[1]Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Munich, Germany, [2]Technical University of Munich, Department of Chemistry, Garching, Germany, [3]Division of Paediatric Endocrinology and Diabetes, Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany.

MON-175

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders affecting key enzymes of cortisol biosynthesis. In the majority of cases the underlying cause are detrimental mutations in the steroidogenic cytochrome P450 enzyme 21-hydroxylase (CYP21A2). Early diagnosis via newborn screening programs in most Western countries and lifelong oral cortisol replacement therapy enable survival, however quality of life often is reduced and co-morbidities are substantially increased. Treatment is a major challenge as disease control can only be achieved with supraphysiological glucocorticoid doses. In addition, the currently available drugs cannot ideally mimic the circadian rhythm and stress adaption of cortisol secretion. Currently, disease severity is classified by residual enzyme activity. The goal of our research is to better understand the specific biophysico-chemical pathomechanism of 21-hydroxylase deficiency in order to enable causative therapeutic approaches. To this end, we investigated the structural and stability properties of six clinically relevant mutant variants of CYP21A2 (V282G/L, P31L, D323G, R484Q/W). Difficulty in purification of these CYP21A2 variants and various biophysical studies suggest that the proteins were less stable than wild-type (WT). Structural and thermal stability assessment by circular dichroism (CD) spectroscopy of recombinant, purified CYP21A2 mutant variants revealed high α-helical content for the WT (65% α-helix) and the mutants at the position 282 (V282G: 60.6 %, V282L: 57.6%). Other mutations (P31L, D323G, R484Q/W) disrupt the α-helical organization of CYP21A2 in exchange for a slight increase in ß-sheet content but mainly for random coil. Temperature dependent CD spectroscopy showed that all mutant variants have reduced thermal stability (T_m: 41.3 - 45,6°C) compared to the WT (T_m: 47.1°C). Tryptophane fluorescence showed that mutant variants of the protein were more prone to local unfolding at the hydrophobic core compared to WT using urea as denaturant. Furthermore, in UV/Vis spectroscopy at 280 nm and 418 nm we could demonstrate that all mutant variants had a reduced heme incorporation (A_{418}/A_{280}: 0.20 - 0.63) compared to WT (A_{418}/A_{280}: 0.88). Our results show that correct structural folding and stability pose a major problem in specific mutations involved in CAH. Therefore we propose that structural protein instability, play a key role in the pathophysiology of CAH and thus might constitute a novel tailored therapeutic target for the treatment of affected patients.

Neuroendocrinology and Pituitary

ADVANCES IN NEUROENDOCRINOLOGY

Effects of Anti-Mullerian Hormone on the Expression of Gonadotropin Subunits in Pituitary Gonadotroph Cell Models

Haruhiko Kanasaki, MD,PHD[1], Aki Oride, MD[2], Tuvshintugs Tumurbaatar, MD[1], Zolzaya Tumurgan, MD[1], Satoru Kyo, MD[1].

[1]Shimane Univ Sch of Med, Izumo Shimane, Japan, [2]Shimane University, Izumo, Japan.

SUN-250

Aim: We examined the effect of anti-Müllerian hormone (AMH) on the expression of gonadotropin subunits in pituitary gonadotrophs.**Methods:** The mouse pituitary gonadotroph cell line LβT2 was stimulated with AMH and the expression levels of gonadotropin subunits were determined by real-time PCR. We also examined the involvement of the Kiss-1 gene (encoding kisspeptin) and the kisspeptin receptor (Kiss-1R) in LβT2 cells. **Results:** A significant increase was observed in the expression level of the FSHβ subunit with AMH but not in the expression levels of gonadotropin α and LHβ subunits. A significant decrease was observed in the expression of Kiss-1 and Kiss-1R genes in LβT2 cells with AMH stimulation. Kiss-1 gene knockdown by siRNA did not alter the basal expression of gonadotropin subunits. When LβT2 cells overexpressing Kiss-1R were stimulated with kisspeptin, there was a significant increase in the gene expression levels of the gonadotropin subunits α, LHβ, and FSHβ. This inductive effect of kisspeptin was almost completely inhibited by AMH pretreatment. The GnRH-induced increase in gonadotropin subunit genes was unchanged in the presence of AMH. **Conclusions:** AMH can increase FSHβ subunit gene expression in pituitary gonadotroph cells. However, AMH decreases Kiss-1 and Kiss-1R gene expression within the gonadotrophs. Because AMH pretreatment abolishes kisspeptin-induced expression of gonadotropin subunit genes, AMH may control kisspeptin-regulated gonadotropin expression by inhibiting the expression and function of Kiss-1R within gonadotrophs.

Adipose Tissue, Appetite, and Obesity

NEURAL MECHANISMS OF OBESITY

Sex-Specific Modifications in MicroRNAs Contained in Exosomes of Astrocytes in Response to Palmitic Acid

Julie Ann Chowen, PHD, Santiago Guerra-Cantera, MS, Roberto Collado-Perez, MS, Jesús Argente, MD, PhD, Laura M. Frago, PhD.

Hospital Infantil Universitario Nino Jesus, Universidad Autonoma de Madrid, CIBEROBN, Madrid, Spain.

SAT-593

Communication between astrocytes and neurons is fundamental for correct functioning of the brain, in both physiological and pathophysiological situations. It is clear that astrocytes play an active role in metabolic control, but much is yet to be learned regarding how these glial cells and the neurons involved in energy intake/expenditure communicate to regulate energy homeostasis. We hypothesized that miRNAs contained in exosomes are an important means of cross-talk between these cells. Our objectives here were to determine whether the miRNA content of exosomes released by hypothalamic astrocytes changes in function of nutrient signals and if these signals are similar between males and females. To this end, primary hypothalamic astrocyte cultures were prepared from 2-day old male and female mice, using a standard protocol, and treated with