Wednesday April 21

Session Co-Chairs: Shannon Stephens and Andy Babwah

5:40 pm Fernando Marquez (Netherlands Institute for Neurosciences)

5:50 pm Lorna Smith (King's College London)

6:00 pm Encarnacion Torres Jimenez (IMIBIC & University of Cordoba)

6:10 pm Noella Di Giorgio (IBYME-CONICET)

6:20 pm Eulalia Coutinho (University of California, San Diego)

6:30 pm Aleisha Moore (Kent State University)

6:40 pm Bahaa Aloqaily (Rutgers University)

(all times EDT [New York time])

ROLE OF CENTRAL KISSPEPTIN AND RFRP-3 IN ENERGY METABOLISM IN THE MALE WISTAR RAT

Fernando Cázarez-Márquez[1,2,3], Jitske Eliveld[2,3], Wayne IGR Ritsema[2,3], Ewout Foppen[2,3], Yvonne Bossenbroek[3], Simone Pelizzari[1,2], Valérie Simonneaux[1], and Andries Kalsbeek[2,3,4]

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Kisspeptin (Kp) and RFRP-3 are two RF-amides acting in the hypothalamus to control reproduction. In the past 10 years, it has become clear that apart from their role in reproductive physiology both neuropeptides are also involved in the control of food intake, as well as glucose and energy metabolism. In order to investigate further the neural mechanisms responsible for these metabolic actions, we assessed the effect of acute intracerebroventricular (ICV) administration of Kp or RFRP-3 in ad libitum fed male Wistar rats on feeding behavior, glucose and energy metabolism, circulating hormones (luteinizing hormone, testosterone, insulin and corticosterone) and hypothalamic neuronal activity. Kp increased plasma testosterone levels had an anorexigenic effect and increased lipid catabolism as attested by a decreased respiratory exchange ratio (RER). RFRP-3 also increased plasma testosterone levels but did not modify food intake or energy metabolism. Both RF-amides increased endogenous glucose production, yet with no change in plasma glucose levels suggesting that these peptides not only provoke a release of hepatic glucose, but also a change in glucose utilization. Finally, plasma insulin and corticosterone levels did not change after the RF-amide treatment. The Kp effects were associated with an increased c-FOS expression in the median preoptic area and a reduction in pro-opiomelanocortin immunostaining in the arcuate nucleus. No effects on neuronal activation were found for RFRP-3. Our results provide further evidence that Kp is not only a very potent hypothalamic activator of reproduction but is also part of the hypothalamic circuit controlling energy metabolism.

A LONG-TERM ROLE FOR KISSPEPTIN IN PANCREATIC BETA-CELL FUNCTION

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We have previously established a role for kisspeptin signaling in mediating the functional adaptation of the pancreatic beta-cells to pregnancy. Using beta-cell specific GPR54 knockout mice (βGPR54ko), we have shown that kisspeptin signaling stimulates beta-cell proliferation and increases glucose-induced insulin secretion during pregnancy, improving glucose homeostasis and compensating for the increased insulin resistance characteristic of pregnancy. Here we investigate whether the islet kisspeptin system may also regulate beta-cell function with aging outside of pregnancy, in males and virgin females. At 18 weeks of age, male and female control or βGPR54ko mice underwent an intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) following 6 h fast. IPGTT and IPITT were repeated at 26 weeks and every subsequent 4 weeks until 50 weeks old. At 18 weeks, IPGTT area under curve values were not significantly different in either sex between control and βGPR54ko mouse groups (males: P=0.13, females: P=0.87), suggesting a minimal role for islet kisspeptin in regulating glucose homeostasis in early life. However, by 26 weeks, βGPR54ko males (P=0.048) and females (P=0.024) had significantly impaired glucose tolerance over time compared to controls. At 38 weeks, βGPR54ko females continued to exhibit a trend for impaired tolerance compared to control over time (P=0.055), whilst βGPR54ko males had significantly impaired glucose tolerance overall (P=0.003). Similarly impaired glucose tolerance was also seen in βGPR54ko females at 46 (genotype: P=0.015) and 50 (time x genotype: P=0.015) weeks, and in βGPR54ko males at 42 (time x genotype: P<0.0001) and 46 (time x genotype: P=0.004) weeks. IPITT in males at 38 weeks showed no significant effect of genotype (P=0.43), whilst βGPR54ko females had significantly improved insulin sensitivity (P=0.037). These results suggest a beneficial role for beta-cell kisspeptin signaling in supporting islet function outside of pregnancy, and consequently maintaining healthy glucose tolerance with advancing age.

