immunoreactive pattern was observed for the 3ß-HSD, 17ß-HSD and CYP19. Moreover, follicular count showed a significant increase in pre-ovulatory follicles in SULP vs CTL group. We did not register differences in the amount of secondary follicles and neither in the CLs between both groups. Our results indicate that, PRL stimulates E2, by favoring steroidogenesis in the pre-ovulatory follicles instead a direct stimulus over the luteal P4 production observed in other rodents. Supported by PIP 110/14, PICT 1281/2014 and Fundación Científica Felipe Fiorellino grants.

0345 - FILAMIN A MODULATES ANTERIOR PITUITARY CELL PROLIFERATION

Jonathan TOLEDO | Pablo Aníbal PÉREZ | Gabriela MOYANO CRESPO | Jorge Humberto MUKDSI | Ana Lucía DE PAUL | Alicia Inés TORRES | Silvina GUTIÉRREZ

CENTRO DE MICROSCOPÍA ELECTRÓNICA-FACULTAD DE CIENCIAS MÉDICAS. INICSA-CONICET

Abstract/Resumen: Recent studies have identified Filamin A (FLNA) as a scaffold protein that plays vital roles in cellular signalling transduction. Mutations in human FLNA gene have been shown to cause developmental defects and aberrant expression has been observed in many tumours. However, its role in pituitary prolactinoma has seldom been discussed. The aim of this work was to analyse the expression levels of FLNA during pituitary tumour development and to determinate the effect on pituitary cell proliferation. To carry out this objective, female rats treated with estradiol benzoate for 20 (20d), 40 (40d) and 60 (60d) days (hyperplasic/adenomatous pituitary model) and transfected GH3 cells for FLNA overexpression (GH3F+) were used. FLNA expression was determined by Western blot (WB) and subcellular localization was visualized by fluorescence and transmission electron microscopy (TEM). Cell cycle progression was analysed by flow cytometry and ki67 index (proliferation marker) by ICQ. Statistical analysis: ANOVA-Tukey. WB analysis showed two FLNA-immunoreactive bands, at ~280 kDa (full-length) and ~90 kDa, (cleaved form). A weak expression of both isoforms was showed in control, with an increase at 40d and a decrease in latter phase (60d). Furthermore, proliferative phase analysis (S-G2/M) by flow cytometry also revealed a peak at initial stages of with a decrease in latter phases. FLNA subcellular distribution was observed to be mainly cytoplasmic in normal pituitary, whereas 40d was found in cytoplasm and also in nucleus with a specific nucleolus mark. The overexpression of FLNA in GH3 cells significantly decreased Ki67 index. Our results suggest an inhibitory role of FLNA in anterior pituitary cell proliferation as part of the mechanisms that limit cell growth. Furthermore, this conclusion is supported by FLNA nucleus and nucleolus localization at late stages of tumour development. We need further studies to elucidate the mechanistic nature of this phenomenon.

0448 - TGFB1 IMPROVE THE OCTREOTIDE INHIBITORY EFFECTS IN FUNCTIONING AND NON-FUNCTIONING PITUITARY TUMOR CELLS

Florencia PICECH(1) | Liliana SOSA(1) | Pablo PEREZ(1) | Gabriela MOYANO CRESPO(1) | Laura CECENARRO(1) | Juan DE BATISTA(2) | Hugo COCCA(2) | Silvina GUTIERREZ(1) | Jorge MUKDSI(1) | Alicia TORRES(1) | **Juan Pablo PETITI** (1)

INICSA CONICET (1); HOSPITAL PRIVADO UNIVERSITARIO DE CÓRDOBA (2)

