ABSTRACTS

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01. Cellular and Molecular Pharmacology

01.001 Angiotensin II induces extracellular vesicle production in vascular smooth muscle cells. Frony AC[1], Renovato-Martins M[2], Moraes JA[3], Barja-Fidalgo C[1]
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Atherosclerosis is the major cardiovascular disease (CVD) and it is characterized by vessel hardening due to the formation of the atherosclerotic plaque. In this scenario, vascular smooth muscle cells (VSMC) play a major role during atherogenesis, adopting a proliferative and migratory role. Among the atherogenic mediators highlight angiotensin II (Ang II) which is generated through the activation of the renin-angiotensin system and plays a role in different CVD. In addition, in these diseases there is an increase of membrane fragments release, called Extracellular Vesicles (EV). Furthermore, the EV derived from VSMC represents an expressive number of vesicles in human atheroma plaques, however their participation in CVD is not yet known. In this work, we evaluated the pathways involved in EV generation from VSMC stimulated with Ang II, as well as their content and their effects on vascular cells. A7r5 (VSMC) were incubated with Ang II (100 nM) for 1h and the supernatants were collected and submitted to an ultracentrifugation for EV purification. Calcium release was measured by Fura-2 probe. Cell migration was performed in Boyden chamber and cell proliferation was assessed by MTT assay. ROS production was analyzed by DCF or lucigenin probes. Cell invasion was measured in transwell gelatin 1% -coated membrane. We have shown that Ang II was able to induce a redox-dependent calcium release in VSMC. This effect was accompanied by EV generation, which was dependent of ROCK / MLC pathway. Interestingly, these EV presented the characteristic markers of lipid raft (caveolin and flotilin), $p47^{phox}$ (subunit of NOX2) and MMP9. Our results suggest that the EV of Ang II-activated VSMC could influence the effects of Ang II, once the pre-treatment with the vesicles was able to decrease the rate of migration and proliferation of VSMC treated with Ang II. We can suggest that this EV effect occurs in a redox sensitive manner, since these vesicles reduced Ang II-induced intracellular ROS production. In addition, we observed that these EV transfer MMP9 to non-treated VSMC, which could be responsible for the increased invasion of these cells in gelatin. Furthermore, we have also observed that EV of Ang II-activated VSMC were also able to induce the production of extracellular ROS in non-treated VSMC, in a NOX2 dependent manner, which can be explained by $p47^{phox}$ presence in these EV. Finally, we observed that EV of Ang II-activated VSMC were also able to affect endothelial cells, once we observed that they were able to increase the endothelial permeability. Our data indicate that EV derived from activated VSMC can directly affect vascular cells which could be correlated with plaque instability and would worsen the prognosis of atherosclerosis. Together, this new approach could turn possible the development of better strategies for the treatment of atherosclerosis. **Funding support:** CAPES, CNPq, FAPERJ

01.002 H_2S is a key antisecretory molecule against cholera toxin-induced diarrhoea in mice: evidence for non-involvement of the AC/CAMP/PKA pathway and AMPK. Sousa FBM[1], Souza LKM[1], Araújo TSL[1], Sousa NA[1], Araújo S[2], Silva RO[3], Nicolau LAD[3], Souza FM[1], Silva IS[4], Souza MHLP[3], Medeiros JVR[2] [1]Renorbio – Farmacologia, [2]UFPI – Farmacologia, [3]UFC – Farmacologia e Fisiologia, [4]UFMG – Farmacologia e Fisiologia

Introduction: H_2S is a gasotransmitter that participates in various physiological and pathophysiological processes within the gastrointestinal tract. We studied the effects and possible mechanism of action of H_2S in secretory diarrhoea caused by cholera toxin (CT). **Methods:** Swiss mice were pretreated p.o with L-cysteine 10 or 50 mg/kg alone or with DL-propargylglycine (PAG 100 mg/kg), NaHS 3 or 27µmol/kg, or Lawesson's regent 3 or 27µmol/kg. After 30 min was induced of the diarrhea for CT (1µg) in isolated intestinal loop. After 4 h, the loops were removed to evaluate the fluid secretion, determination of Cl^-, H_2S levels and intestinal expression of CSE. The animals also were pretreated with PAG p.o followed by CT (0.5 µg/loop). The effect of H_2S in the intestinal absorption was also evaluated. To identify the participation of the AC/cAMP/PKA, mice were pretreated with PAG, p.o. and the loops received SQ22536 0.01M (inhibitor AC), Bupivacaine 100µM (inhibitor of cAMP production) or KT5720 1µg (PKA inhibitor) before administration of CT. We also tested Forskolin (20 µM/loop), an AC activator, followed of the intraluminal administration of the NaHS or Lawesson's reagent (both 27 µM) and CT. To evaluate the role of AMPK the loops were treated with an activator or a specific inhibitor of AMPK: AICAR 1mM and Dorsomorphine 30µM, respectively. **Results**: CT shows marked in intestinal fluid accumulation (0.16±0.01g/cm). The pretreated with L-cysteine and H_2S donors shows a reduction significant with maximal inhibitory effects observed at dose: L-cysteine 50mg/kg (0.05±0.01g/cm), NaHS 27µmol/kg (0.05±0.01g/cm) and Lawesson's regent 27µmol/kg (0.05±0.01g/cm). These doses were chosen for subsequent studies. Pretreatment with the PAG reversed the effect of L-cysteine and caused severe intestinal secretion (0.12±0.02g/cm). L-cysteine (57.3±11.4 mEq/L), NaHS (41.5±15.1 mEq/L) and Lawesson's regent (63.2±20.1 mEq/L) shows a reduction significant the levels of Cl^- as compared to CT (156.5±14.5 mEq/L). Co-treatment with PAG and CT (0.5 µg) increased intestinal fluid secretion. Corroborating with these results CT also increased the expression of CSE and the production of H_2S. L-cysteine (56±8%), NaHS (39±13%) and Lawesson's regent (41±7%) did not affect intestinal fluid absorption as compared to glucose (83±7%). SQ-22536 (0.08±0.01g/cm), bupivacaine (0.08±0.02g/cm), KT5720 (0.07±0.01g/cm) and AICAR (0.07±0.02g/cm) decreased the intestinal fluid accumulation, but the pretreated with PAG did not reverse the effect of these. The treatment with Forskolin also does not reverse the effects of the H_2S donors. Co-treatment with either NaHS or Lawesson's reagent and Dorsomorphin did not reverse the effect of the H_2S donors. **Conclusions**: H_2S has antisecretory activity and is an essential molecule for protection against the intestinal secretion induced by CT. This effect occurs possibly by a different AC/cAMP/PKA pathway and AMPK. However, more studies are needed to elucidate the possible mechanism of action. **Support:** CNPq. CEP UFPI: Protocol Nº 79/2015

