electrolyte exchange and fluid balance are required. GR and MR act as ligand-activated transcription factors which, following interaction with co-regulators and DNA responsive elements, either promote or repress gene transcription. The affinity for the same ligands, structural homology, and binding to the same DNA regions suggest GR and MR can compensate for each other’s actions. Yet, there are specific glucocorticoid and mineralocorticoid-mediated responses indicating GR-MR functional diversity. To investigate this interplay, we developed U-2 OS (human osteosarcoma) cell lines stably expressing GR, MR, and both GR and MR (GRMR). Immunofluorescence analysis showed that treatment of these cell lines with 1 nM of the synthetic glucocorticoid dexamethasone (Dex) induced nuclear translocation of GR and MR. Conversely, treatment with 1 nM of aldosterone (Aldo) promoted nuclear translocation of the MR only. Moreover, Proximity Ligation Assay revealed that, in the absence of ligand, GR associated with MR in the cytoplasm and, upon 1 nM Dex exposure, GR-MR dimers were detected in the nucleus of GRMR cells. Surprisingly, nuclear GR-MR dimers were also detected in the presence of Aldo, suggesting that it is necessary to activate at least one receptor to induce nuclear translocation of the heterocomplex. To decipher the functional contribution of GR-MR dimers in the transcriptional response of GR to Dex and MR to Aldo, we performed RNA-seq in GR, MR, and GRMR cells treated with 1 nM of Dex or Aldo. Transcriptome analysis revealed that Dex-activated GR regulated the transcription of 6180 genes. Co-expression of MR resulted in a blunted Dex-mediated gene response which affected only 1608 genes, suggesting a functional antagonism of MR. Aldo-activated MR regulated the transcription of 1660 genes. However, co-expression of GR expanded the Aldo-mediated gene response to 3150 genes. Strikingly, 74% of these genes were also regulated by Dex via GR, suggesting that GR-MR dimers in the presence of aldosterone are able to mimic the glucocorticoid transcriptional response. Our data suggest that the role of distinct GR and MR homo- and hetero-dimers is relevant for regulating gene expression. Dissecting the mechanism and investigating the cross-talk between GR and MR may be useful to understanding these two receptors in health and disease.

**Neuroendocrinology and Pituitary**

**PITUITARY TUMORS I**

**Beta-Arrestin 2 Is Required for Dopamine Receptor Type 2 Inhibitory Effects on Akt Phosphorylation and Cell Proliferation in Pituitary Tumors**

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**SAT-312**

β-Arrestin 2 is Required for Dopamine Receptor Type 2 Inhibitory Effects on AKT Phosphorylation and Cell Proliferation in Pituitary Tumors

Dopamine receptor type 2 (DRD2) agonists are the first-choice treatment for PRL-secreting pituitary tumors but are poorly effective in non-functioning pituitary neuroendocrine tumors (NF-PitNETs). DRD2 reduces AKT phosphorylation in lactotrophs, but no data are available in NF-PitNETs. DRD2 effects on AKT are mediated by a β-arrestin 2-dependent mechanism in mouse striatum. The aim of this study was to investigate DRD2 effects on AKT phosphorylation and cell proliferation in human primary cultured NF-PitNET cells and in rat tumoral lactotroph cells MMQ, and to test β-arrestin 2 involvement. We found that DRD2 agonist BIM53097 induced a reduction of p-AKT /total-AKT ratio in MMQ (-32.8±17.6%, p<0.001 vs basal) and in a subset (n=15/41, 36.6%) of NF-PitNETs (subgroup 1). In the remaining NF-PitNETs (subgroup 2), BIM53097 induced an increase of p-AKT. The ability of BIM53097 to reduce p-AKT correlated to its antiinflammatory effect, since the majority of subgroup 1 NF-PitNETs was responsive to BIM53097 and nearly all subgroup 2 NF-PitNETs were resistant. β-arrestin2 was expressed in MMQ and in 80% of subgroup 1 NF-PitNETs, whereas it was undetectable in 77% of subgroup 2 NF-PitNETs. In MMQ, β-arrestin 2 silencing prevented DRD2 inhibitory effects on p-AKT and cell proliferation. Accordingly, β-arrestin 2 transfection in subgroup 2 NF-PitNETs conferred to BIM53097 the ability to inhibit both p-AKT and cell growth.

In conclusion, we demonstrated that β-arrestin 2 is required for DRD2 inhibitory effects on AKT phosphorylation and cell proliferation in MMQ and NF-PitNETs, paving the way for a potential role of β-arrestin 2 as a biomarker predicting NF-PitNETs responsiveness to treatment with dopamine agonists.

