152 IS GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE AN ADEQUATE NORMALIZER GENE DURING CEREBELLAR DEVELOPMENT?

Conforti RA, Sánchez SI, Fuentes LB. Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. ro.conforti64@gmail.com

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been referred as a "housekeeping" gene by remains constant under changing cellular conditions. Recently, GAPDH has been considered as a pro-apoptotic agent in cerebellar granule cells. Angiotensin II exerts its physiological effects through binding to two receptor subtypes: AT_1 and AT_2 receptors. AT_2 is located in the Purkinje cells membrane and its expression is highly modulated during cerebellum development. In view of the emerging evidence, the aim of this study was to analyze GAPDH mRNA levels in neonatal cerebellum in control and AT_2 receptor antagonist-treated animals. Mini-osmotic pumps with PD123319 (AT_2 antagonist, 1.0 mg/kg/day) or saline solution were implanted in pregnant Wistar rats during the last week of pregnancy. GAPDH expression was evaluated in cerebellum of both groups by RT-PCR at different postnatal ages: P3, P5, P8 and P15. In control animals, GAPDH expression gradually decreased during cerebellar development (P3 vs P5, P8; P5 vs P8, $p<0.001$). In PD123319-treated animals, GAPDH expression decreased significantly at P3 (<0.05) and P5 ($p<0.01$), and then increase at P8 (<0.001) and P15 (<0.05), respect to control. Accordingly, we observed a significantly enlarged external granular layer (EGL) in PD123319-treated P15 pups (base of fissure: <0.001; crown of folia: $p<0.01$). These results suggest that, despite its wide use, GAPDH would not be always an adequate normalizer. In addition, our findings indicate that AT_2 is involved in granule cell apoptosis mediated by GAPDH, probably due to crosstalk between these cells and Purkinje cells in developing cerebellum.

153 DAILY PATTERNS OF APO E ARE MODIFIED BY AN I.C.V. INJECTION OF AMYLOID BETA PEPTIDE IN THE RAT TEMPORAL CORTEX

Coria Lucero C, Delgado SM, Anzulovich AC, Navigatore Fonzo L. IMIBIO-UNSL-CONICET. lorenavigfz@yahoo.com.ar

Alzheimer's disease (AD) is a devastating disease characterized by loss of synaptic and neural cells in the elderly. Accumulation of the β-amyloid peptide (Aβ) in the brain is thought to be central to the pathogenesis of AD. ApoE plays a key role in normal and physiological clearance of Aß, since it facilitates the peptide intra- and extracellular proteolytic degradation. Besides the cognitive deficit, AD patients also show alterations in their circadian rhythms. The objective of this study was to investigate the effects of an i.c.v. injection of Aβ (1-42) peptide on the 24h rhythms of Apo E, BMAL1, RORa and Aβ in the rat temporal cortex. Four-month-old males Holtzman rats were used in this study. Groups were defined as: control (CO) and Aβ-injected (Aβ). Rats were maintained under 12h-light:12h-dark conditions before the sacrifice. Apo E, BMAL, RORa, and Aβ protein were analyzed by immunoblotting, in temporal cortex samples isolated every 6 h throughout a 24h period. We found that i.c.v. injection of Aβ (1-42) phase shifted ApoE, BMAL 1, RORa and Aβ daily rhythms in the rat cortex. These findings might constitute, at least in part, molecular and biochemical basis of altered daily beta clearance in AD.

154 MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF TWO TREMATODE WORMS IN THE GASTROPOD *Asolene pulchella* (Anton, 1859)

Dellagnola FA[1], Montes MM[2], Sandri MB[1], Martorelli SR[2], Vega IA[1].1Instituto de Histología y Embriología (IHEM – CCT CONICET Mendoza). Universidad Nacional de Cuyo. 2Centro de Estudios Parasitológicos y Vectores (CCT CONICET La Plata). Universidad Nacional de La Plata. dellagnolaf@gmail.com