01.003 Antinociceptive effect of imidazole alkaloids in inflammatory nociception: Role of cyclooxygenase. Rocha TM[1], Silva MGV[1], Moreira R[2], Guedes RC[2], Sousa JAC[1], Leite JRSA[3], Lima DF[4], Leal LKAM[1] [1]UFC, [2]Universidade de Lisboa, [3]UnB, [4]UNIVASF

Epiisopiloturine (EPIT) and epiisopilosine (EPIL) are imidazole alkaloids found in the leaves of *Pilocarpus microphyllus*, a species that has high importance for pharmaceutical industry of the northeast in Brazil. Preliminary studies with epiisopiloturine have demonstrated antinociceptive and anti-inflammatory activity in mice and human neutrophils. In the present study, we investigated further the antinociceptive effect of the alkaloids epiisopiloturine and epiisopilosine in acute inflammatory nociception animal models, evaluating the role of cyclooxygenase. The alkaloids EPIT (99.7% purity) and EPIL (≥ 90% purity) were obtained from waste produced by pilocarpine extraction from *P. microphyllus* leaves. Male Swiss mice (25−30 g) were pretreated with EPIT or EPIL (0.3 and 1 mg/kg, i.p.); indomethacin (INDO) (20 mg/Kg i.p.), non-selective COX inhibitor, and vehicle - 1% DMSO (i.p.) 30 minutes before to administration of the carrageenan (300 µg/50 µL of saline) in the right hind paw of each mouse. The subplantar tissue was harvested 3h after the intraplantar injection of inflammatory stimuli. Immunohistochemical analysis of COX-2 expression was performed using the streptavidin-biotin-peroxidase method. The sections were incubated with rabbit primary antibody (anti-COX-2). Molecular modeling studies were performed with MOE 2014.1 (Canada). The crystallographic structures of COX-1 and COX-2 were obtained from the RCSB Protein Data Bank (PDB) (PDB code: 1HT5 and 1CX2, respectively). A structure-based virtual screening based on molecular docking was performed with GOLD 5.1.0 software (UK), using the default options for virtual screening and ChemPLP was selected as the fitness function. The binding affinity of each compound was established through binding scores and PLP energy (piecewise linear potential), an approximation of Gibbs free energy. The pre-treatment of the animals with EPIT and EPIL (0.3 and 1 mg/kg, i.p.) decreased after the injection of carrageenan (Cg) when compared to the Cg group (control group). In the counting of COX-2 immunoreactive cells by field, a significant reduction in the number of immunolabelled cells was observed in the EPIT (1 mg/kg), EPIL (0.3 mg/kg) and indomethacin (20 mg/kg) when compared to the carrageenan group, which correspond to inhibitions of 88.6; 60.9 and 78.3%, respectively. The docking into the PDB-entries 1HT5 and 1CX2 was successfully validated. The virtual screening procedure for COX-1 with EPIL and EPIT showed values of higher scores and PLP energy (PLP: -56.16, -67.89, -67.64 and -49.45, -49, 17; -47.17, respectively) for the amino acid residues tested when compared to the reference compound, indomethacin (PLP: -48.08; -54.26 and -49.02). The results for COX-2 generated even more promising results, scores for EPIL and EPIT (PLP: -77.86; -68.08 and -64.58; -62.08, respectively) were also higher than indomethacin (PLP: -55.82 and -56.01), in which the docking was performed under the same conditions. Thus, these results indicated that the antinociceptive effect of EPIT and EPIL seems to be associated not only with the reduction of COX-2 expression but also with direct interaction with the cyclooxygenase isoforms. **Financial support**: CNPq. Approved by the Ethics Committee in Research of the Federal University of Ceara (protocol no. 62/2014).

01.004 Putative alpha$_{1d}$ / alpha$_{1a}$-Adrenoceptors heterodimerization in the abdominal aorta from preeclamptic rats. Silva KP[1], Kiguti LRA[1], Lim L[2], Kocan M[2], Summers RJ[2], Caldeira-Dias M[1], Sandrim V[1], Pupo AS[1] [1]IBB-Unesp – Farmacologia, [2]Monash University – Drug Discovery Biology

