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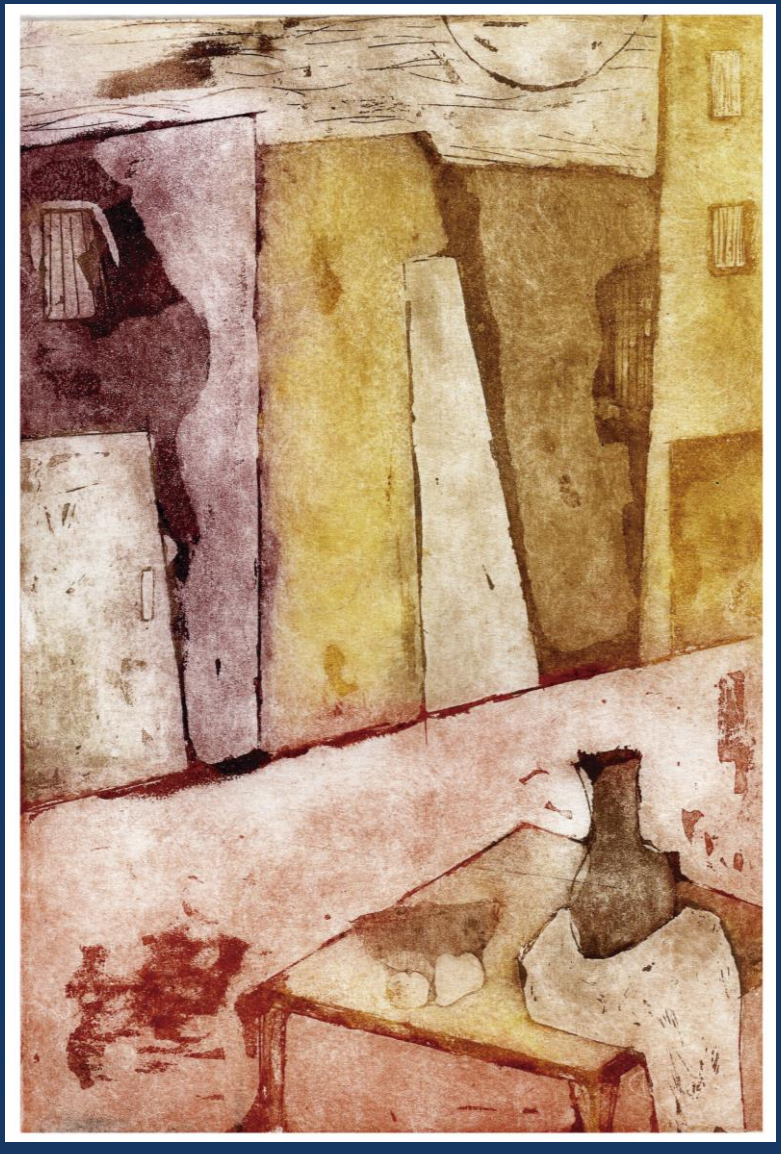

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rhythmically control the proliferation and hormonal secretion of different endocrine cell populations in response to endogenous factors such as Estrogen (E2) acting through estrogen receptors (ER). Also, it has been shown that exogenous agents as phthalates, substances used for plastics manufacture, might act as endocrine disruptors in several tissues, simulating E2 effects. The purpose of this study was to analyze the Di-2-ethylhexyl phthalate (DEHP) effect on pituitary ERa expression and its impact on cell proliferation. We used Wistar female rats prenatally treated with DEHP. At 21 days of postnatal life pituitary glands were extracted and intracellular ERa (ERa) and membrane ERa (mERa) positive cells were quantified by flow cytometry (FC). Also, primary pituitary cell cultures were stimulated with DEHP, E2 or DEHP+E2 for 72 h. The lactotroph and gonadotroph proliferation was quantified by double immunostaining (ki67+PRL or Ki67+bLH). The percentages of ERa+ and mERa+ cells were quantified by FC. ANOVA Fisher ($p<0.05$). Our results showed that DEHP prenatal exposition significantly reduced the cell number that express ERa (DEHP: 73 ± 4.6 vs. control: 86.4 ± 0.5 %) and mERa (DEHP: 5.7 ± 0.8 vs. control: 8.9 ± 0.2 %). In vitro, DEHP decreased ERa+ pituitary cells (DEHP: 79.2 ± 1.1 vs. control: 86 ± 1.2 %) and mERa+ pituitary cells (DEHP: 8.2 ± 1.3 vs. control: 11.5 ± 1.5 %). In lactotrophs, DEHP inhibited the Ki67 expression (DEHP: 21.1 ± 2.2 vs. control: 27.1 ± 2.1 %). Also, DEHP reversed the E2 proliferative effect observed in this cell type (DEHP: 25.4 ± 1.2 vs. 30.7 ± 1.9 %) In gonadotrophs, DEHP inhibited Ki67+cells (DEHP: 20.0 ± 4.2 vs. control: 28 ± 2.5%), without modifying E2 effect. These observations suggest that DEHP impacts on the pituitary gland during the development, downregulates ERa expression and affects the lactotroph and gonadotroph proliferation in adulthood.

