Poster Abstracts Group B

FURTHER STUDIES ON THE ROLE OF PI3K AND PI4K IN ERK1/2 ACTIVATION DURING GnRH ACTION

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Gonadotropin-releasing-hormone (GnRH) is a hypothalamic decapeptide that serves as a key regulator of the reproductive system. Interaction of GnRH with its receptor (GnRHR), a member of the G-protein-coupled-receptor (GPCR) family, leads to activation of the PLCβ-Ca^{2+}-PKCs-MAPKs cascade, which mediates gonadotropins (LH and FSH) synthesis and release. We investigated the role of PI3K and PI4K in GnRH-induced ERK1/2 activation in αT3-1 and LβT2 gonadotropes. Initially, αT3-1 and LβT2 cells were incubated for 1h with wortmannin (10nM and 10μM) or LY294002 (10μM and 100μM), doses known to inhibit PI3K and PI4K, respectively, followed by stimulation with GnRH (100nM) or PMA (100nM) for 10 or 5min. Wortmannin, gave a significant inhibition of ERK1/2 activation by GnRH or PMA at the two doses examined, with a more pronounced inhibition observed in the more mature LβT2 cells. LY294002 also gave a significant inhibition of ERK1/2 activation by GnRH or PMA at the two doses examined, with more pronounced inhibition observed in αT3-1 cells. Inhibition of ERK1/2 activation by GnRH and PMA wasn't evident when cells were incubated with wortmannin or LY294002 for 30min. Wortmannin (10nM and 10nM) also inhibited GnRH induced MEK phosphorylation (Ser298), an upstream effector of ERK1/2, in LβT2 cells. We also examined GnRH-induced Akt activity which is a downstream target of PI3K. To that end, αT3-1 cells were treated with GnRH (100nM) for increasing time period up to 240min. The basal phosphorylation of Akt in both sites (Ser473, Thr308) was markedly high and was reduced rapidly by GnRH within 5min of stimulation. The lack of inhibition by the drugs at 30min raises the possibility that the role of PI3K/PI4K in GnRH induction of ERK1/2 is indirect and exerted by a factor which needs to be identified. Furthermore, the involvement of PI3K/PI4K in ERK1/2 activation by GnRH is independent of Akt activity.

TRACES OF STRESS: CORTISOL RELATED SUSTAINED ENHANCEMENT OF AMYGDALA-HIPPOCAMPAL FUNCTIONAL COUPLING

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Stressful experiences generate intricate neurocircuit modulations, and it is the temporal trajectory of these alterations, elapsing from early disturbances to late recovery, that ultimately assembles the interface between resilience and vulnerability to stress. Such interface may depend on resting-state processing such as introspection, drawing on past experience and envisioning future events, all known to involve the default mode network (DMN). By inducing social stress, and acquiring rest-fMRI before stress, immediately following it, and two hours later, we were able to expand the time-window for examining the trajectory of the stress response. Throughout the study repeated cortisol sampling and self report of stress level were obtained from 62 healthy young individuals. Post-stress alterations were investigated by whole brain resting-state functional connectivity of two central hubs of the DMN: the posterior cingulate cortex and hippocampus. Results indicate a 'recovery' pattern of DMN connectivity, in which all alterations, ascribed to the intervening stress, balanced back to initial level. The only exception was the stress-induced rise in amygdala-hippocampal coupling which was sustained even two hours later. Furthermore, this sustained enhancement of limbic coupling was inversely correlated to individual cortisol responsiveness and characterized only the group lacking the increased cortisol response (i.e., cortisol *non-responders*). Our observations provide evidence of prolonged post-stress response profile, characterized by both the comprehensive balance of most DMN functional connections and the distinct, time and cortisol dependent ascent of limbic coupling. These new insights into neuro-endocrine relations may add another milestone in the ongoing search for individual markers in stress-related psychopathologies.

ELEVATED WHITE BLOOD CELL COUNTS IN CUSHING'S DISEASE - ASSOCIATION WITH DISEASE ACTIVITY

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Background: Glucocorticoid receptors are expressed in white blood cells (WBC) and are known to play a role in cell adhesion and WBCs recruitment from bone marrow. In Cushing's disease leukocytosis is frequently mentioned as laboratory finding. However, there is no data on the prevalence of this aberration among patients, or correlation to disease activity.

Aim: To investigate the prevalence of leukocytosis in patientswith Cushing's disease, alterations in other blood count parameters and correlation to disease activity.