Introduction: The alpha$_1$-adrenoceptors (alpha$_1$-ARs) play an essential role in the regulation of arterial pressure. Dimerization of GPCRs (Gwo-Ching S., *et al*., BJP, 172, 2507, 2015) and/or receptor up-regulation (Oliver E., *et al*., JPET., 328, 982, 2009) contribute to the development and maintenance of hypertension. However, the involvement of alpha$_1$-ARs subtypes in preeclampsia, a gestational hypertensive syndrome, is not fully understood. **Methods**: Female Wistar rats (90-120 days old) were divided into 3 groups: Control (virgins rats), Sham (pregnant rats) and RUPP (pregnant rats that underwent a surgical procedure to reduce uterine perfusion pressure, mimicking the gestational hypertensive syndrome in humans). Blood pressure was monitored and rings (0.5cm length) of abdominal aorta were isolated to in vitro record of contractions to A61603 (alpha$_{1A}$-AR selective agonist) or Buspirone (alpha$_{1D}$-AR selective agonist) in the absence and presence of RS100329 (alpha$_{1A}$-AR selective antagonist) or BMY7378 (alpha$_{1D}$-AR selective antagonist). Agonist (pD$_2$) and antagonist potencies (pA$_2$) were determined. alpha$_{1A}$-, alpha$_B$- and alpha$_{1D}$-AR mRNA expression was analyzed by *RT-qPCR.* Interaction between the human Rluc8-alpha$_{1A}$ and eGFP-alpha$_{1D}$-ARs was investigated in BRET assays in HEK293 cells. Maximal BRET transfer (BRETmax) and the Rluc8/eGFP ratio at with half-maximal BRET is attained (BRET50) were determined. **Results**: Mean arterial pressure was 110±1 mmHg, 96±2 mmHg and 120±1 mmHg in Control, Sham and RUPP, respectively (n= 18-21). The pD$_2$ values for A61603 were: 7.7±0.1 (Control, n=5); 7.0±0.1 (Sham, n=6); 7.2±0.1 (RUPP, n=7). Contractions of the abdominal aorta of Control and Sham rats to A61603 were antagonized by RS100329 (Control: pA$_2$=9.5±0.04; Sham: pA$_2$=9.3±0.1; n=5-7) but not by BMY7378. Nonetheless, both RS100329 and BMY 7378 rightward shifted the A61603 curves in aorta from RUPP. The pD$_2$ values for Buspirone were: 6.7±0.04 (Control, n=4), 6.6±0.06 (Sham;n=6) and 8.0±0.1; (RUPP; n=5). Contractions of aorta from Control and Sham to Buspirone were antagonized by BMY7378 (Control: pA$_2$=8.6±0.04; Sham: pA$_2$=8.7±0.1; n=4-7) but not by RS100329. On the other hand, subnamolar pA$_2$ values for BMY 7378 (pA$_2$=9.8±0.01; n=5) and RS 100329 (pA$_2$=9.6±0.01; n=5) were estimated in the RUPP. Expression of alpha$_{1A}$-AR and alpha$_{1B}$-AR mRNA was not different between the three experimental groups, but mRNA encoding for alpha$_{1D}$-AR was significantly upregulated in aorta from RUPP. Co-expression of Rluc8-alpha$_{1A}$/eGFP-alpha$_{1D}$ ARs resulted in saturable hyperbolic BRET curves consistent with alpha$_{1A}$/alpha$_{1D}$ oligomerization (BRETmax 0.086±0.011; BRET50: 0.328±0.046, n=3). **Conclusion**: Heterogeneous population of alpha$_{1A}$- and alpha$_{1D}$-ARs coexists in aortae from female rats. Additionally, a complex interaction between alpha$_{1A}$/alpha$_{1D}$ was observed in aorta from RUPP and this could be explained by alpha$_{1A}$/alpha$_{1D}$ heterodimerization, as shown in recombinant system. A putative native alpha$_{1A}$/alpha$_{1D}$ heterodimerization and its contribution to the pathophysiology of preeclampsia would give important insights on the role of heterodimerization in the health and disease. **Financial support**: CAPES and FAPESP Local Ethics Committee for the Use of Experimental Animals #634

01.005 Melanoma-derived microvesicles switch neutrophils phenotype in vitro
Guimarães-Bastos D, Frony AC, Saldanha-Gama R, Barja-Fidalgo C, Moraes JA UERJ

The understanding on the mechanisms involved in tumor growth runs through the acknowledgement on its microenvironment, formed by extracellular matrix components, growth factors, cytokines and different cell types. Immune cells present in tumor microenvironment have their pro-inflammatory functions modified to support tumor growth. Recent studies have provided evidence on two phenotypes of tumor-associated neutrophils (TAN): a protumoral, TAN-N2 or an antitumoral, TAN-N1. Evidence also show that tumor microenvironment is rich in membrane-derived microvesicles (MV), which carry molecular information from their cellular origin and are capable of modulating activity of target cells. Although tumor-derived MV was shown to interact with different cells in the microenvironment, so far there are no reports on its contribution to modulate TAN. We investigated whether MV produced by a human melanoma cell line would modulate human neutrophils activity, shifting them to a N2-like phenotype in vitro. MV were obtained from conditioned media of human melanoma cell line MV3 cultures and quantified by annexin-V staining and Nanoparticle Tracking Analysis. Human neutrophils (PMN), isolated by Percoll gradient, were incubated with MV (10% v/v) or LPS (10 µg/mL) at different times. Chemotaxis was assayed in modified Boyden Chamber. Apoptosis was assessed through cytometry and morphological analyses. Neutrophil extracellular traps (NETs) formation was evaluated by DNA quantification, immunofluorescence and elastase activity. Intracellular ROS, NO and peroxynitrite production were measured through the oxidation of DCF, DAF and HPF probes, respectively. Viability of MV3 cells co-cultured with PMN was assessed by MTT assays. Gene expression analyses were obtained by qRT-PCR. CXCR4 and arginase protein contents were obtained by cytometry and immunoblotting, respectively. MV induced PMN chemotaxis, and this effect was inhibited by pertussis toxin and is dependent on PI3K-AKT pathway activation. We also observed that MV effect on PMN chemotaxis relies on CXCR2 pathway, once its inhibitor, SB225002, abrogated PMN migration. MV delayed PMN spontaneous apoptosis, triggered NETs formation and induced intracellular ROS production. Interestingly, NET induced by MV showed elastase activity in a different manner than PMN incubated with LPS, which induced cytotoxic NETs. MV decreased NO basal production, and increased CXCR4 and arginase expression and also augmented the mRNA content of several TAN-N2 molecular markers, such as arginase, CXCR4 and VEGF in PMN. On the other hand, PMN incubated with LPS expressed TAN-N1 markers. Finally, PMN challenged with MV did not affect melanoma cells viability in co-culture, while PMN challenged with LPS decreased melanoma viability by peroxynitrite generation. Our data indicate that melanoma-derived MV may have an important role on shaping the phenotype of PMN in tumor microenvironments towards a TAN-N2 phenotype, which shows an increased expression of pro-tumor molecular markers and do not present cytotoxic properties.

01.006 Zymosan promotes IL-1β processing through NLRP3/ASC/Capsase-1 inflammasome independently of phagocytosis. Silva RL, Lopes AHP, Fonseca MD, Colon D, Saraiva ALL, Zamboni SD, Cunha FQ, Cunha TM FMRP-USP