DIRECT KISSPEPTIN SIGNALING IN ASTROCYTES AS REVEALED BY PROTEOMIC AND FUNCTIONAL GENOMIC ANALYSES

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Reproduction is safeguarded by sophisticated regulatory networks. Kisspeptins and their receptor, Gpr54, play essential roles in the control of reproductive function. However, important aspects of kisspeptin roles and mechanisms of action remain unsolved, including characterization of the whole set of pathways conveying kisspeptin effects in the hypothalamus. We describe herein a novel pathway for direct kisspeptin actions in astrocytes, revealed by combination of proteomic, expression and functional genomic analyses. To identify novel targets of kisspeptins, proteomic approaches were applied to preoptic hypothalamic samples of Kiss1 KO mice following icv injection of kisspeptin-10. Protein-Protein-Interaction (PPI) network and gene ontology (GO) analyses of proteomic data revealed that, among other pathways, glial and synaptic plasticity markers are regulated by kisspeptin. This putative glial-kisspeptin pathway was validated by expression and functional studies, which demonstrated expression of *Gpr54*, but not *Kiss1*, in astrocyte cultures from rats and mice, and the capacity of kisspeptin to activate canonical intracellular signaling pathways in astrocytes. Co-expression of Gpr54 and the astrocyte marker, GFAP, was demonstrated by *in situ* hybridization, with differences in the percentage of co-expression according to the brain area. Moreover, GFAP expression was altered in models of disrupted kisspeptin signaling. Finally, a novel mouse model with conditional ablation of Gpr54 in GFAP-positive cells was generated. This line, termed G-KiRKO, displayed effective ablation of Gpr54 in astrocytes but only modest alterations in the functioning of reproductive axis in basal conditions, denoted mainly by enhancement of LH secretion after kisspeptin stimulation. However, some reproductive responses to metabolic stress induced by high-fat diet were perturbed in G-KiRKO mice, including altered female pubertal timing and estrous cyclicity, and attenuated indices of reactive gliosis. Our data unveil a non-neuronal pathway for kisspeptin actions in astrocytes, which may cooperate in the fine tuning of the reproductive axis and glial responses to metabolic stress.

DELETION OF GABABR IN KISS1 CELLS AFFECTS GLUCOSE HOMEOSTASIS IN YOUNG ADULT MALE MICE.

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Kisspeptin (encoded by *Kiss1* gene) neurons co-express GABAB receptors (GABABR) and GABA is an important regulator of their physiology. *Kiss1* expression is a key factor in the control of reproduction and is involved in metabolic control. We developed a new strain of mice with specific deletion of GABAB1R in *Kiss1* cells/neurons (KO). KO males have similar body weight compared to WT from birth to adulthood; however, they have an increase in postnatal ano-genital index (AGI) between PND7 and PND21. Here, we confirm the specific deletion of GABAB1R by double immunofluorescence and we evaluate the neuroendocrine/reproductive axis and different parameters of glucose homeostasis in WT and KO young adult males: a) AGI, hormonal levels and fertility; b) *Kiss1* expression in hypothalamic/extra-hypothalamic nuclei and testis; c) non-fasted (NFG) and fasted (FG) glycaemia; d) glucose (GTT) and insulin (ITT) tolerance tests. We found that AGI, LH, testosterone levels and fertility were similar between genotypes. However, we found that *Kiss1* was increased in testis without differences in arcuate nucleus or medial amigdala. On the other hand, while KO males had normal NFG, they had higher FG, altered response to glucose overload (GTT) and lower insulin sensitivity (ITT). In conclusion, prebubertal AGI and adult *Kiss1* expression alteration in testis do not impact on fertility. However, the lack of GABABR in *Kiss1* cells alters glucose homeostasis in young adult male mice. We need to investigate whether these alterations have a central or peripheral origin, or both.

THE ROLE OF KISSPEPTIN NEURONS IN ABNORMALLY HIGH LH PULSATILITY IN A PCOS-LIKE MOUSE MODEL

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Polycystic ovary syndrome (PCOS) is a common reproductive disorder in women characterized by hyperandrogenemia, anovulation, cystic follicles, and abnormal LH hyper-pulsatility, but the pathophysiology of this condition is incompletely understood. We previously reported a PCOS-like mouse model using chronic letrozole (LET; aromatase inhibitor) initiated at puberty. LET female mice demonstrate multiple PCOS-like phenotypes, including polycystic ovaries, anovulation, high testosterone, and elevated basal LH, assayed in "one-off" measures at sacrifice. Due to prior technical limitations, *in vivo* LH pulsatile secretion, which is abnormally elevated in PCOS women, was not previously studied. We therefore first examined *in vivo* LH pulse dynamics of awake, freely-moving LET female mice. Compared to control females, LET females exhibited elevated *in vivo* LH pulsatility, with increased pulse frequency, amplitude, and basal LH, similar to PCOS women. Importantly, the hyperactive LH pulses of LET mice were not as elevated as in OVX control females. We next assessed whether changes in important neural reproductive regulators, such as kisspeptin and neurokinin B, may be altered in LET females. LET mice had greatly elevated *Kiss1* and *Tac2* expression in the ARC nucleus, as well as increased ARC *Kiss1* neuronal activation, correlating with their elevated LH pulses. This suggests that elevated kisspeptin synthesis and neuron activation may help drive elevated LH pulses in this PCOS model. To functionally test this, we generated mice with inhibitory DREADDs receptor expressed in *Kiss1* neurons ("Kiss1Cre/DI"). Kiss1Cre/DI and control mice were exposed to the LET paradigm and then underwent repeated sampling for LH before and after acute CNO treatment. Chemogenetic inhibition of kisspeptin neurons reduced the *in vivo* hyperactive LH pulses in LET Kiss1Cre/DI females but not in LET controls. These findings suggest that kisspeptin neuron activity likely contributes to the elevated LH pulses in LET females, thereby driving the hyperandrogenemia in this PCOS-like condition.

