Within the PVN expression of *CLOCK*, *CRY1*, *ARNTL*, and *PER2* was less than that of *CRY2*, *NPAS2*, and *NR1D1* ($P < 0.01$). In the AP, with the exception of *PER1*, no other clock gene differed in degree of expression. In the AC, expression of *CLOCK* and *NPAS2* was greater than *CRY1*, *ARNTL*, *PER2*, and *NR1D1* ($P < 0.05$), whereas *CRY2* expression exceeded only *CRY1* ($P < 0.05$). Within the AM, *CLOCK* and *CRY2* expression was greater than *CRY1* and *ARNTL* ($P < 0.05$). Overall, clock gene expression among tissues differed ($P < 0.01$) for each individual clock gene. The AC and AM had similar clock gene expression, except expression of *CRY2* and *PER2* was greater in AM ($P < 0.05$). The AC and AM had greater expression of *CLOCK* than the PVN and AP ($P < 0.01$), with PVN having greater expression than AP ($P < 0.01$). The AP had greater expression of *NPAS2*, followed by PVN, with the least expression in the AC and AM ($P < 0.01$). Both PVN and AP had greater *CRY1* and *NR1D1* expression than AC or AM ($P < 0.01$). The AP had greater *PER1* expression than PVN, AC, and AM ($P < 0.01$), whereas PVN, AC, and AM had greater *ARNTL* expression than AP ($P < 0.05$). Both AP and AM had greater expression of *PER2* than PVN or AC ($P < 0.01$). The PVN had greater expression of *CRY2* than the AP, AC, and AM ($P < 0.01$). These results indicated that ***within*** each tissue the various clock genes were expressed in different quantities. Also, the clock genes were expressed differentially ***among*** the tissues of the bovine neuroendocrine adrenal system. Temporal relationships of these genes with the primary endocrine products of these tissues should be investigated to define the roles of peripheral clock genes in regulation of metabolism and health.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Dose Escalating and Bioavailability Phase 1 Studies Assessing Safety and Tolerability and Pharmacokinetics of Tildacerfont, a Small-Molecule CRF1 Receptor Antagonist

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Background: Tildacerfont (SPR001; LY2371712), a second generation, potent, selective, nonsteroidal, oral small-molecule antagonist of corticotropin-releasing factor type-1 (CRF1) receptors in the pituitary gland, is in late stage development as a potential treatment for adults with **c**ongenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD). We report the results of 3 randomized Phase 1 studies of safety, tolerability, and pharmacokinetics (PK) of tildacerfont in healthy adults and compare the relative bioavailability of 2 tildacerfont formulations.
Methods: Two Phase 1 studies were double-blind, placebo-controlled and evaluated escalating doses (2–800 mg QD) as single doses (SAD; n=18) and 50-200mg as multiple doses (MAD; n=47). The studies also included pilot food effect and drug-drug interaction sub studies, respectively. A randomized, balanced, open-label, crossover study (n=42) examined the relative bioavailability (BA) of single oral doses of 2 tildacerfont formulations, 200 mg powder-in-capsule (PIC) compared to 200 mg low-drug load tablet.
Results: In the SAD and MAD studies, the most common, non-procedural adverse events were headache and cough. In the BA study, the most common, non-procedural adverse events were constipation and headache. There were no study drug-related serious adverse events (SAEs) in any study. In the SAD and MAD (PIC formulation), tildacerfont concentrations declined in a multi-exponential manner with C_{max} occurring moderately late, ~5 hours post dose, and a high degree of variability was observed in AUC and C_{max} (CV range: 54–122%). A food effect was observed in the SAD. Fourteen days of dosing achieved near steady state exposure with an AUC accumulation ratio of 2.51 to 3.65 in the MAD study. Midazolam, a sensitive probe substrate for CYP3A4 exhibited ~2-fold increase in AUC and a 1.8 increase in C_{max} when co-administered with tildacerfont suggesting moderate CYP3A4 inhibition. The tablet formulation reduced the variability (AUC and C_{max} CV range: 69–72%) compared to the PIC formulation resulting in a more predictable pharmacokinetic profile. The half-life of the tablet was ~60 hrs. Bioequivalence in AUC was established with the tablet and PIC formulations (fed state) with C_{max} ~20% higher for tablet than PIC due to a reduction in and more consistent T_{max}, median [range], from 6 [4.5, 24] hours to 3 [2, 6] hours.
Conclusions: These results suggest that tildacerfont is safe and generally well-tolerated in healthy adults and suitable for further study in patients with CAH. The tablet formulation provided a more consistent pharmacokinetic profile with reduced variability in all parameters and is being evaluated in late stage studies of tildacerfont in adult CAH.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Effects of L-3,4-dihydroxyphenylalanine (L-Dopa) Treatment in the Neuroendocrine Response to Stress