Methods: Data of 21 patients diagnosed and followed for Cushing's disease at our clinic was reviewed. Two patients had disease relapse after complete remission and were studied as 2 separate events.

Results: Of the 21 patients, 15 were women (71.4%), with a mean age of 39±12.6 years. Mean baseline WBC count was 10,460±2,800 cells/µl and dropped to 8,800±1,970 cells/ µl ($P < 0.005$) after treatment, lymphocyte count was 1,940±600 cells/µl and raised to 2,230±570 cells/µl ($P<0.005$), hemoglobin was 13.6±1.2 g/dl and dropped to 12.8±1.5 g/dl ($P<0.005$), whereas platelet number did not change. There was no correlation between WBC count and disease severity. High WBC count was present in 9/23 cases (39%). Those patients with normal baseline WBC (mean, 8,700±1,500 cells/µl) dropped also to 7,700±1,400 cells/µl after treatment ($p<0.005$).

Conclusions: Patients with Cushing's disease present with leukocytosis in approximately 40% of cases, with no correlation to disease severity. In most cases, including those without elevated baseline count, the WBCs decreased with remission, emphasizing the possible effect of glucocorticoids on these cells

CORTISOL RESPONSE AND DESIRE TO BINGE FOLLOWING PSYCHOLOGICAL STRESS IN OBESE PATIENTS WITH AND WITHOUT BINGE EATING DISORDER

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About 30% of obese people are diagnosed with Binge Eating Disorder (BED). While stress

and negative affect is known to precede "emotional eating" and binge eating, this relationship is not

fully understood. The objective of this study was to explore the relationship between induced

psychological stress, HPA axis and eating behavior in Binge Eating Disorder (BED). The Trier Social

Stress Test (TSST) was applied in obese participants with (n=8) and without BED (n=8), and normal

weight controls (n=8). Subjective psychological state and eating-related symptoms were evaluated and

cortisol secretion was assessed throughout the stress test. Baseline stress, anxiety and cortisol

measures were similar in all groups. At baseline desire to binge was significantly higher among the

BED group. While the TSST induced an expected increase in cortisol levels, a blunted cortisol response

was observed in the BED group. Furthermore, in the BED group, a positive correlation was found

between the change in cortisol levels during the TSST and the change in VAS scores for desire to binge

and sweet craving. Post-TSST desire to binge and sweet craving were significantly higher in the BED

group and correlated positively with stress, anxiety, and cortisol response in the BED group only.

These results suggest chronic downregulation of the HPA axis in participants with BED, and a

relationship between psychological stress, the acute activation of the HPA axis, and food craving.

ECTOPIC PITUITARY TISSUE OF THE NASOPHARYNX AND SPHENOID SINUS EXPRESSING MULTIPLE HORMONES

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Introduction: Nasal obstruction is a common complaint, but an unusual presentation of ectopic pituitary adenoma (EPA), located in the nasopharynx and sphenoid sinus region.

Case report: A62 year old woman presented to the Otolaryngology Clinic with nasal obstruction. On MRI a soft tissue lesion of the nasopharynx and sphenoid sinus was seen, involving the anterior wall of the sphenoid bone. A normal pituitary gland was clearly detected and separated from the intra-sphenoidal mass. The mass in the nasopharynx was endoscopically removed. Histologic examination revealed a round cell tumor consistent with EPA with positive staining to all of the anterior pituitary hormones except ACTH. Clinical and laboratory evaluation revealed no pituitary hormone excess or deficiency. Two years following the operation the patient is asymptomatic with a normal hormone profile. A follow-up MRI revealed mild enlargement of the residual mass in the sphenoid sinus. Somatostatin receptor scan with Ga68-DOTATOC PET was positive.

Review of the literature: An EPA is defined as an extra-seller adenoma, not connected to the normal pituitary gland. It has been proposed that EPA arise along the migration path of Rathke's pouch, or from aberrant anterior pituitary cells. Approximately 40% of these tumors are located in the sphenoid sinus, presenting with epistaxis or nasal obstruction. Most of these tumors produce excess of ACTH, while the rest may secrete prolactin, GH or TSH, causing clinical syndromes. Extremely rare cases are associated with empty sella.