PRENATAL ANDROGEN EXPOSURE ALTERS KNDy NEURONS AND THEIR AFFERENT NETWORK IN A MOUSE MODEL OF POLYCYSTIC OVARIAN SYNDROME

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Polycystic ovarian syndrome (PCOS) is the leading cause of infertility in women of reproductive age worldwide. PCOS patients display increased luteinizing hormone (LH) pulse frequency, indicative of increased pulsatile gonadotropin-releasing hormone (GnRH) release due to impaired responsiveness to progesterone negative feedback. The site of impaired feedback remains unclear; however, arcuate nucleus (ARC) neurons that co-express kisspeptin, neurokinin B and dynorphin (KNDy cells) are the neural generator responsible for GnRH/LH pulses and are implicated in steroid hormone feedback. We utilized a prenatal androgen-treated (PNA) mouse model of PCOS to determine whether changes in KNDy neurons or their afferent network underlie altered negative feedback. Fluorescent multiplex *in situ* hybridization on slide-mounted brain sections identified RNA transcripts encoding the androgen receptor were significantly increased in ARC Kiss1 cells of PNA mice (n=6) compared to prenatal vehicle-treated (PNV) controls (n=4), whereas RNA for dynorphin and the progesterone receptor was significantly reduced, suggesting elevated androgens in PCOS may disrupt progesterone negative feedback via direct actions upon KNDy cells. Second, immunofluorescent labelling for synaptophysin and vesicular GABA transporter (vGAT) or vesicular glutamate transporter (vGluT2) in brain sections from Kiss1-Cre/eYFP mice showed significantly reduced GABAergic and glutamatergic synaptic inputs to ARC Kiss1 neurons in PNA mice (n=5) compared to PNV controls (n=5). Retrograde monosynaptic rabies-mediated tract-tracing from ARC Kiss1 cells in Kiss1-Cre PNV male (n=6), PNV female (n=6) and PNA female (n=7) mice revealed reduced synaptic input in PNA mice originating from sexually dimorphic afferents within the preoptic area, anteroventral periventricular nucleus, anterior hypothalamic area and lateral hypothalamus. Together, these results reveal two sites of neuronal alterations that may be responsible for defects in progesterone negative feedback in PNA mice: changes in gene expression of steroid receptors and peptides within KNDy neurons, and changes in synaptic inputs onto KNDy cells arising from other steroid hormone-responsive hypothalamic regions.

HEPATIC KISSPEPTIN REGULATES GLUCOSE METABOLISM AND FAT MASS

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Kisspeptin, a neuroendocrine protein critical for the control of pubertal development and fertility, has been shown to be modulated by nutritional signals. While the secretion of kisspeptin from specific hypothalamic nuclei is well-known to regulate GnRH-mediated pubertal maturation and reproduction, it remains unclear what role peripheral kisspeptin, specifically of hepatic origin, plays in regulating metabolism and glucose homeostasis. To define the role of kisspeptin in the liver, we developed a novel Kiss1$^{f/f}$ mouse line and targeted liver-specific Kiss1 ablation by injecting a AAV8-TBG-iCre virus via the tail vein (LKiss1KO). Control mice consisted of Kiss1$^{f/f}$ male and female mice injected with AAV-GFP (LKiss1WT). To clarify the effects of liver-specific KISS1 knockout on insulin action and glucose homeostasis *in vivo*, we conducted hyperinsulinemic-euglycemic clamp studies three weeks after tail injections. We noted a sexual dimorphism in the glucose infusion rate (GIR), female mice have a higher GIR to maintain euglycemia associated with an elevated glucose consumption rate, suggesting that female mice are more insulin sensitive than male mice. However, deletion of liver kisspeptin did not affect the glucose production rate in either sex. Indirect calorimetry assessment was conducted 4 weeks post-injection. Both male and female LKiss1KO mice showed significantly higher oxygen consumption, carbon dioxide production, and increased energy expenditure as compared to the LKiss1WT groups. However, there were no differences in either the respiratory exchange ratio or total ambulatory activity among treatments. Furthermore, both male and female LKiss1KO mice displayed significantly lower body weight combined with a significant decrease in fat mass as compared to the LKiss1WT groups. These results demonstrate that hepatic KISS1 is required for normal insulin secretion, glucose homeostasis, and basal metabolism.