negative feedback with age. Additionally, RBM plasma CORT was further reduced in all time groups versus NM, accompanied by a return to baseline CORT after 90 min recovery, suggesting a parity-dependent effect on the HPA axis. Changes in CORT levels were correlated with c-FOS-*ir*. MMS increased PVN c-FOS-*ir* in all groups compared to controls and c-FOS-*ir* in NM was significantly greater than PVN c-FOS of RBM. Further, while c-FOS-*ir* in the NY females was reduced to baseline 30 min after MMS, the return to baseline was more gradual in NM. No effect of parity or age was seen in *Crh* mRNA. Collectively, our findings show that activation of the HPA axis in females involves interactions between age- and parity- dependent function. Our findings further show activation and inhibition of the HPA axis in females involving long-term changes that occur after pregnancy, which may increase risk for stress- or postpartum- related disorders. Supported by NIDDK 1-R01 DK105826

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Analysis of the Relationship Between Learning and Synaptophysin in Obese Rats Treated With DPP4 Inhibitor

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In this study, it is aimed to investigate possible changes in cognitive functions in obesity by using targeted treatment hypothesis. Accordingly, the effects of DPP4 inhibitor, which is actively used in the clinic in the treatment of diabetes, and the effect of exercise, which has been proven to be effective in the treatment of obesity, on the change of learning performance and the relationship of these effects with the synaptophysin molecule were investigated. In our study, 42 Wistar albino rats were used. The animals were randomly divided into seven groups as obese, control, obese+DPP4i, control+DPP4i, obese+exercise, control+exercise, control+NaCl. To create experimental obesity, the animals that are targeted to be obese were separated and fed on a high fat diet for 8 weeks. After the obese model was created, sitagliptin was applied to the DPP4i groups and swimming exercise was applied to the exercise groups for obesity treatment. The last week of the study was performed reference memory learning test to the whole group with Morris water maze. Then, the hippocampus tissues were removed from the animals under anesthesia. mRNA and protein isolations were performed from the extracted tissues. Synaptophysin gene expressions were determined from mRNA samples by Real-Time PCR method. Synaptophysin protein levels were determined from protein lysates by Western Blot method. In the learning test, in the obese groups, there was a statistically significant difference between the average escape time of the DPP4i and exercise groups and the groups that did not ($p<0.05$). As a result, in groups where obesity is treated with DPP4i and exercise; It was concluded that cognitive performance was better than obese groups. There was a evident decrease in synaptophysin gene expression levels in obese groups compared to the control group. In the treatment groups, an increase was observed in synaptophysin gene expression levels in the DPP4 inhibitor and especially in the exercise groups compared to the control groups (P> 0.05). Gene expression results were similar in analyzes performed at the protein level. According to these results, in terms of performance in cognitive function due to obesity and synaptophysin gene relationship; DPP4 inhibitor showed as effective a result as exercise. This provides a resource for advanced molecular and metabolic research.
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Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Antisense Oligonucleotides as a Novel Therapy for Cushing's Disease

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Background: Cushing's disease (CD) is caused by high levels of blood cortisol resulting from excess secretion of ACTH from a corticotroph adenoma in the anterior pituitary gland. Clinical features include hypertension, diabetes, osteoporosis, and depression. If untreated CD has an increased mortality of five-fold owing to cardiovascular comorbidities, stroke or raised vulnerability to infection. Transsphenoidal surgery is considered the first-line treatment but remission is achieved in only 65% of cases and the relapse rate is high. Furthermore, medical treatments are often accompanied by unpleasant side-effects. Antisense therapy is a technique for suppressing gene expression at the level of translation using antisense oligonucleotides (ASOs) against the mRNA of interest. **Aims:** To investigate antisense therapy as a treatment for CD by targeting ASOs against ACTH-encoding POMC mRNA thereby reducing secretion of the hormone. To transfect mouse AtT20 cells (cells that secrete high levels of ACTH) with ASOs against POMC at varying doses to determine which is the most effective at reducing ACTH secretion. **Methods:** AtT-20 cells that secrete high levels of ACTH were used as the model system. ASOs were designed to specifically target exon 3 of the POMC gene. Transfection of AtT-20 was carried out using Lipofectamine. FACS was used to determine transfection efficiency. ACTH levels secreted by AtT-20 cells were determined by immunoassay. Statistical analysis was done using ANOVA with P values < 0.05 considered significant. **Results:** ASOs that targeted POMC exon 3 (ASO-2 and ASO-3) were transfected into AtT-20 cells at 10 and 100 nM. Control ASOs were ASO-1 (matched to POMC sense strand) and ASO-4 (a scrambled version of ASO-3). Experiments included untreated AtT-20 cells and AtT-20 cells treated with transfection reagent or ASOs alone. The results of six experiments indicated that ACTH secretion from AtT-20 cells was reduced after transfection with ASO-2 and ASO-3 at 100 nM (ANOVA, $P = < 0.05$) and 10 nM (ANOVA, $P < 0.05$) when compared with untreated AtT20 cells. ASO-1 and ASO-4 had no effect on ACTH

secretion by AtT-20 cells (ANOVA, $P > 0.05$). **Conclusions:** Initial experiments have shown that ASOs against POMC can reduce ACTH secretion from AtT-20 cells and may be useful as a novel therapy for CD.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Canine Pituitary Organoids as 3D** In Vitro **Model for Cushing Disease

