1–34 maintained iCa levels for 2 hours after administration above that of Cinacalcet-HCl (AUC±SD (mmol/L).hr from baseline, 0.076 ±0.047 and 0.168±0.0874, t-test P=0.0289) but then levels fell and recovered as for Cinacalcet-HCl alone. Subcutaneous doses of both fusions were able to abrogate the effects of Cinacalcet-HCl from 4hrs post dose onwards giving a prolonged response, with iCa levels quicker to return to baseline levels at 48hrs compared to Cinacalcet-HCl. The AUC±SD (mmol/L).hr from baseline for iCa over 72 hours was 3.93±1.4 for Fusion-1, 5.0±2.7 for Fusion-2 & 10±2.8 for Cinacalcet-HCl and were significantly reduced for both fusions compared to Cinacalcet-HCl alone (t-test P = 0.0028 & P = 0.019, respectively) and not significantly different from vehicle only.

**Conclusions:** Cinacalcet-HCl behaved as expected in terms of iCa lowering (2). PTH maintained iCa but only for 2 hours. Both PTH fusion molecules showed a delayed and prolonged response and reduced the impact of Cinacalcet-HCl induced low iCa levels from 4hrs to 24hrs. These data provide proof of concept for long acting biological activity of these novel PTH fusion proteins.

References:
1. Shimizu M, Joyashiki E, Noda H, Watanabe T, Okazaki M, Nagayasu M, et al. Pharmacodynamic Actions of a Long-Acting PTH Analog (LA-PTH) in Thyroparathyroidectomized (TPTX) Rats and Normal Monkeys. J Bone Miner Res. 2016;31(7):1405–12.
2. Nemeth EF, Heaton WH, Miller M, Fox J, Balandrin MF, Van Wagenen BC, et al. Pharmacodynamics of the type II calcimimetic compound cinacalcet HCl. The Journal of pharmacology and experimental therapeutics. 2004;308(2):627–35.

**Pediatric Endocrinology**

**UNDERSTANDING AND TREATING PEDIATRIC GROWTH DISORDERS**

**Diagnosis of Severe GH Deficiency in Newborns: New Reference Range for the Preterm and Confirmation of the GH Cut-Off**

Gerhard Binder, MD, Karin Weber, no degree, Nora Rieflin, no degree, Louis Steinruck, no degree, Axel Franz, MD.

University Children’s Hospital, Tuebingen, Germany.

**OR10-02**

Introduction
Inborn severe GHD is caused by rare disorders of pituitary morphogenesis or function and frequently associated with additional pituitary hormone deficiencies. Affected newborns commonly present with recurrent hypoglycemia; therefore early diagnosis and therapy is warranted. The GH content of the newborn screening card is a reliable indicator of severe neonatal GHD. Here, we studied the GH content in screening cards and the history of 25 newborns with severe GHD. In addition, we determined the reference range of the GH content in screening cards from 282 healthy preterm newborns.

Patients and Methods
Since 2010, a total of 110 screening cards from hospitalized ill newborns were sent to our laboratory for measuring GH content. Using a questionnaire we obtained relevant clinical information from senders in 61 cases. Severe GHD was defined by the presence of recurrent neonatal hypoglycemia with either a significant cerebral MRI morphology or two additional pituitary hormone deficiencies. In addition, the GH content of screening cards from 282 healthy newborns born preterm with a gestational age at birth from 34.0 to 37.9 weeks was prospectively analyzed. The GH concentration of the eluate from the screening card was measured by a highly sensitive ELISA (Mediagnost, Germany); the GH serum concentration was calculated.

**Results**
In 25 patients, the definition of severe GHD of the newborn was fulfilled; based on recurrent hypoglycemia in combination with ectopia of the neurohypophysis in 17, septum pellucidum agenesis plus opticus hypoplasia in two, severely hypoplastic pituitary gland in two, and combined TSH and ACTH deficiency with no cMRI findings in four newborns. Five newborns with severe GHD were preterm. The median GH concentration of the term newborns with severe GHD (n=20) was 3.9 µg/l (range: 1.1 to 11.8). This was significantly below the previously reported reference data from healthy term newborns (n=269) (median 16.4 µg/l; 95% reference range 7.0 to 39.4) (p=0.001). Using ROC plot analysis a GH serum concentration of 7.0 µg/l was identified as cut-off with the highest accuracy (90.0% sensitivity and 98.7% specificity). The median GH concentration of the 5 preterm newborns with severe GHD was 7.7 µg/l (range; 2.1 to 9.9). The newly determined 95% reference range for healthy newborns born preterm with a gestational age from 34.0 to 37.9 weeks (n=282) spanned from 7.9 to 41.1 µg/l with a median of 20.3 µg/l.

