Oral Presentation Schedule and Abstracts for Session 2:
Saturday, August 6, 10:45AM-12:15PM

10:45-10:55AM
Abstract #5: The regulation of reproduction and metabolism by IGF-1 receptor and insulin receptor in Kisspeptin neurons

Speaker: Jennifer W. Hill
Department of Physiology and Pharmacology, University of Toledo College of Medicine, Toledo, Ohio, USA and Department of Obstetrics and Gynecology, University of Toledo College of Medicine, Toledo, Ohio, USA

11:00-11:10AM
Abstract #6: Obesity induces central hypogonadism and metabolic comorbidities via miRNA-137/325 mediated repression of hypothalamic kisspeptin

Speaker: Maria Soledad Avendaño
Instituto Maimonides de Investigación Biomédica de Córdoba (IMIBIC); University of Cordoba; and Spanish Network of Research on Obesity and Nutrition (CIBEROBN), Cordoba, Spain

11:15-11:25AM
Abstract #7: Arcuate Kiss1 neurons mediate melanocortin action on energy expenditure in male mice

Speaker: Rajae Talbi
Brigham and Women's Hospital, Division of Endocrinology, Diabetes and Hypertension. Boston, MA 02115 and Harvard Medical School. Boston, MA 02115

11:30-11:40AM
Abstract #8: Lesions of KNDy and Arcuate Kiss1R neurons produce different effects on LH pulse patterns

Speaker: Robert Goodman
West Virginia University, Morgantown, WV

11:45-11:55AM
Abstract #9: Activation of Kiss1 neurons in the preoptic hypothalamus stimulates testosterone synthesis in adult male mice

Speaker: Mònica Girona del Pozo
Institute of Neurosciences – Autonomous University of Barcelona (UAB)

12:00-12:10PM
Abstract #10: Kisspeptin Administration Increases Penile Tumescence and Sexual Brain Processing in Men with Low Sexual Desire

Speaker: Edouard Mills
Imperial College London, London, UK

Oral Presentation Abstract #5:

The regulation of reproduction and metabolism by IGF-1 receptor and insulin receptor in Kisspeptin neurons

Mengjie Wang[1], Seamus M. Pugh[1], Judy Daboul[1], **Jennifer W. Hill**[1, 2]

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Introduction/Aim:

The neuropeptide kisspeptin, encoded by the *Kiss1* gene, is critical for puberty and fertility, but the factors that regulate kisspeptin neurons need to be clarified. The anabolic factors insulin and insulin like growth factor-1 (IGF-1) may signal nutritional status to these neurons. Our lab has previously shown that deletion of insulin receptors (IRs) in *Kiss1* neurons delays the initiation of puberty but does not affect adult fertility or glucose homeostasis, although others have reported decreased fasting insulin levels in IR^{Kiss1} males.

Methods/Results:

To test whether IGF-1 action specifically in *Kiss1* neurons affects reproductive and metabolic functions, we have now generated transgenic mice lacking IGF-1 receptors (IGF1Rs) exclusively in *Kiss1* neurons ($IGF1R^{Kiss1}$ mice). Because IGF1R and IR signaling induce overlapping activation of the phosphoinositide 3-kinase (PI3K)-Akt pathway, we also generated mice with simultaneous deletions of IGF1Rs and IRs in *Kiss1* neurons ($IGF1R/IR^{Kiss1}$ mice). Both $IGF1R^{Kiss1}$ mice and $IGF1R/IR^{Kiss1}$ mice of both sexes experienced delayed puberty and adult reproductive deficits, and $IGF1R/IR^{Kiss1}$ mice had decreased gonadotropins. These results indicate that both IGF1R and IR in *Kiss1* neurons are required for the maintenance of normal reproductive function. Female $IGF1R^{Kiss1}$ mice exhibited a "metabolically healthy" phenotype with decreased body weight and food intake but increased energy expenditure and physical activity. These findings highlight the impact that IGF-1 has on metabolic function via *Kiss1* neurons. Notably, compared to controls and $IGF1R^{Kiss1}$ mice, $IGF1R/IR^{Kiss1}$ mice of both sexes had significantly increased fat mass percentage and disrupted glucose homeostasis, which suggests that IGF1R and IR may cooperatively regulate body composition and glucose homeostasis.