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Cushing disease (CD) is a serious endocrine disorder that is most often caused by an ACTH-secreting pituitary adenoma. Patients can be treated medically when surgery is not an option or was unsuccessful. However, currently used pituitary-targeting drugs are effective in only 40% of patients. To efficiently identify new pituitary-targeting treatment options, we need an *in vitro* system that closely mimics *in vivo* conditions. We therefore aimed to establish organoid cultures of normal anterior pituitary and corticotroph adenomas. Organoids or tumoroids are miniature three-dimensional (3D) structures grown from stem cells, that closely resemble the organ or tumor they originate from. Because CD is a thousand times more prevalent in dogs than in humans, and hypophysectomy is the treatment of choice, we used canine tissues. Normal anterior pituitary glands were collected from three healthy dogs that were euthanized for reasons unrelated to the current study. Corticotroph adenomas were collected from six dogs that underwent transsphenoidal hypophysectomy at our University Clinic. The dogs were diagnosed with CD based on clinical signs, endocrine testing, and CT scan imaging. Normal anterior pituitary and corticotroph adenoma cells were cultured in a 3D matrix (basement membrane extract) with anterior pituitary organoid medium containing specific growth factors and ligands, which was refreshed twice a week. The organoids and tumoroids were characterized with histopathology and RT-qPCR. Structures resembling organoids or tumoroids grew from all nine samples (3 normal, 6 adenoma) that were put in culture. Both cystic and dense structures were observed. The organoids and tumoroids expanded rapidly, and could be passaged once every week. The organoids and tumoroids were successfully cultured up until passage number 10, and were then frozen down. Histopathology showed that the organoid or tumoroid cells morphologically resembled healthy anterior pituitary or corticotroph adenoma cells. All organoids cultures expressed mRNA of pituitary stem cell markers SOX2 and SOX9. This study shows that corticotroph adenomas can be cultured as tumoroids *in vitro*, something not previously published in any species. Based on the many opportunities in organoid culture (e.g., high-throughput drug screenings, gene editing, studying developmental processes), we expect that this *in vitro* model will pave the way to efficiently and reliably identify new treatment options for CD. Not only for humans, but also for our best friends: dogs.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Chronic Excess of Non-Aromatizable Androgens From Puberty Does Not Drive a Neuroendocrine Phenotype Observed in Other Preclinical Rodent Models of PCOS

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Polycystic ovary syndrome (PCOS) is the most common form of anovulatory infertility in women of reproductive age, characterised by androgen excess, polycystic appearance of the ovary and irregular menstruation. PCOS is also frequently associated with metabolic abnormalities, including increased adiposity and insulin resistance. The origins of PCOS are unknown, however recent findings in animal models strongly implicate androgen signalling in the brain in the development of PCOS pathophysiology. Exposure to androgen excess, either acutely during prenatal development or chronically from a peripubertal timepoint, can drive the development of PCOS-like features in adulthood. Prenatally androgenized (PNA) mice exhibit the cardinal reproductive features of PCOS and increased luteinizing hormone (LH) pulse frequency. This phenotype is associated with increased GABAergic innervation of gonadotropin-releasing hormone (GnRH) neurons, postulated to drive elevated GnRH/LH release and downstream effects. Chronic exposure to di-hydrotestosterone (DHT) from 3 weeks of age drives both reproductive and metabolic PCOS-like features that are ameliorated by selective AR loss from the brain. Here, we aimed to determine whether chronic exposure to DHT drives a similar increase in LH pulsatility and elevated GABAergic innervation to GnRH neurons as seen following prenatal exposure to androgen excess. GnRH-green fluorescent protein (GFP) female mice received either DHT or blank capsules for 90 days from postnatal day (PND) 21 (N = 6-7/group). Serial tail tip blood sampling was used to measure pulsatile LH and fixed brains were collected and immunolabelled for vesicular GABA transporter (VGAT) to assess putative GABAergic terminals associated with GFP-labelled GnRH neurons. Chronic androgen excess from the peripubertal period resulted in acyclicity and increased body weight as expected. However, LH pulsatility was not different between DHT-treated females and controls. Similarly, the density of VGAT appositions to GnRH neurons was not different between groups. Therefore, the programmed changes in the GnRH neuronal network and hyperactive LH secretion that result from prenatal androgen excess are not affected by chronic DHT exposure initiated at 3 weeks of age. These findings suggest that unique central mechanisms are involved in the reproductive impairments driven by exposure to androgen excess at different developmental stages.