

Neuroendocrinology and Pituitary

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Chronic Undernutrition Inhibits KNDy Neurons in Ovariectomized Ewe Lambs

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Undernutrition negatively impacts reproductive success, at least in part, through a central suppression of GnRH secretion. Given that GnRH neurons are devoid of receptors for peripheral metabolic hormones such as leptin and insulin, nutritional regulation of GnRH secretion must be through afferent input. Neurons which co-express kisspeptin, neurokinin B (NKB), and dynorphin, termed KNDy neurons, are a unique population of cells in the arcuate nucleus (ARC) of the hypothalamus and are believed to play a critical role in GnRH/LH pulse generation. With our recent evidence that chronic feed restriction reduced kisspeptin and NKB in young, castrated male sheep, we hypothesized that nutrient restriction would inhibit expression of the KNDy neuron peptides kisspeptin and NKB, while increasing dynorphin expression in young, ovariectomized female sheep. Fifteen ewe lambs were ovariectomized and were fed to maintain body weight (n=7; Fed) or feed-restricted to lose 20% of pre-study body weight (FR; n=8). Blood samples were taken weekly every 12 minutes for 4.5 hours via jugular venipuncture and plasma was stored at -20°C until assessment of LH using radioimmunoassay. Body weights were recorded weekly and feed amounts were adjusted to achieve desired body weights. Following blood collection at Week 13, animals were euthanized, brain tissue was perfused with 4% paraformaldehyde, and tissue containing the hypothalamus was collected for assessment of KNDy neuropeptide mRNA abundance by *in situ* hybridization (RNAscope). At Week 13, the average percent change in body weight was clearly evident (Fed, 7.35 +/- 2.2% vs FR, -21.36 +/- 0.9%), and mean LH concentrations were lower in FR ewes (10.70 +/- 3.1 ng/ml) compared to Fed controls (20.98 +/- 3.8 ng/ml). Data analyzed to date for kisspeptin, NKB, and dynorphin in the ARC show that feed restriction reduced the number of kisspeptin mRNA-expressing cells (Fed, 165 +/- 25 vs FR, 9.25 +/- 6), the number of NKB mRNA-expressing cells (Fed, 141 +/- 28 vs FR, 24 +/- 5), and the number of dynorphin mRNA-expressing cells (Fed, 109 +/- 41 vs FR, 29.5 +/- 24). Together, these findings demonstrate that chronic feed restriction suppresses KNDy neurons and supports a role for these key reproductive neurons in the central mechanism governing GnRH/LH secretion during undernutrition in female sheep.

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Circulating LEAP-2 Levels in Adult Growth Hormone Deficiency and Their Relationship With IGF-1

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Liver enriched antimicrobial peptide-2 (LEAP-2) is an endogenous antagonist of ghrelin, which acts as an allosteric modulator of growth hormone secretagogue receptors. It is expressed predominantly in liver, followed by kidney, jejunum, duodenum and stomach. Its expression in conditions of metabolic impairment, obesity for instance, may be upregulated, usually pairing with a concomitant reduction in ghrelin secretion. Weight gain and hyperglycaemia seem to be the main trigger factors for LEAP-2 production. Adult growth hormone deficiency (aGHD) is known as a pathological condition characterized by metabolic impairment (insulin resistance, weight gain, increased fat mass, decreased lean mass). To the best of our knowledge, no study in literature deals with the problem of circulating LEAP-2 levels in GHD. Therefore, the primary endpoint of this cross-sectional observational study was to evaluate circulating LEAP-2 levels in GHD in comparison to healthy controls whether the secondary endpoint was to evaluate any possible correlation with IGF-1 and metabolic parameters in such condition.

30 patients were included in the study. Group A included adult GHD: 15 patients, 7 females and 8 males, mean±standard error of the mean (SEM) age 54.6±3.51 years, BMI 29.95±1.63 kg/m². The etiologies of GHD were empty sella (n=6), idiopathic (n=6), post-surgical hypopituitarism (n=2), pineal cyst (n=1). Group B was formed by controls: 15 patients, 11 females and 4 males, mean±SEM age 40.33±2.61 years, BMI 24.19±1.33 kg/m². They were evaluated for: serum glucose and insulin, HOMA-index, QUICKI-index, Total/LDL/HDL cholesterol, triglycerides, IGF-1 and LEAP-2 (measured using Human LEAP-2 ELISA kit, Phoenix Pharmaceuticals Inc, according to manufacturer's instructions).

Circulating LEAP-2 is significantly higher in GHD than in control group (26.98±3.12 vs 18.7±1.9 ng/ml, p=0.03). LEAP-2 levels in our cohort was not influenced by BMI, while a significant direct correlation between LEAP-2 and age was detected in aGHD group. A strong significant inverse correlation between LEAP-2 and IGF-1 was evidenced in aGHD (r²=0.5). Finally, in this group, LEAP-2 inversely correlates with total cholesterol (r²=0.45).

As expected circulating LEAP-2 levels, in a condition of metabolic impairment such as GHD, were higher than controls even if no correlation with BMI was evidenced in our cohort. The inverse correlation between LEAP-2 and IGF-1 in GHD patients may suggest a body adjustment in a worse clinical condition. LEAP-2 may act as a brake for ghrelin production, thus preventing further weight gain and fat mass increase, although losing its secretagogue effect.