Abstract/Resumen: Octreotide (OCT), somatostatin analog that binds with high affinity to receptor SSTR2, is widely used to inhibit GH secretion and cell proliferation in GH-secreting adenomas. However, a significant percentage of patients are resistant to OCT. The aim was to investigate if OCT inhibitory effects are modulated by alterations on SSTR2 expression and/or interaction with TGFb1/Smad2/3 pathway. We determined the expression of SSTR2, SSTR5, TbRI and TbRII by IHC and WB in normal pituitaries (n= 6) and in 16 functioning (4 PRL, 10 GH and 2 ACTH) and 9 non-functioning adenomas (NFPA). Ethics Committee (Repis N° 37/2014). GH3 and human NFPA cells, WT and overexpressing SSTR2, were treated for 24 h with the OCT (100 nM) and/or TGFb1 (4 mg/ml). SSTRs and TbRs mRNA and protein expression were analyzed by qPCR and WB, GH and PRL secretion by WB, cell proliferation by BrdU incorporation. In vivo experiments by xenograft model with nude mice. Protocol approved by CICUAL-FCM-UNC. (t-test or one-way ANOVA-Fisher). Pituitary tumors exhibited a markedly decrease in SSTR2, SSTR5 and TbR2 expression compared to normal pituitary gland. We observed that the combination OCT/TGFb1 lead to a significant reduction in GH and PRL secretion levels compared to OCT treatment and the hSSTR2 overexpression sensitized pituitary tumor cells to the anti-secretory effect of OCT. A significant proliferative reduction in GH3 and NFPA human cells was showed after OCT/TGFb1 treatment compared to OCT alone, effects that were potentiated in hSSTR2 overexpressing cells. These responses were associated with a significant decrease of ERK1/2, AKT and Cyclin D1 proteins and an increase of Smad2/3-mediated anti-proliferative cascade. The in vivo assays showed that the cytostatic effect of OCT was improved in presence of TGFb1 after 11d of treatment. Our results demonstrated that OCT inhibitory effects on GH- and PRL-secretion and proliferation were improved in presence of TGFb1. These responses were reinforced in pituitary tumor cells with higher levels of SSTR2.

0565 - ADRENOCORTICAL RESPONSE TO SEPSIS IN INSULIN RESISTANT ANIMALS

Lilian CALDARERI (1) | Morena WISZNIEWSKI(1) | Carolina VECINO(1) | Esteban REPETTO(1) | Susana GONZALEZ(2) | Cora CYMERYNG(1)

CENTRO DE ESTUDIOS FARMACOLÓGICOS Y BOTÁNICOS (CEFYBO-CONICET), FACULTAD DE MEDICINA, UBA (1); IBYME-CONICET; DEPARTAMENTO DE BIOQUÍMICA HUMANA, FACULTAD DE MEDICINA, UBA (2)

Abstract/Resumen: Adrenal insufficiency in patients with insulin resistance (IR) could affect the body's response to stressful situations. Administration of sucrose in the drinking water (SRD), to rodents, constitutes a valid model for human IR as it reproduces many of its characteristics. The aim of the present study was to analyze the HPA axis response in SRD-treated rats subjected to a ligation and cecal puncture procedure (CLP), a very well characterized sepsis model. Adult male Wistar rats were randomly distributed in six groups: Control, SRD, Control-Sham, Control-CLP, SRD-Sham and SRD-CLP. Rats in SRD groups received 30 % sucrose for 15 weeks. IR was determined during the 10th week by an insulin tolerance test. Sham or CLP surgeries were performed during the 15th week. For the following 24 hours before sacrifice, animals were observed and body temperature was measured. Results indicate that surgery induces a significant increase in serum corticosterone levels in all groups ($p<0.0001$). Compared to Control-Sham, corticosterone levels were higher in the C-CLP, SRD-SHAM and SRD-CLP groups ($p<0.01$) while only the SRD-CLP group showed higher circulating ACTH levels ($p<0.01$). Histological examination of H&E stained tissues showed an area of greater vascularization in the adrenal cortex of the SRD-CLP group compared to others. eNOS mRNA levels, as determined by RT-qPCR were higher in the SRD-CLP group compared to others. In summary, regardless of circulating ACTH levels, SRD treatment prevented the CLP-dependent increase in corticosteronemia. Taking into account the key role of glucocorticoids in the stress response, animals presenting an attenuated glucocorticoid output during sepsis may have a worse prognosis.

0803 - OXIDATIVE DAMAGE RESPONSE ACTIVATION AND MITOCHONDRIAL DYNAMICS ALTERATIONS INDUCED BY 17ß-ESTRADIOL IN NORMAL AND TUMORAL PITUITARY CELLS