Conclusions: We described here a case of ectopic pituitary tissue of the nasopharynx and sphenoid sinus. In the case described, the tumor stained positive for multiple pituitary hormones, but was not hyperfunctioning. This raises the question whether this mass represents hyperplasia rather than a real adenoma. Surgical and medical treatments were considered. The fact that the mass was detected by SRS arises the possibility of Somatostatin analogues therapy.

LONG-TERM CLINICAL, ENDOCRINE AND METABOLIC EFFECTS OF VALPROIC ACID VERSUS CARBAMAZEPINE MONOTHERAPY FOR BOYS AND MALE ADOLESCENTS WITH EPILEPSY

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Background: Both epilepsy and antiepileptic drugs (AEDs), and in particular valproic acid (VPA), have been associated with numerous endocrine abnormalities. Previous studies have focused mainly on female patients.

Objective: to evaluate prospectively the endocrine effects of VPA compared to carbamezapine (CBZ) in males pediatric patients with epilepsy.

Patients and methods: We prospectively studied 37 boys with epilepsy (22 with generalized, 14 with partial, and one with unclassified seizures). Twenty-four were treated with VPA monotherapy (13.9 mg/kg/day) for a mean duration of 7.1 years, and 13 with CBZ monotherapy (13.4 mg/kg/day) for a mean duration of 6.1 years. Upon diagnosis, 14 were prepubertal, 15 pubertal and 8 postpubertal. A control group included 48 age-matched boys with hypothyroidism adequately treated and euthyroid. We compared the data on metabolic, auxological and hormonal parameters before treatment initiation, 6 months later and at last follow-up visit.

Results: Weight-SDS increased significantly during the first 6 months of treatment ($P<0.001$) with no correlation to the AED regime, but decreased between the first and last visit ($P=0.01$). These changes were not observed in the controls. Mean free-T4 significantly declined between the first and last visit in the CBZ group ($P=0.006$), but remained within the normal range, with no change in mean TSH levels. No significant changes were observed between first and second and last visits in insulin resistance or lipid profile for the entire study group, and when analyzed by treatment medication or by partial versus generalized epilepsy. No correlation was found between mean dose of treatment or duration and auxological characteristics and fasting metabolic profile. All patients had normal pattern of puberty and linear growth.

Conclusions: Long-term therapy with VPA or CBZ had no significant clinical or endocrinological effect on boys and adolescents with epilepsy, except for an increase in body weight during the first 6 months of treatment, with a decline thereafter. Further, larger prospective studies are required to corroborate our findings.

THE LONG-TERM EFFECTS OF GROWTH HORMONE THERAPY ON BODY MASS INDEX IN CHILDREN WITH GROWTH HORMONE DEFICIENCY: A RETROSPECTIVE STUDY

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Background: GH therapy has important metabolic effects beyond its role in growth. It has been shown that GH increases lean body mass and decrease body fat mass therefore GH therapy has been suggested for weight reduction. Despite extensive experience with GH therapy in children from the mid-1980s, there are only few studies on its effect on body mass index (BMI).

Objective: In this retrospective study, we examined the effect of GH on BMI over time in children diagnosed with GH deficiency and treated with GH. Subjects and Methods: 77 subjects with GHD were enrolled. Subjects were treated with recombinant GH at a dose of approximately 0.033 mg/kg/d, with adjustments based on IGF-I levels and response to treatment. Height and weight were measured and BMI calculated at regular follow-up visits and converted into age- and gender-specific z-scores based on CDC 2000 Growth Charts.

Results: Overall BMI z-score of most subjects remained stable throughout GH therapy. There was no simple trend in BMI in response to GH. It varies over time, with fluctuations and differences between individuals, between gender and degree of GH deficiency. There were some patients whose BMI increased as well as other patients whose BMI decreased from their individual starting points. When looking at BMI z-score, it appears that children, who either decrease in obesity or remain stable, begin at a normal BMI z-score while children who gained weight started off underweight. Girls' linear growth response to GH therapy was better than that of boys, but their BMI z-score also increases whereas that of boys decreases.

Conclusion: Our results show that there are individual differences in BMI over the course of GH therapy. Some children gain weight or loss weight, therefore when following GHD patients, it is important to follow BMI and to address obesity as a possible side effect of GH therapy.

AKAP4 AS A POTENTIAL SWITCH BETWEEN PKA AND PKC/MAPK IN HUMAN SPERMATOZOA

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The Mitogen-activated protein kinases (MAPK) cascade is a central signaling pathway that regulates a wide variety of cellular processes, such as proliferation, differentiation, survival, apoptosis and stress responses. One of the MAPKs is ERK1/2 that plays a crucial role in signaling pathways in general and in the pituitary-gonadal axis, in particular.

