ABSTRACTS

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13. Systems Pharmacology

13.001 Is body weight gain under regulation of circadian melatonin? Cruz-Machado SS[1], Pereira EP[1], Oliveira AP[2], Silva-Souza E[1], Markus RP[1] [1]IB-USP – Departamento de Fisiologia, [2]UFPI – Biologia e Fisiologia

Introduction: Melatonin, classically known as the 'chemical signal of darkness', orchestrates rhythmic physiological functions and has exquisite roles in the regulation of immune responses. This indolamine is highly relevant for the timing of inflammation, as the activation of the immune-pineal axis transiently switches melatonin synthesis from pinealocytes to activated immune cells (Jockers et al., Br J Pharmacol, 2016). In chronic diseases, such as obesity and diabetes, inflammation is linked to a long-lasting reduction of melatonin levels. Acute high-fat diet feeding (HFD) promotes inflammatory signals and blocks the nocturnal rise in circulating melatonin since the first meal (da Silveira Cruz-Machado et al., Annals of the 49th SBFTE, 2016). Because activation of pineal microglia regulates melatonin production (da Silveira Cruz-Machado, PloS One, 2012) we evaluated whether blocking microglia activation inhibits HFD effect. We also approached whether melatonin suppression leads to chronodisruption of hormones linked to feeding and inflammatory responses, as well as the relevance of melatonin reposition. **Methods:** Rats kept under light/dark cycles were fed *ad libitum* by low (4.5% kcal from fat) or HFD (42% kcal from fat). Rats were euthanized every 3 hours along 24 hours after 7 days of diets. Body weight and food intake were followed daily. Plasma level of hormones and cytokines were measure by Milliplex® immunoassay. Institutional ethical committee (CEUA/IB 198/2014) approved this project. **Results:** Reduction of plasma melatonin as well as hyperphagia was abolished by blocking microglia activation (minocycline, 100 mg in 5 ml, i.c.v.). Circadian variation of glucose, glucagon, corticosterone, GM-CSF and MCP1 levels was not altered by HFD. Leptin and insulin maximal amplitude was significantly increased, whilst melatonin was significantly reduced. HFD induced a 27-fold increase of melatonin levels in the liver but not in the spleen or adipose tissue, suggesting a selective extra-pineal site of melatonin production. Imaging flow cytometry showed that hepatocytes express MT2 melatonin receptor. Blocking endogenous signaling of melatonin by DH97 (170 ng/mL, drinking water), a selective competitive antagonist of MT2 receptor, increased liver steatosis. In addition, significant increase in glucose, food intake and body weight was observed in DH97-treated animals fed under HFD. DH97 also disrupted the levels of circulating IL4 and IL12p70 cytokines. Reposition of melatonin in the nocturnal drinking water (25 ng/mL) reversed weight gain induced by HFD without changing leptin or insulin plasma levels. Interestingly there were also no changes in hyperphagia. Therefore, when animals are fed to HFD and keep melatonin signaling they eat the same amount of food of animals receiving vehicle, but do not gain weight. **Conclusion:** Here we highlight, for the first time, a network of interaction played by melatonin to keep the biological organization of metabolism. Our data shows that endogenous melatonin, acting via MT2 receptors, plays a protective role in the regulation of body weight and inflammation induced by HFD overfeeding. The above findings uncover novel roles for melatonergic signaling in energy metabolism and provide new insights into the interactions between circadian regulation of metabolism and immune responses. **Financial Support**: FAPESP, CNPq and CAPES.

13.002 Early-life stress promotes alterations in intestinal permeability and hippocampal 5-HT $_{1A}$ mRNA expression in juvenile rats. Bravo JA[1], Astudillo-Guerrero C[1], Rossi-Vargas G[1], Escobar-Luna J[1], Barrera-Bugueño C[1], Gotteland M[2], Julio-Pieper M[1] [1]Pontificia Universidad Católica De Valparaíso – NeuroGastroBioquímica, [2]Universidad de Chile – Nutrición

Introduction: Early-life stress, such as maternal separation (MS) in rodents has been used to model stress-related psychiatric disorders and alterations in bowel function, however most reports focus on the effects seen at adulthood. Methodology: We evaluated the effect of MS (3h / day from post-natal day (PND) 2 to PND12) on colon barrier function in male Sprague-Dawley rats at PND21 and PND35, and compared them to non-separated controls. Additionally, through *in situ* hybridization we Evaluated topographical changes in hippocampal 5-HT$_{1A}$ mRNA expression, which is the marker of stress-related psychiatric disorders. To evaluate colin permeabilty, FITC-conjugated 4.4kDa dextran (FD4.4) was applied on the mucosal side, and then measuring fluorescence in the serosal side for up to 3 hours, while transepithelial electrical resistance (TEER) was determined using Ussing chamber studies. All animal studies were approved by Pontificia Universidad Católica de Valparaíso's Bioethical Committee. Results and Discussion: There was no difference in permeability to FD4.4 and TEER between MS and NS at PND21. However, at PND35 there was an increase in permeability to FD4.4 in MS rats when compared to NS, while no differences in TEER where found between both groups. Additionally, when MS rats at PND35 were subjected to 5min swim stress, they showed a blunted corticosterone response in comparison to swim-stressed NS rats. Furthermore, FD4.4 permeability in swim-stressed MS rats was lower than non-swim stressed MS rats. In addition, hippocampal 5-HT$_{1A}$ mRNA expression was higher in both layers of the dentate gyrus and cornus ammonis 1 and 3 of MS rats in comparison to their controls at PND21 and PND35. These data show that early-life stress affects gut permeability to macromolecules at PND35, and that this phenomenon is sensitive to corticosterone. Moreover, as opposed to what has been reported in adulthood, early-life stress affects hippocampal 5-HT$_{1A}$ mRNA expression in young animals and is observed at early PND21, suggesting an alteration in the brain-gut axis of young animals that might contribute to the development of stress-related psychiatric disorders later in life. Approved by the University's Bioethical committee. Funded by: FONDECYT # 1140776