QT-interval; mean change from placebo was -1.1ms (95CI [-16.0; 13.9], p=0.882), nor were arrythmias registered during the trial period. **Conclusions:** Tesomet was generally well-tolerated, did not affect heart rate, blood pressure or QTc-interval, and resulted in significant reductions in body weight compared to placebo in this cohort of hypopituitary patients with acquired hypothalamic obesity.
The study was sponsored by Saniona A/S

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Acute Transcriptional Effects of Dexamethasone on Mouse Adrenal Gland Transcriptome

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Researchers have long known that dexamethasone causes cellular and functional changes in the adrenal gland. For example, long-term dexamethasone treatment leads to reversible adrenal cortex atrophy. In the adrenal medulla, dexamethasone treatment alters the maturation and function of the neural crest-derived chromaffin cells. Here we aim to study the acute transcriptional effect of dexamethasone on mouse adrenal gland at the transcriptome level. Our data suggested that a one-hour dexamethasone treatment had a cell type-specific effect on the adrenal transcriptome. There were 922 dexamethasone-induced genes and 853 dexamethasone-suppressed genes. GO analysis showed that the upregulated genes were primarily linked to neuronal cell function. Clustered heatmaps further showed that many genes involved in the catecholamine synthesis were upregulated by dexamethasone treatment, whereas most genes involved in the steroidogenesis pathway were downregulated. Interestingly, steroidogenic factor 1 (SF1, encoded by *Nr5a1*), the critical transcription factor that regulates steroidogenesis, had a >2-fold decrease under the one-hour dexamethasone treatment, suggesting a possible mechanism of the acute suppression of steroidogenic activity. Our findings indicate that the acute effects of dexamethasone stimulate catecholamine synthesis in the medulla, whereas steroidogenesis in the cortex is suppressed by dexamethasone.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Androstenedione Is the Preferred Substrate for Cytochrome P450 11β-hydroxylase Leading to the Production of 11β-Hydroxyandrostenedione in the Adrenal Gland

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Adrenal cytochrome P450 11β-hydroxylase (CYP11B1) is a mitochondrial enzyme that catalyzes the final step of glucocorticoid synthesis, converting 11-deoxycortisol (S) and deoxycorticosterone (DOC) to cortisol (F) and corticosterone (CORT), respectively. CYP11B1 is predominantly located in the zona fasciculata of the adrenal gland with studies also showing that CYP11B1 catalyzes the conversion of androstenedione (A4) and testosterone (T) to 11β-hydroxyandrostenedione (11OHA4) and 11β-hydroxytestosterone (11OHT), respectively. Adrenal vein sampling in women has shown that 11OHA4 turnover is high, with a marked increase after treatment with ACTH which is known to upregulate CYP11B1 expression. We hypothesized that CYP11B1's affinity for A4 as a substrate may be favored over glucocorticoids since 11OHA4 is one of the major androgens produced in the adrenalThis study aimed to elucidate the kinetic parameters (K_m and V_{max}) for A4 and T with respect to CYP11B1. A4 and T (0.2 μM to 5 μM) were assayed in HEK-293 cells transiently transfected with CYP11B1 and the adrenodoxin redox partner. Data was used to generate progress curves and fitted to the Michaelis-Menten equation. A4 had the lowest K_m (0.21 μM) with a significantly higher V_{max} (315.77 pmol/min/mg protein) in comparison to T, S and DOC. Results suggest that A4 binds more readily to CYP11B1 resulting in the high turnover of substrate. The androgenic activity of CYP11B1 catalyzed steroid products was determined using a luciferase assay conducted in CV1 cells. Both A4 and 11OHA4 showed no androgenic activity, however, the 11βHSD2 product of 11OHA4, 11-ketoandrostenedione (11KA4), elicited a response at 100 nM. 11OHT was an androgen receptor (AR) agonist, however, exhibiting a lower response when compared to T and 11-ketotestosterone over all concentrations tested (1, 10 and 100 nM). As confirmation of CYP11B1 activity in the adrenal, circulatory steroid levels were determined in healthy female subjects (n=88). CORT, F and 11OHA4 were measured at a frequency of 70–100%, compared to 11OHT (40–70%), while 11βHSD2 activity produced 11KA4 (<10%). In conclusion, the catalytic efficiency of CYP11B1 towards A4 is higher compared to T and the classical substrates. The high substrate affinity and turnover provides evidence for 11OHA4 in adrenal vein samples. Concurrently, our analysis suggests that agonism is diminished by the presence of the C11- hydroxyl group, while AR agonistic activity is gained upon its conversion to a keto group. Furthermore, these androgens could perhaps modulate cortisol production in the adrenal due to potential competition between precursor substrates.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