We have recently reported that ERK 1/2 is a positive regulator of human spermatozoa motility. In addition, we have found that ERK1/2 phosphorylates A-kinase anchoring protein 4 (AKAP4), which is one of the major components of the sperm fibrous sheath and is known to be crucial for sperm motility. Furthermore, we have also found that cAMP attenuated the activation of ERK1/2 by PMA in human spermatozoa. Therefore, we decided to examine whether AKAP4 is a switch molecule that links between PKA and PKC pathways in human spermatozoa. At first we found that AKAP4 is phosphorlated by ERK1/2 in human spermatozoa and identified the phosphorylation site as threonine 265. Then we examined whether the phosphorylation of AKAP4 is important for its cellular localization. Since mature sperm do not have active transcription machinery, we used a heterologous system, i.e. in HEK293T cells expressing AKAP4. Indeed, PMA treatment led to translocation of AKAP4 from the cytosol to the Golgi in the HEK293T cells. The effect was abolished in HEK293T cells expressing AKAP4-T265A, which has a point mutation in the ERK1/2 phosphorylation site.

In order to check whether AKAP4 has a role in cAMP inhibition of ERK activation by PMA, we transfected the cells with tGFP-AKAP4, or with tGFP alone as a control. We found that cAMP and a phosphodiesterase inhibitor, IBMX decreased ERK1/2 activation by PMA in the HEK293T cells expressing AKAP4, while no inhibitory effect was noticed in the tGFP expressing cells.

Thus, AKAP4 seems to play a role as a switch molecule that links between PKA and PKC pathways in human spermatozoa. The physiological significance of AKAP4 phosphorylation by ERK1/2 in human sperm is under investigation.

PLEIOTROPIC EFFECTS OF FGF2 IN CELLS OF THE DEVELOPING CORPUS LUTEUM

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Corpus luteum (CL) formation is characterized by rapid growth, accompanied by intense angiogenesis, which is driven by LH and hypoxia. We have previously shown that FGF_2, present in endothelial and steroidogenic cells, is highly expressed in the developing CLs. This growth factor is further up-regulated by administering prostaglandin F2α (PG), specifically in young CLs refractory to PG luteolytic action. Therefore, at this early stage, FGF_2 may play a luteo-protective role. We sought here to better understand how FGF_2 manifests its protective effects by acting on endothelial and steroidogenic cells.

FGF_2 increased the proliferation of luteinized granulosa (LGC) and luteal endothelial cells (LEC) in a dose-dependent manner. In both cell types FGF_2 also induced phosphorylation of MAP kinase. Stimulation of LEC with varying doses of FGF_2 increased mRNA expression of hypoxia-inducible factor-1α (HIF1α), VEGF, and glucose transporter type 1, (SLC2A1). As expected, cobalt chloride (hypoxia mimicking agent) increased of HIF1α protein in LEC; notably, also FGF_2 elevated HIF1α protein these cells. In LGC, FGF_2 (20 ng/ml) elevated the mRNA levels of key regulators of angiogenesis : FGF_2 and VEGF. There was an autocrine upregulation of FGF_2 (by twofold), whereas VEGF was much less induced (about 130%). Although proangiogenic genes were increased, the steroidogenic capacity was compromised. The two major steroidogenic proteins, cholesterol side-chain cleavage enzyme (CYP11A1) and steroidogenic acute regulatory protein (STAR), were significantly down-regulated to levels ranging between 20% and 30% of their initial levels. In accordance, FGF_2 reduced progesterone production.

This study proposes amultitude of cooperating mechanisms by which FGF_2 could regulate CL development : it can promote angiogenesis by activating LEC proliferation, inducing HIF1α- a known mediator of angiogenic responses, and by stimulating pro-angiogenic factors. FGF_2 further contributes to the proliferative phenotype of the gland by augmenting LGC numbers and increasing their survival while maintaining lower levels of steroidogenesis.

