advances following 4 shifts. This sex-specific disruption was supported by preliminary tissue explant Per2::luciferase rhythms, which suggest that shift-light alters tissue level circadian phase synchrony in female, but not male, HPG axis tissues. Importantly, females exhibited shortened estrous cycling during shift-light, suggesting the altered HPG axis synchrony could be directly impacting reproductive function. We are currently working to extend this work to determine how this desynchrony impacts hormone release, including luteinizing hormone and follicle stimulating hormone. Together, this work provides insight into how shiftwork may influence circadian rhythms in reproductive tissues and suggests that females may have increased vulnerability to reproductive deficits from shiftwork.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Activation of Norepinephrine Neurons in the NTS (A2 population) is Sufficient to Suppress Pulsatile LH Secretion in Female Mice

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The overarching goal of this work is to identify neural pathways underlying inhibition of pulsatile luteinizing hormone (LH) secretion during stress. Stress-induced suppression of LH secretion is mediated, at least in part, by suppression of arcuate kisspeptin (ARC^{Kiss1}) neurons. The mechanisms by which acute stress suppresses ARC^{Kiss1} cell activity are largely unknown; however, several lines of evidence support the hypothesis that A2 neurons (norepinephrine [NE] neurons in the nucleus of the solitary tract [NTS] of the brainstem) are involved. First, A2 cells are activated during several reactive stress paradigms. Second, NE administered into the paraventricular nucleus, which is innervated by A2 neurons, suppressed pulsatile LH secretion. Finally, ablation of brainstem NE neurons restored estrous cyclicity following chronic glucoprivation (chronic metabolic stress model). The present study employed chemogenetics to test the hypothesis that A2 neurons are sufficient to suppress pulsatile LH secretion in ovariectomized female dopamine beta-hydroxylase (DBH) Cre positive and negative (wild type) mice. Mice received bilateral injections of either a Cre-dependent stimulatory Designer Receptor Exclusively Activated by Designer Drugs (DREADD) virus (AAV1-DIO-hM3Dq-mCherry) or a control virus (AAV1-DIO-mCherry) into the NTS. Mice were randomly assigned to receive either clozapine N-oxide (CNO, specific DREADD agonist; 1mg/kg, i.p.) or saline and blood samples were collected at 6-min intervals for 60 min before and 90 min after injection. Two weeks later, mice received the alternate treatment in a cross-over design (n= 5-10/grp). During the pre-injection period, all mice had clear LH pulses (mean: 6.0 ± 0.2 ng/mL, pulses/60 min: 3.4 ± 1.5). In DBH Cre- (wild type) mice with hM3D virus and DBH Cre+ with mCherry virus (both control groups), neither CNO nor saline altered mean LH or LH pulse frequency. However, DBH Cre+ mice with hM3D virus had a 54% reduction in mean LH ($p < 0.05$) and 59% reduction in pulse frequency ($p < 0.05$) following CNO; neither LH metric was altered in response to saline. To assess transduction efficiency, fixed neural tissue was collected. In tissue analyzed thus far, DBH Cre+ mice have mCherry labeling in ~70% of DBH-immunoreactive neurons in the NTS and >90% of mCherry neurons contained DBH immunoreactivity. Three DBH Cre+ mice with hM3D virus mice had no LH response to CNO and may represent missed viral injections, which will be determined when tissue is analyzed. These data demonstrate that activation of A2 neurons is sufficient to impair pulsatile LH secretion in female mice. Moreover, these data support the broad hypothesis that the A2 population of neurons is critical for modulating neuroendocrine function during stress and raises the possibility that A2 neurons directly or indirectly influence ARC^{Kiss1} cell activity.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Age and Parity Effects in the Hypothalamic Pituitary Adrenal Axis' Response to Multimodal Stress in Female Mice

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The hypothalamic pituitary adrenal (HPA) axis responds to environmental perturbations to maintain homeostasis. Pregnancy demands extensive modifications in HPA axis function to prepare for increased energy and metabolic demands required to meet the needs of mother and offspring. Short-term effects of pregnancy on the HPA axis have been shown, but data is lacking regarding the long-term effects in middle-aged female mice no longer breeding. Since changes of the HPA axis are further found with age, in this study, we examined both parity- and age-related interactions on the HPA axis in female mice. Wildtype C57bl/6N females were divided into nulliparous young (NY) (3-6 mo) and nulliparous middle-aged (NM) or multiparous retired-breeder middle-aged (RBM) (8-10 mo) groups. RBM mice were killed at least 4 weeks after their last litter was weaned. Control mice were euthanized directly out of the home cage and experimental groups were euthanized at 0 min, 30 min, or 90 min recovery (n=8-10/ group) after 2h of multi-modal stress (MMS; restraint, noise, shaker, light). (Paraventricular nucleus of the hypothalamus (PVN) neuronal activity was quantified by c-FOS immunoreactivity *(-ir)*, and plasma corticosterone (CORT) levels were measured by radioimmunoassay. Corticotropin releasing hormone (CRH) mRNA was assayed by *in situ* hybridization. Two-way ANOVA showed effects of age ($p<0.0248$), parity ($p<0.0021$), time ($p<0.0001$), and interaction ($p<0.0009$) on CORT levels. Specifically, basal CORT levels were reduced in NM/RBM versus NY mice. In all groups, CORT levels were significantly elevated by MMS. There was no difference between CORT levels immediately after MMS in NY or NM groups, but CORT levels after 30- and 90- min recovery from MMS remained elevated in NM, indicating reduced

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

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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 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