**Conclusions**
A GH content below 7.0 ng/ml in the newborn screening card identified severe GHD with 90% sensitivity and 98.7% specificity. In preterm newborns, the lower limit of the 95% reference interval was by 0.9 µg/l higher than in term newborns. The newborn screening card is a valuable source for the diagnosis of GH deficiency in newborns and young infants.

**Neuroendocrinology and Pituitary**

**HYPOTHALAMIC-PITUITARY DEVELOPMENT AND FUNCTION**

**Osteocalcin and Exercise Improve Mood and Cognition in Female Mice with High-Fat Diet Induced Type 2 Diabetes.**

Jesse Rentz, BSc.H, Jordan Winberg, BSc.H, Walter Swardfager, PhD, Jane Mitchell, PhD.

University of Toronto, Toronto, ON, Canada.

**SAT-293**

The skeleton has been characterized as an endocrine organ, demonstrating a capacity to modulate cognition, mood and energy homeostasis (1,2). These endocrine actions of the skeleton have been attributed to the osteoblast-derived peptide osteocalcin. In mice, uncarboxylated osteocalcin (ucOCN) decreased the acquisition of type 2 diabetes melitus (T2DM) and ameliorated depressive- and anxiety-like behaviours (1,2). Clinically, T2DM patients present with reduced serum osteocalcin levels and approximately 1 in 4 also suffer from co-morbid depression (3,4). The cognitive and metabolic benefits of ucOCN are similar to the
beneficial effects of exercise that is recommended in treatment of both depression and T2DM. Here we compared the effects of ucOCN or exercise in female C57-BL/6J mice under two different metabolic conditions. Mice were fed either a high-fat diet (60% calories from fat) to induce T2DM or a control diet (10% calories from fat). Groups of mice were either sedentary or exercised daily by 30 min treadmill running for two months, with or without daily administration of ucOCN (30 ng/g/day). Mice with T2DM displayed depressive behaviours marked by a higher immobile time in tail suspension tests compared to control mice (97±25 n=11 vs 207±9.0 n=12; t_{19}=-4.21, P=0.0004). Exercise and osteocalcin both improved depressive behaviour (137±8 n=12; t_{18}=-5.85, P<0.0001 & 127±15 n=12; t_{14}=-4.46, P=0.0002). Anxiety, measured by the elevated-plus maze revealed the mice with T2DM displayed increased anxiety spending less time in the open arms and had a lower ratio of open to closed arm entries than the control group (0.37±0.03 n=10 vs 0.21±0.032 n=11; t_{17}=-3.56, P=0.0021). Neither exercise nor osteocalcin improved anxiety in the T2DM mice. The puzzle box test revealed the negative effects of the high-fat diet in problem solving and memory, where the sedentary mice displayed greater latency to solve each task compared to control mice. Exercised and mice receiving osteocalcin displayed performances comparable to that of the control group. Under normal metabolic conditions (low fat diet), neither osteocalcin nor exercise altered responses in any of the behavioural tests. Together, these results: 1. The effects of osteocalcin on behaviour and cognition are comparable to that of the effects of exercise in female mice with T2DM; 2. Behaviour and cognition did not improve from exercise or osteocalcin in female mice on a low-fat diet.

References: (1) Ferron et al., Bone. 2012 Feb;50(2):568–575. (2) Oury et al., Cell. 2013 Sep 26;155(1):228–241. (3) Liu et al., Horm Metab Res. 2015 Oct;47(11):813–9. (4) Khaledi et al., Acta Diabetol. 2019 June;56(6):631–650.