0158 - LONG-TERM EFFECTS OF SUCROSE CONSUMPTION ON GLYCEMIA AND RAGE EXPRESSION IN JUVENILE VERSUS ADULT RATS.

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Abstract/Resumen: Excessive consumption of sucrose in early stages of development has deleterious neurobiological and behavioral effects in adulthood. Among these disturbances, difficulties in memory retrieval by early sucrose exposure were previously described. Here, we examined the glycemic levels of the animals and their response to an intraperitoneal glucose overload. We found that sucrose consumption increased the AUC (area under the curve derived from the oral glucose tolerance test) in both young and adults (two-way ANOVA, FTreat(1,34)= 7,658; p= 0.0091. Fisher's LSD post hoc test, p= 0.047 and p= 0.040, respectively) but only consumption during youth maintained this effect in the long term (two-way ANOVA, FTreat(1,34)= 4,445; p= 0.0429. Fisher's LSD post hoc test, p= 0.020). RAGE expression was also assessed by Western blot. Age differences were detected by two-way ANOVA in the two brain areas examined, the mPFC and the vHIP, but only in the mPFC differences by treatment were observed (two-way ANOVA, FTreat-Age(1,20)= 2,327; p= 0.001). Surprisingly, sucrose exposed animals in their youth showed decrease of RAGE while adults raised these values in the mPFC (Fisher's LSD post hoc test, p= 0.037 and p= 0.002, respectively). When all animals were pulled together, there was a negative correlation of the exploration ratio of the memory recognition test and the RAGE levels in the mPFC (F(1,14)= 7,225; p= 0.0434; r^2= 0.59) indicating that higher RAGE values relate with poorer novelty recognition on the final task of the memory test. Similarly, the basal plasma glucose levels also correlated with lower exploration ratio on the final recognition task (F(1,21)= 5,744; p= 0.0264; r^2= 0.223). When basal glycaemia was analyzed together with mPFC RAGE levels, a positive correlation was observed (F(1,18)= 5,693; p= 0.0289; r^2= 0.251). In summary, these results suggest that sucrose induced-hyperglycemia is detrimental for the memory a phenomenon that is related with the RAGE pathway in the mPFC.

0210 - THE KALLIKREIN-KININ SYSTEM IN THE PITUITARY GLAND IS INVOLVED IN LACTOTROPH FUNCTION AND CONTROLLED BY DOPAMINE AND ESTRADIOL