**Pediatric Endocrinology**

**UNDERSTANDING AND TREATING PEDIATRIC GROWTH DISORDERS**

**Interpretation of Insulin-like Growth Factor-1 (IGF-1) Levels Following Administration of Somatrogon (a Long-acting Human Growth Hormone - hGH-CTP)**

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**OR10-04**

IGF-1 is often used as a biomarker to evaluate the efficacy and safety of hGH replacement therapy. Typically, the mean IGF-1 SDS level during the dosing interval, rather than the peak value, guides clinical decision-making: sustained mean values > +2 may require hGH dose modifications. With long-acting formulations (administered weekly), the IGF-1 evaluation paradigm needs to take into account when the sample was obtained relative to the last administered dose. Previous studies with OPKO’s once weekly Somatrogon (hGH-CTP), demonstrated that IGF-1 SDS peaked ~ 48 hours post-dose and that values at ~ 96 hours best approximated the mean IGF-1 SDS throughout the dosing interval [1]. Data from the pivotal Phase 3 non-inferiority study comparing treatment with Somatrogon to...
Genotropin allowed further evaluation of the IGF-1 SDS analysis paradigm. Enrolled subjects were randomized to receive treatment with either once weekly Somatrogon (0.66 mg/kg; N=109) or once daily Genotropin (0.034 mg/kg; N=115). IGF1 was sampled ~ five times during 52 weeks of treatment with Somatrogon, providing a total of 557 samples obtained after the first dose of Somatrogon. IGF-1 SDS values were calculated using Billingmaier’s equations [2]. Analysis of IGF-I SDS data from the Phase 3 study showed that the previously-developed model, with adjustments to two parameters (baseline IGF-1, EC<sub>50</sub>) and adapted to fit IGF-1 values in the absence of Somatrogon concentration data, fit the IGF-1 data for Somatrogon with minimal bias. This allowed prediction of IGF-1 SDS values at timepoints throughout the dosing interval as well as calculation of the mean value during a dosing interval. Of the samples obtained between 48–72 hours post-dose (representing peak IGF-1 SDS), approximately 17% had an IGF1 SDS > +2. At 96 hours (corresponding to mean IFG-1 SDS), fewer than 2% of modeled values were > +2. Mean IGF-1 SDS over the dosing interval was between -1 and +1 for all subjects. These findings indicate that IGF-1 SDS values need to be interpreted in the context of when the sample was obtained relative to the last dose of Somatrogon. Our results indicate that samples obtained 96 hours post-dose best represent mean IGF-1 levels and that values obtained between 48–72 hours post-dose represent values closer to peak IGF-1 concentrations. In our Phase 3 study, of the 557 samples collected from 114 patients during the 12-month Somatrogon treatment period, fewer than 2% of the corresponding values at 96 hours postdose (estimated from a pharmacokinetic/pharmacodynamic model) had IGF-1 SDS levels > +2.

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Bone and Mineral Metabolism
PARATHYROID HORMONE TRANSLATIONAL AND CLINICAL ASPECTS
A Non-Surgical Animal Model of Hypoparathyroidism for Testing PTH Analogues

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SAT-396
Background:
In vivo animal models for testing the pharmacokinetics and bioactivity of PTH and its analogues require parathyroidectomy by surgery (1, 2). As the parathyroid glands of rodents are very small the surgery often includes thyroidectomy, making this animal model time-limited, single use, complex, and expensive. We have developed a non-surgical rodent model of hypoparathyroidism using the Type II calcimimetic compound, Cinacalcet-HCl, to suppress PTH and thereby serum calcium levels.

Methods:
Normal male Wistar rats were gavaged with 30 mg/kg Cinacalcet-HCl (or vehicle only). To test the effect of PTH 1–34, animals were dosed immediately after Cinacalcet-HCl gavage with either a single subcutaneous injection of PTH at 20 nmol/kg or given as same dose repeated every hour for 6 hrs or vehicle only. Serum samples were analysed for ionised calcium (iCa) using an EasyLyte, fully automated electrolyte analyser (Medica Corporation) and phosphate using a Phosphorus Detection Assay Kit (Pars Azmun, IRAN) and an Hitachi 917 Clinical Chemistry Analyser.

Results:
Rats gavaged with 30 mg/kg Cinacalcet-HCl produced a significant reduction in iCa levels between 2-24hrs returning to baseline at 48-72hrs post dose with the nadir at 8 hours (ANOVA P < 0.0001). This equated to a 25% reduction in iCa at 8 hrs: mean±SD, iCa 1.19 ± 0.09 mmol/L at predose and 0.891 ± 0.04 mmol/L at 8 hours (t-test P < 0.0001). For phosphate there was an initial lowering within the first 2 hrs in all test groups but then a rise such that phosphate was at higher levels than control from 8–24 hrs (ANOVA, ns), returning to baseline at 48 hrs. PTH at 20 nmol/kg given as a single sc dose abrogated the Cinacalcet-HCl induced fall in iCa for up to 2 hrs (AUC±SD (mmol/L).hr, 0.076 ± 0.047 versus 0.168±0.0874, t-test P=0.0289).

Conclusions:
We have shown that the administration of Cinacalcet-HCl provides a robust and reproducible lowering of calcium which is line with current published data (3). These studies demonstrate that the use of Cinacalcet-HCl in normal rats produces a hypocalcemic state that can be abrogated by the addition of PTH. This non-surgical animal model of hypoparathyroidism will be of value in testing the pharmacodynamics of PTH analogues.

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Adrenal
ADRENAL - TUMORS
Evaluation of Life Style and Anthropometric Parameters in Patients with Nonfunctional Adrenal Incidentalomas

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SAT-160
EVALUATION OF LIFE STYLE AND ANTHROPOMETRIC PARAMETERS IN PATIENTS WITH NONFUNCTIONAL ADRENAL INCIDENTALOMAS