UNDERSTANDING THE ROLE OF GNRH IN ACTIVATING TRANSCRIPTION OF THE LHB GONADOTROPIN SUBUNIT GENE THROUGH ITS EFFECTS ON NUCLEOSOMAL DYNAMICS

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Luteinizing hormone (LH) is an anterior pituitary gonadotropin heterodimeric glycoprotein, composed of two subunits: α and β. It plays a distinct role in regulating development and function of the gonads, where the β-subunit imparts biological specificity. The synthesis and release of the LH β-subunit is positively regulated by the gonadotropin-releasing hormone (GnRH) which overcomes LHβ gene repression. To overcome this repression, the promoter region has to be accessible for the transcription machinery. Accessibility is achieved by removal of nucleosomes or their repositioning in this region. Various factors influence nucleosome positions, including DNA sequence, histone modifications and transcription factor binding. It has been previously demonstrated that GnRH upregulates various specific transcription factors including Egr-1, which along with SF-1 and Pitx-1 activates LHβ gene transcription. Additionally, it has been shown that GnRH regulates gonadotropin subunit gene transcription at the chromatin level, through displacement of histone deacetylases (HDACs), thereby allowing subsequent histone acetylation. We hypothesize that transcriptional activation of the LH β-subunit gene by GnRH causes nucleosome repositioning in its regulatory region through upregulation of gene specific transcription factors and subsequent histone modifications.

Here, we used MNase-qPCR analysis to characterize the nucleosome positioning in the LHβ promoter area in cells that do not express the LHβ gene (αT3-1) and in cells that express the gene abundantly (LβT2). We compared nucleosome patterns in both cell types without treatments, and found that there is less nucleosome occupancy at the Egr-1 and SF-1 transcription factor binding site region in LβT2 cells. In αT3-1 cells treated with GnRH for 8 hr, or the HDACs inhibitor Trichostatin-A for 24 hr, and also in αT3-1 cells in which the SF-1, Egr-1 and Pitx1 transcription factors were over expressed, the same occupancy pattern was seen as in non-treated LβT2 cells. Taken together, our results suggest that GnRH regulation of LHβ subunit gene transcription involves the nucleosome repositioning at the binding sites of these key transcription factors at the promoter.

HDACS ARE INVOVLED IN NUTRITIONAL CATCH-UP GROWTH

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Background: The association between children's growth and nutrition status is well established, but the exact mechanism by which nutrition affects growth has not been completely elucidated. Using a food restriction protocol, we have previously found global changes in gene expression within the growth plate, suggesting epigenetic regulation of the growth processes.

Methodology: 1. *In-vivo* model- Prepubertal rats were subjected to 10 days of 40% food restriction, followed by a renewal of the regular food supply. After one day animals were sacrificed and bones, liver, kidneys, lungs and heart were excised and studied. Changes in the protein level of histone deacetylase (HDAC)s were monitored. 2. An i*n vitro* model consisting of Huh7 cells was established to study the mechanism of starvation induced growth attenuation.

Results: After the first day of restriction removal, the weight of the animals increased significantly by 18% ($P<0.001$) with a similar increase in the weight of the heart (127%; $P<0.05$) and kidneys (123%; $P<0.01$) while the weight of the lungs and the length of the tibias were not significantly changed. The most dramatic effect was noted in the weight of the liver (186%; $P<0.01$). Protein level of several HDACs were significantly increased by food restriction and reverted to normal levels already after one day of catch up. Similar results were obtained in the *in vitro* model.

Conclusion: HDACs act as chromatin modifiers to alter global gene expression and also exert a broad range of functions outside the nucleus by deacetylating non-histone target proteins. Understanding the role of HDACs in the regulation of the growth process may help us design new treatment strategies.

IS OXYTOCIN A LUTEOTROPHIC OR LUTEOLYTIC AGENT IN THE EQUINE CORPUS LUTEUM?

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The main function of the corpus luteum (CL) is progesterone (P4) production, which is essential for conceptus survival in early pregnancy. In contrast, oxytocin (OT) is suggested as a main inductor of pulsatile prostaglandin F2alpha output from the uterus during luteolysis in mares. However, in the early luteal phase, when peripheral OT level is relatively low, systemic OT administration prolongs equine CL lifespan, suggesting a luteotrophic/luteosupportive role of this peptide. The aim of this study was to determine (i) OT concentrations profile in the equine CL during the estrous cycle, and (ii) putative OT actions on CL secretory function in the mid-luteal phase.