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Abstract/Resumen: TGFß1 is a potent inhibitor of lactotroph cell proliferation and prolactin (PRL) secretion and Tissue Kallikrein (KLK1) was described as an important activator of latent TGFß1 in vivo. The Kallikrein-Kinin System (KKS) is complex; kininogens are cleaved by KLK1, releasing kinins, which exert their effect throw its receptors B1R and B2R. Whereas B2R is constitutively expressed, B1R is inducible in pathological conditions. We have previously found that the pituitary expression of most components of the KKS, as well as local TGFß1 activity, is reduced in prolactinomas. Then we postulate that the recovery of pituitary KKS, could improve local TGFß1 activity counteracting prolactinoma development. To this end, we first deepen the study of the pituitary KKS regulation by dopamine and estradiol and the pituitary cell types expressing kinins receptors. Female mice lacking the dopamine receptor type 2 (Drd2KO, prolactinoma) vs. WT counterpart were used. 1- Double immunofluorescences were performed to assay B2R expression in different pituitary cell-types in WT females. We found that lactotrophs, somatotrophs and gonadotrophs express B2R. 2- Adult females were injected with E2 valerate (0.2 mg/kg, s.c.), cabergoline (DA agonist, 2mg/Kg, i.p.), sulpiride (DA antagonist, 5mg/kg, i.p.) or vehicle (castor oil or saline) and were sacrificed after 3 hours. Pituitary expression of KKS components was evaluated by RTqPCR. We found that E2 exerts a negative regulation of klk1, b2r and b1r expression in WT females, but this control is lost in Drd2KO. On the contrary, DA exerts a positive regulation of klk1 expression but negatively regulates b2r expression in WT females. We conclude that the positive DA-regulation exercised on the pituitary KLK1 expression is lost in the Drd2KO pituitary, reducing KLK1 local activity, reducing TGFß1 activation, and contributing to prolactinoma development. The improvement of pituitary KKS activity could represent a novel treatment for resistant prolactinomas.

0226 - DEREGULATION OF THE PITUITARY SMAD-INDEPENDENT ACTIVIN SIGNALLING PROMOTES PROLACTINOMA DEVELOPMENT.

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Abstract/Resumen: Activins are known inhibitors of lactotroph function. Pit-1 is a pituitary-specific transcription factor that plays an important role in regulating PRL expression. Despite pSMAD2/3 is known as the activins-canonical intracellular signalling, it was described that activin represses Pit-1 expression in lactotroph cells in a Smad-independent mechanism. We have previously demonstrated that decreased pituitary activin expression is involved in prolactinoma development. In the present work we have studied the activin-signalling pathways involved. We used two different animal models of prolactinomas: female mice lacking dopamine type 2 receptor (Drd2-/-) and female mice overexpressing the ß subunit of the human chorionic gonadotrophin (hCGß+). We used wild-type (wt) mice as controls. Despite the reduced activin expression found in prolactinomas vs wt pituitaries, we found unexpectedly increased pSMAD3 expression (Western blot) in

prolactinomas. But nevertheless, by using double immunostaining, we observed that pSMAD3 co-localizes mainly in FSH+ cells, but not in PRL+ cells. Then we focused on the activin alternative pathway involved in prolactinoma development. Our results show that wt female pituitaries present high activin expression concomitant with strong expression of p-p38 in lactotroph population (double IHC). However, activin expression is decreased in prolactinomas concomitant with decreased p-p38 expression in PRL+ cells, increased Pit-1 mRNA expression (q-RT-PCR) and tumor development. This highlights the importance of the activin inhibitory action on lactotroph function and places the activin system and the p38 MAPK pathway as new targets in the treatment of dopamine agonist resistant prolactinomas. Key words: pituitary, prolactinoma, activins, p-p38 pathway

0268 - EFFECTS OF THE CHRONIC TREATMENT WITH L-3,4-DIHYDROXYPHENYLALANINE (L-DOPA) ON THE NEUROENDOCRINE STRESS RESPONSE

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Abstract/Resumen: Stress evokes a complex response mediated by two systems: the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic-adreno-medullar (SAM) axis. Glucocorticoids and catecholamines secreted from the adrenal glands and sympathetic nerves are the main hormone effectors of the physiological adaptations observed during stress. Moreover, prolactin (PRL) is another pituitary hormone secreted under stressful stimuli. Catecholamines are synthesized from the hydroxylated precursor L-Dopa. This agent is also used with therapeutic purposes, e.g. in Parkinson's disease. In the present research, we studied the effects of L-Dopa on the response to a stressor. Adult male Wistar rats (300 g) were chronically treated (24 days) with LEBOCAR (L-Dopa (75 mg/day) - Carbidopa (7.5 mg/day)) orally in drinking water and stressed by immobilization during the last 9 days of treatment. We first explored the activity of the SAM axis. Circulating noradrenaline increased in rats treated with LEBOCAR ($p<0.05$; HPLC), while its content in the adrenals showed no significant alteration. Serum adrenaline (A) levels augmented by LEBOCAR treatment or stress ($p<0.05$; HPLC). Also, the adrenals from stressed animals showed higher content of A ($p<0.05$). Next, we studied the reactivity of the HPA axis. 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) peaked 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 ($p<0.05$). In summary, our results suggest that L-Dopa alters the neuroendocrine stress response by enhancing SAM axis activity and declining HPA axis reactivity and PRL expression.