Conclusions:

In summary, our study shows that IGF1R and IR signaling in *Kiss1* neurons have unique and cooperative roles in the regulation of reproductive and metabolic functions in mice.

Oral Presentation Abstract #6:

Obesity induces central hypogonadism and metabolic comorbidities via miRNA-137/325 mediated repression of hypothalamic kisspeptin

Maria Soledad Avendaño*, Cecilia Perdices-Lopez, Yolanda Guerrero, Alexia Barroso, Marco A. Calzado, Veronica Sobrino, Maria Jesús Vazquez, Manuel Tena-Sempere*

Instituto Maimonides de Investigación Biomédica de Córdoba (IMIBIC); University of Cordoba; and Spanish Network of Research on Obesity and Nutrition (CIBEROBN), Cordoba, Spain. *Equal senior authors

Introduction/Aim:

Obesity is a life threatening condition associated with numerous comorbidities. Among them, central hypogonadism, defined by low circulating testosterone, has been recently suggested to contribute to metabolic complications of obesity, especially in men. Yet, the mechanisms for obesity-induced hypogonadism (OIH), and its actual role in the generation/evolution of the metabolic alterations linked to obesity remain ill defined. Recent data suggest that OIH may be bound to suppression of the hypothalamic Kiss1 system, which is a major activator of the reproductive axis. Yet, the mechanisms for Kiss1 suppression in obesity remain unknown. Considering our recent evidence for a role of microRNAs (miRNAs) in the modulation of the Kiss1 system, here we aimed to identify novel miRNAs capable of regulating kisspeptin expression and evaluated their potential contribution to OIH.

Methods/Results:

Bioinformatic analyses on the KISS1 gene were conducted with different algorithms to seek for potential miRNA regulators. Selection of miRNA candidates was based on the following criteria: 1) to be identified in at least two databases; 2) to show an evolutionary-conserved seed region (rat, mouse, human); and 3) to be deregulated by metabolic alterations. Using these criteria, the miR-137-3p/miR-325-3p tandem was selected, as putative modulators of a common, evolutionary conserved seed region at KISS1 3'-UTR. A repressive action of miR-137-3p/miR-325-3p at the human KISS1 3'-UTR was documented using luciferase reporter assays. In addition, central injection of a miR-137-3p mimic induced a decrease in kisspeptin levels, together with a drop in circulating testosterone in lean male rats. A Target Site Blocker (TSB), tailored to selectively block the repressive interaction of miR-137-3p/miR-325-3p at the Kiss1 3'-UTR, was centrally injected to obese male rats, displaying reduced testosterone (T) and gonadotropin (LH) levels. TSB infusion increased hypothalamic kisspeptin levels and enhanced LH and T concentrations. Of note, this model of OIH suffered also marked cardio-metabolic alterations, including increased systolic blood pressure, altered vascular reactivity, glucose intolerance and insulin resistance, that were ameliorated by TSB administration. Notably, reversal of OIH by TSB was more effective than that induced by chronic kisspeptin or testosterone treatments in obese rats. Conversely, over-expression of miR-137 in Kiss1 neurons reduced Kiss1/kisspeptin levels and partially replicated reproductive and metabolic alterations of OIH in lean mice.

Conclusions:

Our data provide conclusive evidence for a relevant role of miR-137-3p/miR-325-3p, as repressors of kisspeptin, in the pathophysiology of OIH, and strongly suggest that central hypogonadism is a key contributor for the metabolic complications of obesity.