Zymosan (ZY) is an insoluble preparation of cell wall from *Saccharomyces cerevisiae*. It is widely used to study the innate immune system and to induce the inflammatory response in pre-clinical studies. Although ZY induces production of several cytokines by the innate immune system, the mechanisms by which ZY induces IL-1β are still unknown. The production of mature IL-1β depends on two stages: 1) the production of pro-IL-1β, which is triggered by PRRs activation, 2) and IL-1β maturation, which is dependent on inflammasome activation (an intracellular multiprotein complex). Here, we show the PRRs and inflammasome complex as well as molecular mechanisms by which ZY promotes IL-1β production and maturation in macrophages. **Methods:** Peritoneal naive macrophages (MΦ) were harvested from C57BL/6 wide type (WT), TLR2$^{-/-}$, MyD88$^{-/-}$, Dectin-1$^{-/-}$, NLRC4$^{-/-}$, NLRP3$^{-/-}$, ASC$^{-/-}$, Caspase-1$^{-/-}$, Pannexin-1$^{-/-}$ and P2X7$^{-/-}$ mice cultured at 5% CO_2, 37 °C, and incubated with ZY (10-100 µg/mL) from 6 to 12 hours. MΦs from WT mice were pre-incubated with KCl, glyburide (potassium channel inhibitor), cytochalasin D or latrunculin (phagocytosis inhibitor), acetylcysteine (scavenger total ROS), MnTMP (inhibitor of mitochondrial ROS). Culture MΦ supernatants were used to measure the levels of IL-1β and caspase-1 by Western blot or IL-1β and TNF by ELISA. IL-1β mRNA levels were quantified by PCR-RT. Mitochondrial (mROS) and total ROS (tROS) were measured using fluorescent probes MitoSox and CM-H2DCFDA, respectively. ATP levels were measured by ATPlite Luminescence Assay. This study was approved by Local Ethical Commission in Animal Research: Protocol n° 055/2016. **Results:** ZY promotes the release of active IL-1β from WT MΦ in a concentration-dependent manner. The production of IL-1β and TNF induced by ZY was reduced in MΦ from TLR2$^{-/-}$, MyD88$^{-/-}$ and Dectin-1$^{-/-}$. In contrast, IL-1β release induced by ZY was impaired in MΦ obtained from NLRP3$^{-/-}$, ASC$^{-/-}$ and caspase-1$^{-/-}$. On the other hand, TNF levels released by MΦ obtained from all inflammasome knockout mice and WT did not diverge. The absence of NLRC4 (NLRC4$^{-/-}$) in MΦ does not change levels of IL-1β or TNF compared to WT. ZY-induced IL-1β release by WT MΦ was inhibited by latrunculin or cytochalasin D. ZY induces the release of ATP in a time-dependent manner and MΦ from P2X7$^{-/-}$ mice has impaired release of IL-1β. Likewise, pre-incubation of MΦ from WT with KCl or glyburide prevents the release of IL-1β, but not TNF. Additionally, ZY induces production of mROS and tROS, which can be reduced by MnTMP and acetylcysteine, respectively. However, the reduction only in tROS decreases IL-1β releasing, without the change in TNF levels. The concentration of IL-1β released, but not TNF, was partially reduced in MΦ obtained from pannexin-1$^{-/-}$ compared from MΦ WT. **Conclusions**: ZY induces production of IL-1β by activation of the Dectin-1/TLR2/MyD88 pathway. Nonetheless, ZY promotes the maturation/release of IL-1β through a mechanism dependent of potassium efflux, production of tROS, P2X7 channel, pannexin-1 leading to NLRP3/ASC/caspase-1 inflammasome activation, independently of phagocytosis. Supported by: CAPES, CNPq, FAPESP.

01.007 PPARg blockage do not restore insulin sensitivity in pioglitazone treated adiponectin knockout mice possibly by a miR-222 dependent mechanism.
Mendonca M[1], Paixão AO[1], Sousa E[1], Rodrigues AC[1] [1]ICB-USP – Farmacologia

Introduction: Pioglitazone(PIO), an agonist of peroxisome proliferator-activated receptor (PPAR) alpha(α) and gamma(γ), induces the production of adiponectin and ameliorates insulin resistance in diet-obese mice and a study from our group showed that treatment with PIO in obese mice leads to microRNA(miR) expression regulation in skeletal muscle[1]. Recent data from our laboratory studying the effect of PIO in diet-obese adiponectin knockout mice (adipoKO) showed that PIO ameliorates insulin resistance by a adiponectin-independent manner, and it was associated with decreased expression of miR-23b and miR-222, and an increase in phosphorylated AMP-activated protein kinase (pAMPK) and Sirtuin 1 (Sirt1) protein expression in skeletal muscle. Since PIO is a ligand of PPAR γ and α, and Sirt1 is involved in a regulatory loop of these two PPARs[2,3], the aim of this study was to evaluate in adipoKO mice the effect of PPAR α or PPARγ block during PIO treatment. We also evaluated in vitro the effect of inhibition of the expression of miR-23b and miR-222 in C2C12 cells. **Methods:** Forty male C57BL/6 adipoKO mice, 8 weeks old with ±20g were fed a balanced diet (BD) or high fat diet (HFD) for 6 weeks and part of the mice of the HFD group was treated for 2 weeks with PIO (35mg/kg/day) (group HFD+P), or with PIO+ PPARα antagonist (group HFD+P+GW6471) or with PIO +PPARγ antagonist (group HFD+P+GW9662). At the end of treatment, animals were euthanized and soleus muscles were collected for analysis. For in vitro experiments, anti-miRs (Exiqon) for miR-23b and miR-222 were obtained which were transfected into the differentiated C2C12 cells, treated with 0.75mM palmitic acid(PA) or vehicle for 16h and stimulated with 100 nM insulin for 15 min. Analysis of miR-23b and miR-222 expression by real-time PCR and protein expression by western blot were performed in soleus muscle and in C2C12 cells. The data were analyzed by ANOVA One-way/Two-way followed by Tukey's/ Bonferroni post-test. **Results:** miR-222 expression was not restored in HFD+P+GW9662 group (BD: 1.00 ±0.1 vs HFD: 1.98 ±0.13 vs HFD+P: 1.1 ±0.05 vs HFD+P+GW6471: 1.22 ±0.06 vs HFD+P+GW9662: 1.48 ± 0.03; $p<0.05$), however miR-23b expression was not altered by antagonists treatment. Expression of pAMPK (thr172) was increased expression in HFD+P and HFD+P+GW6471 groups, compared to C and HFD group, but not in HFD+P+GW9662 group (BD: 1.00 ±0.07 vs HFD: 1.64 ±0.11 vs HFD+P: 0.67 ±0.05 vs HFD+P+GW6471: 0.67 ±0.05 vs HFD+P+GW9662: 0.67 ±.05; $p<0.05$). In vitro, differently from PA treated cells, miR-222 inhibition did not caused decreased phosphorylated protein kinase B (pAKT) protein expression (C: 1.00 ±0.04 vs C+PA: 0.49 ±0.14 vs anti-miR-222+PA: 0.73 ±0.07; $p<0.05$). **Conclusion:** *In vivo* the effect of PIO on the regulation of miR-222 in skeletal muscle in the absence of adiponectin appears to be mediated by PPARγ, and the activation of AMPK might be involved in its mechanism of action. In vitro miR-222 may has a role in insulin resistance development. **Financial support**: FAPESP(2015/24650-0); Animal Research Ethical Committee Process Number: 137/2015. References: 1-Frias F. Front Endocrinol (7):76(2016); 2-Hayashida S. Mol Cell Biochem (339):285(2010); 3-Han L. Nucleic Acids Res (38): 7458(2010)