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Corticosterone Enhances Formation of Neutral but Not Fear Memory During Infectious Illness

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Survivors of critical illness often report traumatic memories of their illness period, and these memories are thought to contribute to development of neuropsychiatric disorders, such as PTSD. Many patients are treated with high doses of glucocorticoids for their vasoactive and anti-inflammatory properties, and glucocorticoids have also been shown to prevent the development of PTSD after trauma. Due to their activity in the hippocampus and amygdala, the putative protective effect of glucocorticoids may occur via memory formation during illness. To examine the effect of glucocorticoids on memory formation during acute infectious illness, male and female C57BL/6 mice (N=80, 40 male/40 female) underwent cecal ligation and puncture and were treated with either corticosterone (16 mg/kg) or vehicle in the early afternoon daily for five days beginning on the day of surgery. All mice were habituated to a neutral object in their home cage for five days and underwent one 30-minute footshock/no shock training session during the illness period. After physiologic recovery (2 weeks), the mice underwent behavioral testing including open field exploration, object recognition testing in which they were presented with both the familiar (habituated) object and a novel object, and testing in the shock context. The results showed that drug treatment had no effect on behavior in the open field, including time spent in the center (VEH: 20.19±10.81 vs CORT: 22.32±12.87 sec; P=0.476). Drug treatment increased overall object exploration (12.28±10.79 vs 19.17±15.88 sec; P=0.049). Corticosterone-treated mice showed a preference for the familiar object (60.9±23.0% of total exploration time with familiar object; P=0.015), while vehicle-treated mice did not (54.1±23.3%; P=0.378). The increase in overall object exploration seen in corticosterone-treated mice could be accounted for by an increase in exploration of the familiar object. History of footshock increased freezing in the training context (3.96±2.54% vs 36.08±15.42%; P<0.0001) and corticosterone treatment had no effect (18.06±17.65% vs 22.16±21.19%; P=0.557). In conclusion, administration of corticosterone during infectious illness facilitated memory of a neutral object from the illness period, and recovered mice exhibited a preference for this object over a novel one. Corticosterone treatment had no impact on fear memory formed during illness. This is consistent with human literature suggesting that hydrocortisone decreases PTSD symptoms without impacting traumatic memories. These findings suggest that glucocorticoids selectively enhance the formation, consolidation, and/or recall of neutral but not fear memories during illness, which may rely on hippocampal circuitry. We further suggest that accurate memories of the illness period may influence patients' perception of this experience and alter their risk for psychiatric sequelae.

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*Development of a Novel Tetracycline-Inducible
Kiss1-Cre Mouse Line for Temporally Controlled Gene
Deletion in Kisspeptin Neurons*

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The tetracycline (Tet)-controlled inducible system is the most widely used reversible system for transgenic expression in mice. Previously, we generated a GnRH-CreTer mouse model, using a first-generation Tet-inducible system to temporally induce expression of Cre recombinase in GnRH neurons. Recently, the Tet-inducible system has undergone several modifications to significantly reduce previous limitations that include leaky background expression and lower sensitivity to tetracycline induction. Therefore, we have developed a novel mouse model bearing a Tet-inducible kisspeptin-Cre allele (iKiss-Cre mouse) that will enable temporal control over the selective deletion of genes from Kiss1 neurons. This temporally controlled gene deletion will eliminate a longstanding technical limitation of conventional steroid receptor knockout models in which steroid regulation of the axis is confounded by steroid developmental and organizational effects in the reproductive axis. Two mouse lines were generated. The first line targets kisspeptin neurons with a third generation Tet-inducible reverse tetracycline transactivator (rtTA, Tet-On 3G) expressed under the control of the Kiss1 allele. Using CRISPR-Cas9 technology, we inserted a cassette containing an internal ribosome entry site (IRES) sequence followed by the rtTA downstream of the Kiss1 coding region as was previously done using Cre recombinase. Transcription of the recombinant Kiss1 allele yields a bicistronic messenger RNA, from which both kisspeptin and rtTA are independently translated. The second mouse line, TRE-Cre mice, was constructed to express Cre recombinase under control of the PTRE3G promoter. The PTRE3G promoter is bidirectional to allow simultaneous monitoring of Cre expression and a second fluorescent protein reporter (ZsGreen1). The Kiss1-rtTA only binds to and activates the PTRE3G promoter in the presence of doxycycline (an analog of tetracycline). Offspring from breeding of these mouse lines, iKiss-Cre, result in a system capable of generating Tet-induced expression of Cre recombinase in kisspeptin neurons. In order to document specificity and sensitivity of this system, we performed immunofluorescent staining and observed colocalization of kisspeptin with Cre recombinase in brain sections of iKiss-Cre mice only after doxycycline treatment. We will use this model to investigate negative feedback actions of E₂ on the adult pulse generator after E₂ exerts its organizational actions on maturing kisspeptin neurons. We expect that this mouse model will become a major tool used by the neuroendocrine community and serve as proof of principle for development of similar inducible knockout models employing the current generation of inducible methodologies.

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*Differences in Menin Expression in Pituitary
Adenomas in Multiple Endocrine Neoplasia Type 1,
Its Phenocopies and Sporadic Acromegaly*