Material and Methods. Experiment 1. Corpora lutea were obtained in early-, mid-, and late luteal stages of the luteal phase (n=6 for each stage). Relative quantification (qPCR) of mRNA transcription of peptidyl-glycine-alpha-amidase (PGA), OT prepropeptide (OT/NP1), and OT receptor (OTR) were performed. Luteal tissue oxytocin was extracted and its concentration determined by EIA method. Experiment 2. Explants from mid-CL were cultured for 24h with OT (10-7M). Concentration of P4 in culture media was determined using EIA. qPCR for 3β-hydroxysteroid dehydrogenase (3βHSD) and steroidogenic acute regulatory protein (StAR) were performed. Results. Exp. 1. The transcription of PGA, OT/NP1 and OTR mRNA showed a tendency to increase throughout the luteal phase, reaching its highest level ($P<0.01$) in the late CL. OT concentration in mid CL was lower with respect to early and late CL ($P<0.05$). In Exp. 2. OT stimulated P4 secretion and StAR gene expression ($P<0.05$), but not 3βHSD ($P>0.05$).

The presence of genes responsible for OT synthesis and peptide secretion, as well as OT receptors expression profiles were shown in the equine CL Additionally to luteolysis regulation, luteal-derived OT seems to play a luteotrophic role during equine CL growth and maintenance, by supporting P4 secretion.

THE ROLE OF PROHIBITIN AND THE Bcl2 FAMILY MEMBERS IN GnRH-INDUCED APOPTOSIS IN MATURE GONADOTROPES

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The gonadotropes are a population of cells in the pituitary that play a pivotal role in the mammalian reproductive system. When exposed to gonadotropin-releasing hormone (GnRH), these cells undergo several intracellular modifications leading to production and secretion of the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH). GnRH is also involved in the gonadotrope development, and we have already reported that it induces cell proliferation in immature, partially differentiated gonadotropes, while leading to apoptosis in mature, fully differentiated gonadotropes. Several MAPK cascades are activated by GnRH in the gonadotropes, but the downstream mechanisms responsible for mediating the GnRH-induced cell proliferation and death have not been elucidated yet. We have previously reported that the protein levels of prohibitin, a protein involved in cell proliferation regulation, are higher in the nuclei of mature compared to immature gonadotropes. We hypothesize that prohibitin, as well as Bax and Hrk, two members of the apoptosis-related Bcl-2 family of proteins, are at least partially responsible for mediating GnRH-induced apoptosis in mature gonadotropes.

Here, we show that the GnRH-induced apoptosis in mature gonadotropes is at least partially mediated by JNK. We also show that GnRH increases the transcript levels of prohibitin both in a mature gonadotrope cell line and in primary gonadotropes, and induces prohibitin nuclear export. Additionally, we show that prohibitin at least partially mediates the GnRH-induced apoptosis in mature gonadotropes, most likely by allowing Bax mitochondrial import. Our results also indicate a role for the pro-apoptotic protein Hrk, in GnRH-induced apoptosis in mature gonadotropes. GnRH increases the transcript levels of Hrk in these cells in a JNK-dependent manner. Collectively, our findings suggest that Bax, Hrk and prohibitin play a role in GnRH-induced cell death in mature gonadotropes.

INVOLVEMENT OF HIF-1α IN GRANULOSA CELL GENE EXPRESSION

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Hypoxia-inducible factor 1α (HIF-1α) is a critical mediator of physiological responses to hypoxia. The active HIF-1α protein initiates transcription of target genes by binding to hypoxia response element (HRE), located in their promoter. Hypoxia plays pivotal roles in tumor growth and angiogenesis through its trans-activation of angiogenic factors. It is also an important physiological cue in the developing corpus luteum (CL), together with LH it orchestrates the robust angiogenic process in this gland. Here we investigated the regulation and the role of HIF-1α in granulosa cells (GC) function using two cell models; primary bovine GC (bGC) and transformed human GC (SVOG).

Hypoxia (reduced oxygen tension) increased VEGF, endothelin-2 and glucose transporter- type1 (SLC21A) in SVOG cells. Similarly, hypoxia-mimicking agent, CoCl2 elevated the mRNA of VEGF and SLC2A1. In bGC CoCl2 also increased the expression of VEGF, endothelin-2 and SLC2A1. Forskolin alone only slightly elevated HIF-1α levels in SVOG cells, but it additively increased its protein levels induced by CoCl2. An analogous effect was observed in bGC incubated with LH and CoCl2; each stimulated HIF-1α protein but there was a synergistic effect when the two treatments were combined. LH was more effective then forskolin in stimulating HIF-1α protein. To further understand the role of HIF-1α we silenced its gene expression with specific small interfering RNA (siRNA). In SVOG, siRNA constructs effectively reduced HIF-1α protein concentrations approximately to 15% of levels present in non transfected cells or cells transfected with negative control siRNA. Along with reduced HIF-1α protein, silenced GC had lower expression of hypoxia induced genes: VEGF, endothelin-2 and SLC2A1.