01.008 Fenofibrate prevents weight gain through hypothalamic leptin resistance improvement in DIO mice Rocha KC[1], Santos BAC[1], Rodrigues AC[1] [1]USP – Farmacologia

Introduction: The hypothalamus is an important brain region that regulates energy metabolism, food intake and weight gain through the effects of hypothalamic neuropeptides, nutrient and hormones[1]. Recently, microRNAs and activation of peroxisome proliferator activated receptor alpha (PPARα) in hypothalamic neurons have also been implicated in food intake and whole-body energy balance control[2,3]. To evaluate metabolic changes via PPARα activation and its consequences on hypothalamic mRNA and microRNA expression we treated obese mice with fenofibrate, a PPARα agonist. **Methods:** Male C57BL/6 mice at 8 weeks' old (n ± 40) were randomly assigned to receive a control (CD) or a high-fat diet (HFD) for 6 weeks. After that, they were subdivided into four groups: C group (fed with CD, untreated), CF group (fed with CD and treated with fenofibrate), H group (fed with HFD, untreated) and HF group (fed with HFD and treated with fenofibrate). Fenofibrate (50 mg/Kg/day) was administered daily by oral gavage for 2 weeks. After 48h of the last dose, they were euthanized. Body weight was determined every week and food intake every 3 days. Insulin Tolerance Test (ITT) was performed on the last week of protocol. Insulinemia and leptinemia were measured by specifics ELISA kit. Visceral fat depots including, mesenteric, epididymal, and retroperitoneal were dissected and weighed. The hypothalamus was immediately flash-frozen in liquid nitrogen for total RNA extraction. Expression of microRNA (miR-9-5p) and mRNAs (Foxo1, Socs3 and Cart) were measured by Real-time PCR assay using miR-30d-5p or B2m, respectively, as normalizers. Statistical analysis was performed using Two-way ANOVA, followed by Bonferroni post-test. Differences at $P < 0.05$ were considered significant. **Results:** Mice fed with HFD showed augment of body weight gain, visceral fat depots weights, fasting leptin and insulin serum levels and reduced constant of glucose disappearance (Kitt). Without changes in food intake, fenofibrate treatment attenuated the weight gain in both CF and HF groups, contributing to reduce the adipose tissue storage, and restored, compared to C group, insulin and leptin levels. Kitt was not different between HF and H groups. Obese mice showed increased expression of Cart (1.34 fold-increase) and Socs3 (0.48 fold-increase). Fenofibrate treatment was able to normalize Cart and Socs3 expression levels and to decrease Foxo1 expression, in comparison to H group and CF group, respectively. Obese mice showed an increased (7.34 fold-increase) in hypothalamic miR-9-5p and fenofibrate restored it levels. **Conclusion:** Fenofibrate prevents weight gain in obese mice by a mechanism that might involve the improvement of hypothalamic leptin resistance through normalization of Socs3, Cart and miR-9-5p expression. Reference: 1. Cifani, C. et al. Front. Neurosci. 9, 187 (2015). 2. Rijnsburger, M. et al. Physiol. Behav. 162, 61 (2016). 3. Schneeberger, M. et al. Front. Neurosci. 9, 41 (2015). **Financial Support**: CNPq and FAPESP. Animal Research Ethical Committee Process Number: 165/2011.

01.009 LPS-induced acute epididymitis changed the functional alpha-1 adrenoceptor subtype in the cauda epididymis smooth muscle Mueller AM[1,2], Silva AAS[1], Silva EJR[1], Pupo AS[1] [1]IBB-Unesp – Farmacologia, [2]UFMT-Campus de Sinop – Farmácia

Introduction: The distal cauda epididymis (CE) is densely innervated by sympathetic postganglionic neurons that release noradrenaline (NA) to contract the smooth muscle layer surrounding the epithelium through alpha-1 adrenoceptors (alpha-1-ARs) activation during ejaculation. As acute epididymitis has been associated to damage of CE smooth muscle, we hypothesize that inflammation chances in the CE sympathetic innervation affect the contractility of the CE smooth muscle, playing a role in the impairment of tissue function induced by epididymitis. Thus, the aim of this study was to investigate the alpha-1-ARs in CE in an experimental model of epididymitis in rats induced by the injection of LPS from *E. coli* into the lumen of the vas deferens. **Methods**: adult male Wistar rats (90-110 days-old) were anesthetized with ketamine:xilazine (10 and 100mg/kg) and intravasal retrograde injection of 25uL of ultrapure LPS from *E. coli* O55:B55 (1mg/ml) or 25uL of sterile saline solution (control) was performed to induce inflammation in the cauda epididymis. Rats were killed by decapitation after 6h (LPS_6h; Sal_6h) of treatment and epididymal ducts were isolated and mounted in 10mL organ baths to record isometric tension. Cumulative concentration-response curves (CRC) to NA were obtained in the absence and presence of prazosin and BMY 7378 (alpha-1 and alpha-1D-ARs competitive antagonist, respectively). All the CRC were performed in the presence of a cocktail of inhibitors to allow experimental conditions to evaluate antagonist potency (pA_2). Agonist potency was expressed as pD_2 values. **Results**: NA induced concentration-dependent contractions of CE in all groups which were competitively antagonized by prazosin with similar potencies (pA_2 □ 9.0, n=4-9), showing the alpha-1-AR participation. BMY 7378 also antagonized NA induced-contractions in a competitive manner, and its potency in Sal_6h (pA2 □ 6.9, n=7) was consistent with alpha-1A-ARs functional role. Conversely, the antagonism of BMY 7378 in LPS_6h suggests alpha-1D participation in NA induced-contractions in CE (pA2 □ 8.7, n=6), with a slope different from unity in Schild regression. Correlated to this, NA was approximately 6-fold more potent in LPS_6h (pD_2=7.26□0.08, n=6) than in Sal_6h (pD_2=6.49□0.07, n=6, $p<0.05$). **Conclusion**: the ongoing functional studies suggest that LPS-induced epididymitis promotes change in the alpha-1-AR subtype in the cauda epididymis smooth muscle, indicating a likely heterogeneous receptor population (alpha-1A and alpha-1D) involved in the contractile response to noradrenaline during the early phases of inflammation. It will be important to investigate the effects of LPS-induced inflammation on the expression of alpha-1-AR subtypes in the CE to further determine subtype(s) involved in the contraction induced by NA and its impact on male fertility after inflammatory events. **Financial support**: Fapesp (2015/08227-0) and CNPq (479546/2013-4). Process number of Local Ethics Committee for the Use of Experimental Animals: 749-CEUA