Oral Presentation Abstract #7:

Arcuate Kiss1 neurons mediate melanocortin action on energy expenditure in male mice

Rajae Talbi[1,2], Todd Stincic[3,4], Kaitlin Ferrari[1,2], Ji Hae Choi[1,2], Achi Gerutshang[1,2], Silvia León[1,2], Caroline Maguire[1,2], Martin Kelly[3,4], Victor Navarro[1,2]

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Introduction/Aim:

Hypothalamic Kiss1 neurons are essential regulators of reproductive function through the direct release of the gonadotropin-releasing hormone (GnRH) secretagogue kisspeptin onto GnRH neurons. Mounting evidence from our lab and others suggest that Kiss1 neurons also have an active role in the control of metabolism, as suggested by the anorexigenic role of kisspeptin in rodent species under certain metabolic conditions and the obese phenotype of kisspeptin receptor knockout (Kiss1rKO) mice. In the control of energy balance, arcuate proopiomelanocortin (Pomc) neurons promote satiety and increase energy expenditure (EE). Because POMC neurons have been described to project to Kiss1 neurons, we set out to characterize the role of Kiss1 neurons in the metabolic action of melanocortins.

Methods/Results:

Using RNAscope we found that Mc4r is expressed in $Kiss1^{ARC}$ neurons, suggesting that these neurons are direct targets for melanocortins. Indeed, high-frequency stimulation results in the development of a slow inward current that is attenuated in the presence of a MC4R antagonist. In addition, low-frequency photo-stimulation of $POMC^{ARC}$ neurons also elicit a mono-synaptic glutamatergic response in $Kiss1^{ARC}$ neurons. To assess the role of Kiss1 neurons in the metabolic action of melanocortins, we generated a conditional mouse model with a specific deletion of Mc4r from Kiss1 neurons (Kiss1cre:Mc4rfl/fl mice). Kiss1cre:Mc4rfl/fl males developed obesity under chow and high fat diet compared to littermate controls, due to reduced EE, i.e. lower O2 consumption and CO2 production, without changes in overall food intake or activity. The decrease in EE correlated with significantly lower expression of the uncoupling protein 1 (Ucp1) gene in the interscapular pad of the brown adipose tissue (BAT), suggesting impaired BAT thermogenesis in KO mice. Viral tracing of $Kiss1^{ARC}$ neurons revealed projections to the dorsomedial hypothalamus (DMH), in line with recent studies showing that $Kiss1^{ARC}$ neurons can activate leptin receptor $(Lepr)^{DMH}$ neurons. Furthermore, through retrograde tracing, we observed that this population of $Lepr^{DMH}$ neurons projects to the raphe pallidus nucleus (RPa), known to regulate BAT activity.

Conclusions:

These data document the involvement of Kiss1 neurons in the metabolic action of melanocortins by serving as direct targets of α-MSH from POMC neurons. In turn, Kiss1 neurons regulate BAT activation through a pathway that involves $Lepr^{DMH}$ neurons and the RPa. These findings offer novel insight into the neurocircuitry underlying the melanocortin control of energy expenditure, which have been ill-defined compared to their role in food intake and suggest a mechanism by which reproductive events may affect metabolism regulation.

Oral Presentation Abstract #8:

Lesions of KNDy and Arcuate Kiss1R neurons produce different effects on LH pulse patterns

Robert Goodman[1], Kayla Onslow[2], Stanley Hileman[1], Steve Hardy[1], Elizabeth Bowdridge[1], Sami Agus[2], Max Griesgraber[1], Eliana Aerts[1], Lique Coolen[2], Michael Lehman[2]

[1]West Virginia University, Morgantown, WV; [2]Kent State University, Kent, OH

Introduction/Aim:

KNDy neurons in the arcuate nucleus (ARC) are critical for the generation of GnRH/LH pulses. Although these neurons do not contain Kiss1r, kisspeptin agonists and antagonists can act in the ARC to alter LH pulse patterns. Therefore, the aim of this study was to assess the contributions of these two neural populations to producing normal LH pulse patterns.

