same number of primordial follicles when compared to the controls, suggesting that POF is not due to a deficit in primordial follicles. However, at 8 weeks of age, FMR1 KO ovaries had higher number of corpora lutea, and KO females had larger litters, indicating that FMR1 KO mice have more follicles recruited in each estrous cycle. FMR1 KO mice have higher FSH, which corresponds to the high FSH in women. Serum estradiol levels and inhibin b expression levels were unaffected by FMR1 mutation suggesting normally functioning negative feedback signals from the ovaries. Analyses of hypothalamic gene expression demonstrated elevated *GnRH* mRNA in KO mice. To further investigate alterations is hypothalamic protein levels, western blot analyses determined that FMR1 KO mice have higher levels of NMDAR1 and higher levels of $GABA_A$ receptor G2 subunit. Dual label immunofluorescence analyses revealed higher number of NMDAR1 and $GABA_A$ receptors specifically in GnRH neurons of FMR1 KO mice when compared to control, suggesting that GnRH neurons themselves are affected by FMR1 mutation. Given that both glutamate and GABA can activate GnRH neurons, alterations in the number of these receptors can potentially cause hyperactivity in the HPG axis at the hypothalamic level leading to elevated FSH and the subsequent POF. In summary, our results reveal a potential mechanism of premature ovarian failure in Fragile X mutation carriers.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Paracrine Signalling From SOX2-Expressing Pituitary Embryonic Cells Is Required for Terminal Differentiation of Hormone-Producing Cells

Saba Manshaei, BSc[1], Thea Willis, BSc, MRes[2], Dominic Withers, PhD[3], Jesus Gil, PhD[3], Cynthia Lilian Andoniadou, BSC, PhD[2], Juan Pedro Martinez-Barbera, PhD[4].
[1]UCL Great Ormond Street Institute of Child Health, London, United Kingdom, [2]King's College London, London, United Kingdom, [3]Imperial College London, London, United Kingdom, [4]University College London, London, United Kingdom.

The pituitary gland is the master regulator of the endocrine system, housing six major hormone producing cell types. This gland is derived from Rathke's Pouch, an invagination of the oral ectoderm. Hormone-producing pituitary cell lineages are derived from a population of embryonic cells expressing SOX2. ZFP36L1/Butyrate Response Factor 1 (BRF1) is an RNA binding protein that binds and targets mRNAs of various cytokines and chemokines for degradation prior to translation, attenuating secretion of inflammatory factors (Herranz et al. 2015). Here, we show that BRF1 is a novel marker expressed in SOX2+ cells in human and mouse pituitaries, suggesting that these cells may have a secretory profile. To investigate this possibility, we have combined molecular and genetic studies *in vivo*. We have used a novel mouse model, $R26^{lsl\text{-}mBRF1}$ that allows the expression of a mutant, constitutively active BRF1 protein upon *Cre*-mediated recombination, alongside our lab's models ($Hesx1^{Cre/+}$ and $Sox2^{CreERT2/+}$), to express mutant BRF1 in HESX1+ and SOX2+ cells during development and postnatally. This approach results in pituitary hypoplasia and severe hypopituitarism due to a failure of cell-lineage specified cells to differentiate into hormone-producing cells.