01.010 New insights on β-defensins: differential expression in the central nervous system. Freitas GA[2,1], Scavone C[1], Pinna G[3], Avellar MCW[2] [1]ICB-USP – Farmacologia, [2]Unifesp-EPM – Farmacologia, [3]University of Illinois

Introduction: β-defensins (DEFBs) are components of host defense, with antimicrobial and pleiotropic immuno-modulatory actions. Increasing evidences suggest that DEFBs have also a variety of other non-immunological activities, such as role in cancer, male reproductive tract function and male fertility. More recently our research group has also broaden the view of DEFB role as regulators of tissue morphogenesis. In the literature, DEFBs have been found in brain areas of patients with Alzheimer disease and others neurodegenerative disorders, suggesting their potential role in the regulation of immune responses in the central nervous system (CNS). However, the expression and functional role of DEFBs in the CNS has been scarcely studied. **Aim**: Herein we evaluated the spatial expression profile of two DEFBs, named SPAG11C (sperm-associated antigen 11, isoform C) and DEFB1 (β-defensin 1), in the mouse brain. **Methods**: Adult Swiss mice (90 days) were used. Total RNA from pre-frontal cortex and hippocampus were extracted and used for RT-qPCRs analysis of *Spag11c* and *Defb1* transcripts, using *Hprt1* as internal reference gene. Brain from mice perfused with 4% PFA in saline were also isolated, included in cryopreserving solution and used for immunofluorescence studies (IF; 20 µm cryosections) with antibodies against SPAG11C and DEFB1. Molecular marker antibodies for neurons (NeuN), glial cells (GFAP, IBA1) and GABAergic neurons (GAD65/67) were used in co-immunofluorescence studies. Positive (IF in adult rat caput epididymis cryosections) and negative controls (absence of primary and secondary antibody) were performed. Image analyses were conducted by fluorescence and confocal microscopy. **Results**: SPAG11C- and DEFB1-positive immunostainings were widely distributed throughout the brain. Strikinly, in areas such as the pre-frontal cortex, hippocampus, third ventricle and cerebellum, both DEFBs were found in neurons ($NeuN^+$ cells), neurites (neuronal processes), but not in glia cells ($GFAP^+$ for astrocyte and $IBA1^+$ for microglia). Part of these $SPAG11C^+$/$NeuN^+$ and $DEFB1^+$/$NeuN^+$ neurons were identified as GABAergic ($GAD65/67^+$ cells). Spatial distribution of SPAG11C- and DEFB1-positive neurons varied along the pre-frontal cortex layers (more abundant between layers III and IV) and hippocampal areas. Curiously SPAG11C, but not DEFB1, was found in the hippocampal dentate gyrus. Besides, we also found immunolocalization of DEFBs in neuroepithelial cells from the choroid plexus. Depending on the brain region and cell type, SPAG11C and DEFB1 soma immunostaining pattern varied from cytoplasmic and/or nuclear and supranuclear localization. RT-qPCR revealed the presence of both *Spag11c* and *Defb1*transcripts in the pre-frontal cortex and hippocampus. **Conclusion**: The present study expands the potential functional repertoire predicted for DEFB in a mammalian tissue. Our findings constitute a baseline for the better understanding of DEFB contributions to CNS function and disease susceptibility, both fields of clinical relevance. **Financial support**: CNPq, CAPES, FAPESP (#2014/19378-6). **Ethics Research Committee Approval**: CEUA UNIFESP-EPM #7991170915/2015.

01.011 Uvaol stimulates endothelial cell migration and fibronectin production in vitro. Carmo JOS[1], Ferro JNS[1], Correia ACC[2], Barreto E[1] [1]ICBS-UFAL, [2]UPE-Campus Garanhuns-PE

Introduction: The use of angiogenic drugs in patients with diseases related to insufficient angiogenesis, such as diabetes and coronary artery disease, improve blood perfusion, deliver survival factors to sites of tissue repair, and restore form and function to the tissue. Uvaol, a natural pentacyclic triterpenoid, has been demonstrated to have anti-inflammatory property and cardioprotective effect, however, its effects on the angiogenic process has not yet been investigated. Therefore, our objective was to evaluate the effect of uvaol in key events during the angiogenesis, such as migration and fibronectin production by endothelial cells in vitro. **Methods**: Endothelial murine cell line (tEnd.1) cultured in supplemented RPMI medium (10% fetal bovine serum, 2 mM glutamine, and 1% gentamicin) were exposed to different concentration of uvaol and incubated for 24 h in a humidified incubator (at 37°C, 5% CO_2). Cell viability was evaluated by means of MTT assay. To assess the effect of uvaol on cell migration, a scratch assay was performed, in which a “scratch” was made by a standard pipette tip in a cell monolayer following uvaol exposure, and closure of the scratch was evaluated. Fibronectin production in uvaol-treated endothelial cells was quantified by immunofluorescence. In another set of experiments, uvaol-exposed endothelial cells were treated with a specific p38-MAPK inhibitor, SB203580, to explore a possible molecular mechanism involved in cells migration by using scratch assay. All assays were performed in three independent controlled experiments. Statistical analysis was performed using two-way ANOVA with post hoc Newman-keuls test. Differences at $p<0.05$ were considered significant. **Results:** Endothelial cells treated with different concentrations of uvaol (1-100 µM) exhibited no statistically significant alterations on viability after 24h in culture. However, treatment with uvaol at 10 or 50 µM significantly increased the endothelial cell migration at 6 h (in 48% and 59%), 12 h (in 24% and 35%) and 24 h (in 29% and 40%). Furthermore, immunofluorescence analysis revealed that endothelial cells treated to uvaol (10 µM) for 24 h exhibited a significant increase ($p<0.05$) in the production of fibronectin as compared untreated cells. Cotreatment with SB203580 (a p38-MAPK inhibitor) abolished the uvaol-induced migration ($p<0.001$), indicating that the increase in cell migration induced by uvaol requires activation of the MAPK. **Conclusion:** These results indicate that uvaol is able to induce fibronectin production and increase endothelial cells migration via stimulation of the p38-MAPK signaling pathway, suggesting a proangiogenic role of uvaol. **Financial support**: CNPq and CAPES.