0298 - SEXUAL DIMORPHISM OF THE KISSPEPTIN AND DOPAMINERGIC SYSTEMS IN THE HYPOTHALAMUS OF THE SOUTH-AMERICAN PLAINS VIZCACHA (L. MAXIMUS).

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Abstract/Resumen: Vizcacha shows the highest sexual dimorphism among rodents. It presents atypical reproductive features as natural poliovulation up to 800 oocytes per estral cycle, inhibition of follicular atresia and ovulation during pregnancy with new corpora lutea formation. Brain dimorphism occurs during the embryogenesis and such process is modulated by the hormonal environment they are exposed, which modifies their structure and neurochemical composition. Within hypothalamic structures, this process affects the anteroventral-periventricular nucleus (AVPV) and the arcuate nucleus (ARC) among others. The aim of this work was to characterize the hypothalamic dimorphic sexual nuclei in the vizcacha focusing on the neurochemistry of cellular composition. Adult non-pregnant female (FM) and male (M) vizcachas were used (n=6/group). The localization of AVPV and ARC was determined by Nissl staining, and estrogen receptor alpha (REalpha), tyroxine hydroxylase (TH) and kisspeptin (Kiss) expression were studied by immunohistochemistry. Sexual dimorphism was observed at macroscopic level, being the brain weight/body weight ratio higher in FM than in M (0.47%, t test, $p<0.05$). In addition, Nissl staining showed a significantly increased AVPV area in FM with a significantly increased number of REalpha, TH and Kiss immunoreactive neurons and immunoreactive cell area ($p<0.05$). In ARC, TH showed similar variations than AVPV, however, Kiss did not show differences between sexes. AVPV of both sexes showed colocalization of REalpha with Kiss and with TH, whereas in ARC, REalpha colocalized with Kiss. Estradiol plays an important function in the establishment of brain sexual dimorphism through the REalpha which plays a key role in the sexual dimorphism maintenance. In consequence, the expression level of REalpha should support TH and Kiss expression in the AVPV nucleus in female vizcachas. The association of REalpha with Kiss and TH would be important as part of the indirect GnRH regulation pathway.

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0311 - PROLACTIN STIMULATES FOLLICULAR STEROIDOGENESIS IN AN INDUCED HYPERPROLACTINAEMIC MODEL IN PLAINS VIZCACHA (LAGOSTOMUS MAXIMUS)

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Abstract/Resumen: Over the past years, our lab has been working with female vizcachas since this autochthonous rodent has atypical reproductive characteristics that position it as an unconventional animal model for endocrine studies. It has been established that prolactin (PRL) plays a key role in the corpus luteum (CL) maintenance and progesterone (P4) production during pregnancy in mice and rats. We have recently developed a model of induced hiperprolactinaemia in vizcachas by treating adult non-pregnant females with sulpiride (a potent inhibitor of D2 and D3 dopaminergic receptors) for 7 consecutive days. To investigate PRL relevance over ovarian performance, we analyzed the ovarian steroidogenic enzymes 3ß-HSD, 17ß-HSD and CYP19, and the ovarian hormone receptors PRL-R, LH-R and FSH-R in hiperprolactinaemic (SULP) and control (CTL) vizcachas. Also, we studied steroid serum levels by ELISA and performed follicular counting in hematoxilin-eosine ovarian stained sections. Circulating P4 levels did not differ between experimental groups. Yet, serum estradiol (E2) was significantly higher in SULP animals ($p<0.05$, n=5). Immunoexpression levels of all PRL-R, LH-R and FSH-R were higher in SULP group, specifically in secondary follicles compared to CLs, and a similar