These results demonstrate that HIF-1α, induced in GC by hypoxia and hormonal treatment, plays a key role in diverse gene expression by mediating their transcriptional activation. These HIF-1α dependent genes may promote angiogenesis and other processes to ensure proper CL development.

IGFALS GENE MUTATIONS IN CHILDREN WITH IDIOPATHIC SHORT STATURE

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Background: The acid-labile subunit (ALS) is a protein that binds to IGF-I and its main role is extension of the half life of IGF-I. To date, only 16 mutations of the *IGFALS* gene were reported in children presenting with short stature. The aim of the present study was to assess whether children with idiopathic short stature (ISS) harbor mutations in the *IGFALS* gene and to characterize the phenotype of the affected patients.

Patients & Methods: 62 children with ISS were enrolled. Serum ALS levels were measured by ELISA and the *IGFALS* gene was sequenced.

Results: A novel mutation of *IGFALS* gene was identified in homozygous state in two siblings of a consanguineous family, c.380T>C mutation in exon 2 resulted in substitution of leucine with proline in position 127 (p.L127P). The proband, 16-y-old was -2.9 SD in height and -4.5 SD in weight. Exaggerated stimulated GH 38 ng/ml, extremely low IGF-I and IGFBP-3 (<25 ng/ml, <500 ng/ml; respectively) and no response to IGF-I generation test indicated GH insensitivity. His sister, 13.8 years old, was -2.8 SD in height and -3.75 SD in weight. She had very low IGF-I and IGFBP-3 (44.5 ng/ml, <500 ng/ml respectively) and exaggerated peak GH 29 ng/ml in response to stimulating test. Low concentration of ALS in both siblings was shown by ELISA (0, 43, respectively; normal range, 122-1020 mIU/l)) and undetectable levels by immunoblast analysis**.** Both had delayed puberty with blunted gonadotrphins response to GnRH. Two previously described sequence changes were identified in 42 subjects.

Conclusions: *IGFALS* gene mutations are not commonly found in children with ISS. Our findings of delayed puberty in two siblings with ALS mutations points to additional effects of IGF-I apart of its known function in growth process and suggest that IGF-I might has an important role in pubertal onset and fertility.

DIFFERENTIAL LOCALIZATION OF ION CHANNELS ENaC AND CFTR IN THE FEMALE REPRODUCTIVE AND RESPIRATORY TRACT EPITHELIA

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A major function of the female reproductive tract (RT) is the transport of the cumulus-oocyte complex to the fallopian tube and from there toward the ampulla where fertilization takes place. This process is mediated by the rhythmic beating of cilia on epithelial surface of the tract. The volume of the periciliary fluid bathing the cilia is determined by the osmolarity of the extracellular and intracellular liquids. As the sodium ion is the major ion that determines volume and osmolarity of extracellular fluids, Epithelial sodium channel ENaC may play a significant role in the RT. The level of Cl- that is the major counter-ion of Na^+ is regulated by the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). Previous studies suggested that in both reproductive and respiratory tracts ENaC and CFTR work in coordination to maintain the osmolarity and hence volume of the luminal fluid. In this study for the first time we examined the tissue distribution and intracellular sites of expression ENaC and CFTR. For this purpose we developed for the first time polyclonal antibodies against the extracellular loop of α-ENaC subunit. Three dimensional (3D) confocal microscopy of immunofluorescence using these antibodies revealed that ENaCs are uniformly distributed on the ciliary surface in all epithelial cells with motile cilia lining the bronchus in the human lung and female RT, all along the fimbria, fallopian tube, the ampulla and rare cells in the uterine glands. In contrast to ENaC, CFTR is located mainly on the apical side but not on cilia. We previously showed that pseudohypoaldosteronism patients with ENaC mutations suffer from recurrent respiratory infections and fail to conceive. These findings reveal the importance of ENaC all along the RT and respiratory tract but contradict previous studies claiming that CFTR influences ENaC function by directly binding to ENaC subunits.