Methods/Results:

We used saporin conjugated to either NKB (NK3-SAP) or Kiss-10 (Kiss-SAP) to lesion KNDy and Kiss1r-containing cells in the ARC, respectively. LH pulses were monitored every 12 mins for 4 hours in ovariectomized ewes before and 2 and 3 weeks after bilateral injections of NK3-SAP (n=8), Kiss-SAP (n = 9), or Blank-SAP as a control (n = 10). Ewes were then euthanized and effects of injections on KNDy and Kiss1r cells assessed using RNAscope in situ hybridization. Effects of Kiss-SAP on GnRH cells and fibers was also determined using immunohistochemistry. There was no effect of Blank-SAP on LH pulse patterns, while NK3-SAP and Kiss-SAP decreased LH pulse amplitude to 16% and 33% of pre-treatment amplitudes, respectively. NK3-SAP also decreased LH pulse frequency, with inter-pulse intervals (IPI) increasing from 71.6 ± 5.0 mins to 126.3 ± 25.6 and 164. 3 ± 25.4 mins 2 and 3 weeks after injections. However, there was no significant effect of Kiss-SAP on IPI (pre: 68.9 ± 5.3 min; post: 91.3 ± 18.7 and 65.2 ± 5.0 min). The regularity of episodic LH release was also assessed by analyzing the variability of IPIs based on their coefficient of variability (CV) for each pattern. Kiss-SAP injections increased this CV from 8.3 ± 2.2% to 36.0 ± 7.0% and 33.7 ± 6.2% at 2 and 3 weeks post injection. In contrast, NK3-SAP did not (pre: 12.5 ± 3.4%, week 2 post: 19.8 ± 3.1%; too few pulses for analysis at week 3). Histological analysis is ongoing, but the results to date indicate that NK3-SAP lesioned almost all *Kiss1* cells, but did not affect *Kiss1r* neurons, while Kiss-SAP produced the opposite effect without altering GnRH in either cells or fibers.

Conclusions:

KNDy neurons are essential for episodic LH secretion in ewes, while ARC Kiss1r neurons are not. However, these Kiss1r neurons play an important role in ensuring regular pulse patterns and normal GnRH/LH pulse amplitude. We thus propose this population is part of a positive feedback loop that reinforces the KNDy neural activity responsible for GnRH/LH pulse patterns.

Oral Presentation Abstract #9:

Activation of Kiss1 neurons in the preoptic hypothalamus stimulates testosterone synthesis in adult male mice

Mònica Girona,[1] G. Stanley McKnight,[2] Albert Quintana,[1,3]* and Elisenda Sanz[1,3]*

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Introduction/Aim:

Hypothalamic kisspeptin (*Kiss1*)-expressing neurons are essential in the mammalian reproductive system. Both humans and mice deficient for kisspeptin signalling system components, which participate in the regulation of the hypothalamic-pituitary-gonadal (HPG) axis, have been reported to manifest hypogona-dotropic hypogonadism, infertility and absence of puberty. Hence, hypothalamic kisspeptin-positive neurons present in the rostral periventricular region of the third ventricle (RP3V) and the arcuate nucleus (ARC) constitute a critical sexually dimorphic neuronal population within the brain and are thought to coordinate differences in physiology and behaviour between males and females. Nevertheless, *Kiss1* mRNA expression in the RP3V seems to be notably reduced in males, in contrast to females. Kiss1^{RP3V} neurons in adult female mice are implicated in the stimulation of the preovulatory surge of GnRH and LH in response to estrogen, but adult male mice do not respond to either estrogen or testosterone with an LH surge.

Methods/Results:

This study uses adult mice (3 to 4 months) of the Kiss1$^{Cre:GFP}$ (Kiss1-Cre) mouse line (Gottsch et al., 2011) which had been backcrossed onto a C57BL/6 background. With the aim of exploring the functional role of the male Kiss1^{RP3V} neuronal population in the regulation of the HPG axis, Kiss1-Cre male mice were injected with a viral vector expressing a Cre-dependent activating DREADD, and neuronal activity of Kiss1 neurons was subsequently manipulated by administering the agonist clozapine-N-oxide (CNO) or vehicle. Administration of CNO resulted in increased serum testosterone levels compared to vehicle-injected mice. Thus, our results show that direct activation of male Kiss1^{RP3V} neurons stimulates the HPG axis by increasing testosterone production, suggesting that these neurons could act as physiological regulators of testosterone levels and may play a role in the reproductive behaviour of male mice. Male Kiss1^{RP3V} neurons will be further characterised using mouse genetics and cell-type specific gene expression analysis by crossing mice from the recently generated mouse line Kiss1-Cre (v2) (Padilla et al., 2018) with RiboTag mice (Sanz et al., 2009) to define the molecular profile of these neurons.