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Stressful stimuli evoke a complex response mediated by two systems: the Sympathetic-Adreno-Medullar (SAM) axis and the Hypothalamus-Pituitary-Adrenal (HPA) axis. Among the factors involved in stress, glucocorticoids and catecholamines secreted from the adrenal glands and sympathetic nerves are the main effectors of the physiological adaptations to stressors. Besides these, prolactin (PRL) is another hormone secreted under stress conditions. Catecholamines are synthesized from the hydroxylated precursor L-Dopa. This agent is commonly used for the treatment of Parkinson's disease and it would act as a neurotransmitter *per se*. On the other hand, it has

been suggested that HPA axis dysregulation is a potential risk factor for the development of depression. In line with this, several studies reported that L-Dopa treatment may alter the serum levels of ACTH, PRL, and glucocorticoids in parkinsonian patients and Parkinson's disease animal models. In the present study, we determined whether the chronic treatment with L-Dopa altered the stress response inducing depressive-like behaviours. Adult male Wistar rats were treated orally during 24 days with LEBOCAR® - commercial formulation of L-Dopa (75 mg/day) and Carbidopa (7.5 mg/day) - in drinking water. Animals were stressed by immobilization during the last 9 days of treatment and depressive-like behaviours were assessed by the sucrose intake and forced swimming tests. Behavioural tests showed no signs of depressive-like behaviours in the LEBOCAR®-treated and/or stressed rats. We next explored the SAM axis reactivity. Circulating noradrenaline and adrenaline increased in rats treated with LEBOCAR® ($p<0.05$; HPLC). Also, the adrenals from stressed animals showed higher content of adrenaline ($p<0.05$). Then, we studied the HPA axis activity. Chronically stressed rats displayed a lower ACTH secretion (ELISA) and a downregulation of POMC expression (qPCR) in the anterior pituitary ($p<0.05$). In addition, LEBOCAR® treatment induced a reduction in serum ACTH and POMC levels ($p<0.05$). As expected, serum corticosterone (ELISA) enhanced under chronic stress, an effect that was inhibited by treatment with LEBOCAR® ($p<0.05$). Finally, pituitary PRL gene expression (qPCR) was downregulated by LEBOCAR® treatment with a more pronounced effect when rats were also stressed ($p<0.05$). Our results suggest that L-Dopa alters the neuroendocrine stress response enhancing SAM axis reactivity and reducing HPA axis activity and PRL expression.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Evaluation of the Molecular Pathogenesis of Adrenocortical Tumors by Whole-Genome Sequencing

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Pathogenesis of autonomous steroid secretion and adrenocortical tumorigenesis remains partially obscure. Our aim was to identify novel genetic alterations in adrenocortical adenomas (ACA) without somatic mutations in known driver genes. Whole-genome sequencing was performed on 26 ACA/blood-derived DNA pairs without driver mutations in *PRKACA*, *GNAS* and *CTNNB1* genes at previous WES (ENSAT study JCEM 2016). These included 12 cortisol-producing adenomas with Cushing syndrome (CS-CPAs), 7 with mild autonomous cortisol secretion (MACS-CPAs), and 7 endocrine-inactive ACAs (EIAs). Seven adrenocortical carcinomas (ACC) were added to the cohort. We developed a bioinformatics pipeline for a comprehensive genome analysis and to reveal differences in variant distribution. Strelka, VarScan2 and ANNOVAR software and an in-house confidence score were used for variant calling and functional annotation. Combined Annotation-Dependent-Depletion (CADD) values were used to prioritize pathogenic variants. Additional focus relied on variants in pathogenically known pathways (Wnt/β-catenin, cAMP/PKA pathway). NovoBreak algorithm was applied to discover structural variations. Two hypermutated CS-CPA samples were excluded from further analysis. Using different filters, we detected variants in driver genes not observed at WES (one p.S45P in *CTNNB1* and one p.R206L in *PRKACA* in two different CS-CPAs). In total, we report 179,830 variations (179,598 SNVs; 232 indels) throughout all samples, being more abundant in ACC (88,954) compared to ACA (CS-CPAs: 31,821; MACS-CPAs: 35,008; EIAs: 29,963). Most alterations were in intergenic (>50%), followed by intronic and ncRNA intronic regions. A total of 32 predicted pathogenic variants were found in both coding (CADD values ≥ 15) and non-coding (CADD values ≥ 5) regions. We found 3,301 possibly damaging and recurrent variants (intergenic mutations removed) (CS-CPAs: 1,463; MACS-CPAs: 1,549; EIAs: 1,268; ACC: 1,660), mostly accumulated in intronic regions. Some of these were detected in members of the Wnt/β-catenin (CS-CPAs: 6; MACS-CPAs: 2; EIA: 1) and cAMP/PKA (CS-CPAs: 6; MACS-CPAs: 7; EIA: 4) pathways (e.g. *ADCY1, ADCY2, GNA13, PDE11A*). We also found a slightly higher number of structural variations in EIA (3,620) and ACC (3,486) compared to CS-CPAs (977) and MACS-CPAs (2,119). In conclusion, still unrevealed genetic alterations, especially in intronic regions, may accompany early adrenal tumorigenesis and/or autonomous cortisol secretion.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Extracellular Vesicles From SDHB Deficient hPheo1 Cells Activate STAT3 in Wild-Type Cells

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Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors that originate from the adrenal medulla and extra-adrenal paraganglia, respectively. Inactivating mutations in succinate dehydrogenase (*SDHx*) genes leads to succinate accumulation, increased HIF1-α levels, and uncontrollable growth of PPGLs. We hypothesized that small extracellular vesicles (EVs) released from progenitor cells derived from pheochromocytoma (hPheo1) with a shRNA mediated knockdown of *SDHB* are enriched in succinate metabolites that play a key role in the activation of various tyrosine dependent