01.012 Inhibitory Apoptosis Proteins (IAPs) as targets for anticancer therapy: Heterologous expression of XIAP Silva CSMR[1], Branco PC[2], Barbosa GH[3], Paula C Jimenez[3], Costa-Lotufo LV[2] [1]UFSCar, [2]ICB-USP, [3]Unifesp

Introduction: Marine pharmacology is a branch of the pharmaceutical sciences that focuses its studies on substances with pharmacological properties in marine organisms. The marine environment is an exceptional warehouse for new bioactive natural products with unique structural and chemical characteristics, including those with anticancer properties. One of the targets of these new anticancer-based marine bioactive products are IAPs. IAPs are apoptosis inhibitory proteins characterized by the presence of the BIR domain and can be found in several organisms. This family of proteins is involved in the regulation of a number of cellular processes including cell death, immune and inflammatory responses, cell proliferation, cell differentiation and cell motility. Their overexpression in tumors is common, however almost or totally absent in normal tissues. The ability to prevent cell death is a characteristic of cancer cells and one of the reasons for resistance is the current anticancer treatments. Therefore, reactivation of cell death is a promising strategy for treatment innovation. This protein has been identified as being important for the inhibition of apoptosis. XIAP inhibits apoptosis by direct binding to three cysteine protease (caspase-9, caspase-3 and 7) inhibiting it. These caspases are important for apoptosis, so by inhibiting them XIAP prevents cell death. XIAP is found overexpressed in many cancer cells such as cholangiocarcinoma and is important in conferring resistance to these cells, so this protein is an interesting target for new anticancer drugs. **Methods for heterologous expression of XIAP:** *E. coli* BL21 and Rosetta bacterial strains were transformed with the plasmid containing XIAP. The transformed bacteria were induced with different concentrations off IPTG. We also evaluated this expression in small and large scale and also in two different temperatures: 16 and 37°C. Validation of overexpression was evaluated on polyacylamide gel. Proteins were purified by immobilized metal ion affinity chromatography (IMAC) and subsequent dialysis. The purified protein XIAP was subjected to the functional chromatography technique described by Lau et al. (2015). **Results:** In the tests for the heterologous expression of XIAP the protein, best results were obtained with the E. coli BL21 strain and the concentration of 0.3 mM of IPTG for induction. The growth of the bacteria was also better on the small and large scale at 16ºC. The purified protein was used for the performance of functional chromatography (FC) with 12 marine extracts, previously selected thanks to the results obtained in the cytotoxicity tests, including marine sediments and ascidia. The marine extracts were composed of 10 raw extracts and 2 pure substances. We also evaluated Embelin that is a natural product extract from plants that has shown that interacts with XIAP. **Conclusion:** XIAP's heterologous expression method was standardized, which will aid in future research with this protein, reducing cost and production time. XIAP is an interesting therapeutic target and for this reason it was chosen for the performance of the functional chromatography with the different marine extracts. The studies involving XIAP and marine extracts present potential in new anticancer therapies. **Financial Support:** CNPq (PróArquipélago and INCTBioNat) and FAPESP (2015/17177-6)

01.013 Influence of antipsychotics on leukocyte telomere length. Polho GB[1], Cardillo GM[2], Kerr DS, Gattaz WF[2], Forlenza OV[2], Brentani H[3], Paula VJR[3] [1]FMUSP – Acadêmico, [2]FMUSP – Neurociências, [3]FMUSP – Psicobiologia

Abstract: Telomeres are tandem repeats of the non-coding sequence TTAGGG at the end of each chromosome. Human telomeres protect the DNA from end-to-end fusion and cellular erosion, but they diminish progressively with cellular division (1). Antipsychotics consist in a class of drugs used for the treatment of several neuropsychiatric conditions, including schizophrenia. The exact mechanism of action of antipsychotics is still debated. In the literature, we can find some evidence for antipsychotic influence on telomere dynamics, but the results are elusive. In this project we test the hypothesis of telomere lengthening by antipsychotics after oxidative stress. **Methods:** Healthy volunteers were strictly recruited among workers at Institute of Psychiatry–University of São Paulo. Mononuclear leukocytes were extracted using Ficoll-Histopaque methodology, and grown in RPMI 1640 media supplemented with 10% fetal calf serum. We induced oxidative stress with H_2O_2 for 3 days. Simultaneously, we tested different concentrations of antipsychotics in cell cultures, for 4 days: aripiprazole 250ng/mL-2000ng/mL, haloperidol 10ng/mL-200ng/mL and clozapine 125 ng/mL-5000ng/mL. Then, we used MTT assay to compare cell viability. DNA was extracted using QIAamp DNA Mini Blood kit (QIAGEN) with QIAcube protocol, and DNA quantity and quality was assessed with Spectophotometry (Nanodrop) and capillary electrophoresis (Fragment Analyzer). Telomere length was assessed using a validated methodology by qPCR(3). For absolute quantification of telomere, we prepared a standard curve using known oligomers. **Results:** The best concentration of H_2O_2 was 200µM with greatest reduction in telomere length as well as tendency to greatest cell survival in MTT assay (ANOVA test, p=0,05). Capillary electrophoresis and spectrophotometry showed slight DNA degradation, but still good DNA quality. In cultures treated with antipsychotics, we did not observe cell viability change with different doses of haloperidol (R^2=0,039; p=0,091), aripiprazole (R^2=0,052; p=0,065) and clozapine (R^2=0,008; p=0,25). The telomeric size is in the final stage of the experiment. **Conclusion:** According to the experiments showed, telomere damage will be simulated with acute oxidative stress using H_2O_2 for 3 days, followed by 4 days of antipsychotic treatment (haloperidol 7ng/mL, aripiprazole 125 ng/mL and clozapine 1000 ng/mL). This protocol showed the greatest initial damage to telomere and provided greatest cell survival and DNA quality for further telomere length analysis. **Financial Support** and **Ethical Committee**: Fundação Amparo à Pesquisa do Estado de São Paulo – FAPESP (2014/27129-6, 2016/01302-9) supported the research. Project was approved by HCFMUSP Ethical Committee (CAAAE 52622616.8.0000.0065). **References**: 1. Eitan E et al. Trends Neurosci. Elsevier. 37,256. 2014 2. Cawthon RM. Nucleic Acids Res. 30,47. 2002 3. Callicot RJ et al. Comp Med.56,17.2006