Asolene pulchella (Anton, 1859) *Asolene pulchella* (Caenogastropoda, Ampullariidae) is a native gastropod from La Plata River. In a previous study we reported the presence of parasite forms of Trematoda (Plathyhelminthes) in the digestive gland of adult snails from Regatas Lake (Buenos Aires). In this work identified two parasites with a morphological and molecular approach. The digestive gland of A. pulchella showed a large amount of larval forms (sporocysts and rediae) with internal cercariae in diverse degree of development. These parasites are placed in the connective tissue and the hemocoelic spaces of the digestive gland. Morphological features of sporocysts and

rediae (obtained from digestive gland homogenates), and cercariae (obtained by isolation of host under constant light) showed two morphotypes of parasites. The first morphotype shared morphological characters with species of the Plagiorchiida order (xiphidiocercariae armatae opisthioglyphe-type) while the second morphotype showed characters of the Echinostomatidae family (belonging Echinostomida order). A sequence corresponding to the ITS1 (an internal transcribed spacer located between RNA 18S and RNA 5.8S ribosomal genes) was amplified (PCR) using DNA extracted from digestive gland as template. Sequencing and phylogenetic reconstruction by maximum likelihood were made. Two sequences were found: one of them (~900 bp) was akin to the Xiphidiata order, while the other (~700 bp) was related to Echinostomatidae family. The latter taxon has parasitic, symbiotic relationships with prosobranch snails. Although the morphological and molecular findings were congruent, other molecular studies are being conducted for the identification of these parasites at species level.

155 AGING ABOLISHES CIRCADIAN RHYTHMS OF RORα AND ANTIOXIDANT ENZYMES EXPRESSION IN TEMPORAL CORTEX OF FREE RUNNING RATS

Deyurka N, Navigatore Fonzo LS, Coria C, Lacoste MG, Anzulovich AC. Laboratorio de Cronobiología, IMIBIO-SL, CONICET, UNSL, FQBF, UNSL ndeyurka@gmail.com

RORα is a transcription factor that binds RORE motifs in the promoter of clock Bmal1 and other target genes. Antioxidant enzymes contribute to the cellular redox state which is crucial for the molecular clock function. Previously, we showed antioxidant enzymes activity follow a daily variation in temporal cortex (TC), which was abolished in aged rats. Herein we aimed: 1) to investigate endogenous rhythms of Cat, Gpx and Nrf2 genes expression and RORα protein levels in rat TC, and 2) to evaluate whether aging could affect those temporal patterns. Three- and 22-month-old male Holtzman rats were maintained under constant darkness for 15 days before the experiment, in order to validate the endogenous nature of circadian rhythms. TC samples were isolated every 4 h during a 24h period. RORα protein levels were assessed by immunoblotting. Cat, Gpx and Nrf2 mRNA levels were determined by RT-PCR. Specific softwares were used to circadian analysis. We observed circadian endogenous rhythms of RORα, Cat and Gpx expression in the TC of young free running rats (Chronos fit, $p<0.05$, $p< 0.001$ and $p<0.05$, respectively). We found Ebox and RORE sites in the Cat and Gpx genes regulatory regions. RORα rhythm's acrophase occurs at the beginning of the subjective day, preceding Cat and Gpx mRNA peaks. Consistent with previous results, aging abolishes RORα, Cat, and Gpx circadian rhythms in TC. Interestingly, Nrf2 gene expression becomes rhythmic in the TC of aged rats. Our observations would contribute to the knowledge of circadian alterations in TC of the aged brain.

156 MORPHOFUNCTIONAL ANALYSIS OF CREB HETEROGENEITY IN THE RAT PINEAL GLAND

Farias Altamirano LE, Muñoz EM. IHEM, UNCuyo, CONICET. munoz.estela@fcm.uncu.edu.ar

Adaptation to environmental changes is facilitated by the endogenous circadian clock. The pineal gland (PG) via the nocturnal melatonin production is a key effector and regulator of the mammalian circadian timing system. The PG is under sympathetic regulation by local norepinephrine (NE) release from the conary nerves at night. In rat, the NE-dependent phosphorylation of the transcription factor CREB initiates the expression of the aa-nat gene, which encodes for one of the pivotal enzymes in the melatonin synthesis. To challenge the well-accepted concept of pineal homogeneity and to determine if a spatiotemporal dynamism of CREB occurs within the PG, we analyzed and quantified the protein levels at different ZTs (Zeitgeber time; L:D 12:12) in adult naive, ganglionectomized (GCSx) and sham-operated rats. We performed immunohistochemistry (IHC) in PG sections followed by confocal microscopy, and morphometric and statistical analyses. CREB was found in pinealocyte nuclei at ZT6, 10, 14, 18 and 22. Immunoreactive granules of variable size and different nuclear distribution patterns were observed. The fluorescence intensity of CREB varied among the ZTs, with higher values during the night-time (ZT14 and ZT18). Although the nuclear area was significantly higher at ZT14, the relative area occupied by CREB within the pinealocyte nuclei increased at night. The disruption of the circadian circuit by GCSx reduced both the abundance and the area occupied by CREB at ZT14. These findings suggest a NE effect over CREB availability and distribution in the pinealocyte nuclei and therefore over its transcriptional capacity.