Conclusions:

Our results demonstrate that Kiss1^{RP3V} neurons regulate circulating testosterone levels and could play an essential role in the normal pulsatile regulation of testosterone in male mice. We expect our future results to give insight into the role of RP3V neurons in reproductive behaviour in adult male mice.

Oral Presentation Abstract #10:

Kisspeptin Administration Increases Penile Tumescence and Sexual Brain Processing in Men with Low Sexual Desire

Edouard Mills[1], Natalie Ertl[1,2], Matt B Wall[1,2], Layla Thurston[1], Lisa Yang[1], Sofiya Suladze[1], Tia Hunjan[1], Maria Phylactou[1], Bijal Patel[1], Beatrice Muzi[1], Dena Ettehad[1], Jonathan Howard[2], Eugenii A Rabiner[2], Paul Bech[1], Ali Abbara[1], David Goldmeier[3], Alexander N Comninos[1,4], Waljit S Dhillo[1,4]

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Introduction/Aim:

Hypoactive Sexual Desire Disorder (HSDD) is associated with dysfunctional brain activation in regions governing sexual responses, resulting in a deficiency/absence of sexual desire with marked distress. It affects up to 8% of men with detrimental effects on quality of life, interpersonal relationships and fertility, but so far has no licensed treatment options. The reproductive neuropeptide kisspeptin offers a putative therapeutic target owing to its emerging role in modulating reproductive behaviour in animal models and healthy men. However, there are no studies examining its effects in HSDD. To address this, we performed the first clinical study of kisspeptin in men with HSDD.

Methods/Results:

We examined the effects of kisspeptin administration (vs placebo) on brain activity during short and long erotic video tasks using functional MRI in 32 men with HSDD (mean ±SEM age 37.9 ±1.5 y, BMI 24.9 ±1.0 kg/m^2). To provide functional and behavioural relevance for the associated fMRI brain responses during the long erotic video, simultaneous penile tumescence and subjective level of arousal were recorded. Participants also completed psychometric and behavioural questionnaires. Standard analysis methods were used for fMRI data from the short videos task, and the long videos task used regressors derived from the subjective arousal and penile tumescence data. The statistical threshold used for both was $Z=2.3$, $p < 0.05$ (cluster-corrected).

In response to visual erotic stimuli, kisspeptin administration significantly increased penile tumescence during the long video task compared to placebo, with kisspeptin increasing penile tumescence by 56% ($p=0.002$). Moreover, kisspeptin increased participant-reported happiness about sex ($p=0.02$). During both video tasks, kisspeptin significantly modulated brain activity, compared to placebo, in key structures of the sexual-processing network. In response to short erotic videos, kisspeptin enhanced left middle frontal gyrus and left anterior cingulate activity, and decreased activity in bilateral parahippocampus (all $p<0.05$). During the long video task, kisspeptin enhanced right fusiform gyrus and bilateral visual cortex activity, and decreased left frontal pole, right posterior cingulate and bilateral precuneus activity (all <0.05). Additionally, we observed positive correlations between kisspeptin's effects on aforementioned brain activity and psychometric parameters of sexual desire and arousal (all $p<0.01$).

Conclusions:

Collectively, we demonstrate for the first time that kisspeptin administration in men with HSDD increases penile tumescence and psychometric measures of sexual desire and arousal by modulating sexual brain processing. Taken together, our data suggest that kisspeptin-based therapeutics may offer a novel, effective and much-needed clinical strategy for men with HSDD.