CTNNB1-Mutant Aldosterone-Producing Adenomas With Somatic Mutations of GNA11/GNAQ Have Distinct Phenotype and Genotype

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Background: We report (this meeting) somatic mutation of *GNA11*/Q in *CTNNB1*-mutant APAs. The recurrent co-driver mutation causes reversible hypertension in puberty, pregnancy, or menopause. We have investigated the molecular mechanism of this association. **Methods:** Gene expression profiles in 3 double mutant APAs were studied by unsupervised hierarchical clustering analysis and enrichment analysis of 362 differentially expressed genes and validated by qPCR, IFC and IHC in 10 double mutant APAs or transfected primary adrenal cells. Multiple region biopsies were performed in 7 adrenals adjacent to double-mutant APAs and 4 APAs with *KCNJ5* or *CACNA1D* mutations. The findings of APA mutations in adjacent adrenals were replicated in each case by ddPCR ± NGS. **Results:** Unsupervised hierarchical clustering analysis showed clustering of the double-mutant APAs, and a high proportion of genes were many-fold upregulated compared to other APAs. *LHCGR*, *TMEM132E, DKK1, C9orf84, FAP, GNRHR* and *MPP3* are among the genes with high expression. A small number of genes are down-regulated in the double-mutant APAs, including *CYP11B1*. qPCR confirmed an average of ~10 to 1000-fold higher expression of the hallmark genes in double-mutants. Enrichment analysis showed significant enrichment of features or terms concerned with cell junction and cell adhesion ($P<10^{-8}$). IFC confirmed *LHCGR* intensity was 31–144 fold higher in primary adrenal cells with *GNA11*-p.Gln209Pro transfection and high *CTNNB1* intensity. *LHCGR* intensity was qualitatively and quantitatively associated with immunofluorescence for *CTNNB1*. IHC of double-mutant APAs showed absent CYP11B1 but strong staining of CYP11B2. qPCR confirmed a lower CYP11B1/CYP11B2 ratio and a higher *LHCGR* expression ($P<10^{-3}$, both). IHC confirmed LHCGR positivity in double-mutant APAs but distribution varied both within and between cells. Adjacent ZG was hyperplastic, with absence of both CYP11B1 and CYP11B2 staining, but weak/moderate staining for LHCGR. The same *GNA11* ± *CTNNB1* somatic mutations were detected in multiple regions of the adjacent adrenals to 3 double mutant APAs. qPCR of hallmark APA genes differed from the APAs. High concordance between ddPCR, NGS and Sanger sequencing of the findings of APA mutations in adjacent adrenals when analysed in the same sample. No mutations were found in 4 adrenals adjacent to APAs with *KCNJ5* or *CACNA1D* mutations, nor in other 4 adrenals adjacent to double-mutant APAs. **Conclusions:** Patients harboring APAs with somatic mutations in both *GNA11*/*GNAQ* Q209 and *CTNNB1* have distinct phenotype in both the APAs and their adjacent adrenals. Same *GNA11* ± *CTNNB1* somatic mutations were found in the adjacent adrenals to double mutant APAs. We infer that a double-hit within related pathways is more likely than a single-hit to cause large increases in expression of *LHCGR*, and of other genes which may influence clinical presentation.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Differential Expression of Circadian Clock Genes in the Bovine Neuroendocrine Adrenal System

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The primary objective of this investigation was to determine whether circadian clock genes were differentially expressed within or among bovine hypothalamic paraventricular nucleus (PVN), anterior pituitary gland (AP), adrenocortical (AC) and adrenomedullary (AM) tissues. The PVN, AP, AC, and AM were isolated from 5-yr-old Brahman cows (n = 8) harvested humanely at an abattoir between 0800-1100 h. Expression of target genes in each sample was evaluated via RNA-sequencing analyses. Gene counts were normalized using the trimmed mean of M values (TMM) method in the edgeR Package from Bioconductor, R. The normalized gene counts of genes important for circadian rhythm were statistically analyzed using the GLM Procedure of SAS. The genes analyzed were circadian locomotor output cycles protein kaput (*CLOCK*), cryptochrome circadian regulator 1 and 2 (*CRY1* and *CRY2*), aryl hydrocarbon receptor nuclear translocator like (*ARNTL*), period circadian regulator 1 and 2 (*PER1* and *PER2*), neuronal PAS domain protein 2 (*NPAS2*), and nuclear receptor subfamily 1 group D member 1 (*NR1D1*). Overall, relative expression profiles of clock genes differed ($P < 0.01$) within each tissue with *PER1* having greater expression in all tissues ($P < 0.01$).