Adrenal

ADRENAL PHYSIOLOGY AND DISEASE

The Role of Filamin A (FLNA) in the Regulation of Insulin-Like Growth Factor System in Adrenocortical Carcinomas

Erika Peverelli, PHD1, Rosa Catalano, PhD student1, Elena Giardino, PHD, Donatella Treppiedi, PHD2, Federico Mangili, PHD student3, Valentina Morelli, MD4, Massimo Mannelli, MD, Anna Spada, MD5, Maura Arosio, MD, PHD4, Giocanna Mantovani, MD, PHD7.

1Università di Milano, Osp. Policlinico, Milan, Italy, 2Università degli studi di Milano, MILANO, Italy, 3Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy, 4POLICLINICO MAGGIORE, Milan, Italy, 5Univ of Florence, Florence, Italy, 6University of Milan, Milan, Italy, 7University of Milano, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Italy.

SUN-213

Adrenocortical carcinomas (ACCs) are rare endocrine tumors with poor prognosis. They overexpress insulin-like growth factor 2 (IGF2), that drives a proliferative autocrine loop by binding to IGF1R and IR, with molecular dynamics still poorly identified. Although promising, IGF1R/IR-targeted therapies have demonstrated a limited efficacy in clinical trials in ACC patients. The cytoskeleton actin-binding protein filamin A (FLNA) was shown to impair IR and IGF1R signalling in melanoma and neural progenitor cells, respectively. The aims of this study were to test in ACC cells: 1) FLNA involvement in regulating IGF1R and IR expression and signalling; 2) FLNA role in modulating responsiveness to IGF1R and IGF1R/IR inhibitors; 3) FLNA expression in ACCs and correlation with IGF system. In ACC cells we found by immunoprecipitation that both IGF1R and IR interacted with FLNA in basal condition, with an increased or decreased FLNA recruitment to IGF1R and to IR, respectively, after IGF2 stimulation. Genetic silencing of FLNA in ACC cell lines H295R and SW13 induced a significant increase of IGF1R expression (1.4- and 2.3-fold, respectively) and a reduction of IR (-85.5±9.1%, p<0.001 and -32±19.1%, respectively, p<0.05), with a downstream effect of increased cell proliferation (130±13.4%, p<0.01 in H295R and 144±23.3%, p<0.01 in SW13 cells) accompanied by an enhanced ERK phosphorylation. Accordingly, in ACC primary cultured cells FLNA silencing increased IGF1R levels (2.9-fold) and enhanced IGF2 effects on ERK phosphorylation by 2.2-fold. In addition, FLNA knockdown potentiated the antiproliferative effects of IGF1R/IR inhibitor Linsitinib and IGF1R specific inhibitor NVP-ADW742 in H295R cells and SW13. This key role of FLNA was even more evident in A7/M2 melanoma cell model, since IGF2 and Linsitinib exerted the expected effects on ERK phosphorylation in M2 cells, lacking FLNA, but not in FLNA-expressing counterpart (A7 cells). Finally, western blot analysis showed significantly lower, although variable, FLNA expression in ACCs (n=10) than in adrenocortical adenomas (ACAs) (n=10) (FLNA/GAPDH ratio 0.37±0.38 and 0.90±0.63, respectively, p<0.05). Interestingly, FLNA/IGF1R ratio inversely correlated with ERK phosphorylation status in ACCs (p<0.05) but not in ACA. In conclusion, we demonstrated that low levels of FLNA enhance both IGF2 proliferative effects and IGF1R/IR inhibitors efficacy in ACC cells, suggesting FLNA as a new factor possibly influencing tumor clinical behavior and the response to the therapy with IGF1R/IR-targeted drugs.

Steroid Hormones and Receptors

STEROID AND NUCLEAR RECEPTORS

When the Glucocorticoid Receptor Meets the Mineralocorticoid Receptor in the Nucleus of Human Cells

Maria G. Petrillo, PhD, Christine M. Jewell, BS, Robert H. Oakley, PhD, John A. Cidlowski, PhD. NIH-NIEHS, Durham-RTP, NC, USA.

OR12-02

Adrenal corticosteroids, such as glucocorticoids and mineralocorticoids, are indispensable for mediating response to stress, development, limiting inflammation, and maintaining energy and fluid homeostasis. These hormones exert their actions via binding to two closely related nuclear receptors, the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). The GR has low affinity for corticosteroids, but is expressed in nearly every cell. In contrast, the MR shows a higher affinity for corticosteroids and its expression is largely confined to those tissues where...