01.014 Increased ROS production and $P2Y_2$ receptor-mediated leukocyte adhesion to mesenteric endothelial cells during schistosomiasis. Monteiro MML[1], Pereira LM[1], Lanzetti M[1], Valença SS[1], Silva CLM[1] [1]UFRJ – Farmacologia e Inflamação

Introduction: Schistosomiasis is a neglected tropical disease caused by *Schistosoma mansoni* that lives inside mesenteric vessels. According to WHO the disease affects million people in the world, and its morbidity is due to chronic inflammation. Under injury endothelial cells become activated favoring leukocyte adhesion. ATP and UTP are released during cell stress and act through $P2Y_2$ purinergic receptors having a pro-inflammatory effect. Another hallmark of cell stress is the production of reactive oxygen species (ROS). The aim of the present work was to evaluate endothelial $P2Y_2$-mediated leukocyte adhesion and production of ROS during schistosomiasis. **Methods:** Male Swiss mice were divided in two groups (control and *S. mansoni*-infected mice). Seventy five day-old mice were anesthetized and euthanized. Mesenteric vessels were removed, minced and plated with DMEM enriched with 20% fetal bovine serum and streptomycin/penicillin, and maintained at 37^{o}C, 5% CO_2. Endothelial cells were plated for leukocyte adhesion and ROS measurement assays. ROS quantification assay used blue nitrotetrazolium (NBT) and was adapted from Choi et al., 2006 (J. Immunoassay Immunochem, 27:31). After confluence, mesenteric endothelium cells (MEC) were incubated with NBT (1h), the formazan crystals were solubilized and read at 630nm. Results were expressed in arbitrary units (a.u.) of ROS/10^3 cells. For leukocyte adhesion, mononuclear cells were isolated from peripheral blood using Percoll gradient (Oliveira et al., 2011 Plos One 6(8):e23547). Confluent MEC were stimulated with 100 µM UTP ($P2Y_2R$) for 5h, in the absence or presence of the antagonist suramin (50 µM). Then, MEC were co-incubated with mononuclear cells for 30 min before wash. Four fields/well were chosen and the number of adherent cells was defined using an optical microscope. One way ANOVA followed by Bonferroni test or Student´s t test were used considering $P < 0.05$. **Results:** In the control group, 100 µM UTP increased leukocyte adhesion to endothelial cells from 2.08 ± 0.1 to 4.4 ± 0.4 cells/field ($n = 4$, $P < 0.001$). In the infected group UTP also increased leukocyte adhesion from 9.4 ± 0.4 to 17.4 ± 0.9 cells/field ($n = 4$, $P < 0.001$), however, the final number of adhered leukocytes in the presence of UTP was higher in the infected than in control group ($P < 0.001$). Suramin (50 µM) blocked the UTP effect in both groups. Endothelial cells from infected group (0.06 ± 0.005 a.u.) produced approximately twice ROS as compared to control (0.03 ± 0.005 a.u.; $P< 0.001$, $n=3$). Previous data from our group showed that endothelial nitric oxide (NO^-) production is compromised in the infected group (Oliveira et al., 2011 Plos One 6:e23547). Considering that the reaction of superoxide anion and NO^- forms peroxynitrite reducing NO^- bioavailability, and that constitutive endothelial NO inhibits leukocyte adhesion, we could suggest that the increased production of ROS contributes to mesenteric inflammation during schistosomiasis. **Conclusion:** Our data suggest that the phenotype of mesenteric endothelial cells is altered in schistosomiasis, resulting in an increased ROS production and $P2Y_2R$-mediated leukocyte adhesion. The upregulation of inflammatory $P2Y_2R$ purinergic signaling during schistosomiasis may contribute to mesenteric inflammation observed in the disease. **Financial Support:** CAPES, FAPERJ and CNPQ. CEUA UFRJ 048/16

01.015 Desensitization of Alpha-1a Adrenoceptors by Dopamine Wandekin RR, Pupo AS IBB-Unesp – Farmacologia

Introduction: The catecholamine dopamine activates alpha-1 adrenoceptors and it is a useful drug to rise blood pressure in shock. However, there is loss of vasoconstriction upon prolonged exposure to dopamine (tachyphylaxis). This tachyphylaxis in the effects of dopamine has been attributed to alpha-1 adrenoceptor desensitization. As the alpha-1A subtype predominates in vessels densely innervated by the sympathetic nervous system, the aim of this study was to investigate the thachyphylaxis in the contractions induced by dopamine in the rat vas deferens, a tissue in which the contractions to adrenoceptor agonists result from the activation of alpha-1A adrenoceptors. **Methods**: Adult male wistar rats (90-120 days-old) were killed by decapitation and *vasa deferentia* were carefully isolated, cleaned from surrounding structures and connective tissue, and mounted in organ baths to record isometric tension. To check for tachyphylaxis, consecutive concentration-response curves (CRCs) to noradrenaline and dopamine at intervals of 5, 10, 15 or 30 min. Were performed in presence of cocktail of inhibitors containing desipramine (0,1 □M, to block neuronal uptake), corticosterone (10 □M, to block extraneuronal uptake), yohimbine (0.1 □M, to antagonize alpha-2 adrenoceptors) and propranolol (0.1 □M, to antagonize beta-adrenoceptors). pEC_{50} and E_{max} were determined to estimate agonist's potencies and efficacies, respectively. Data are expressed as mean±s.e.m of 6 independent experiments and differences between mean values were checked by Student's "t" test; statistical significance was accepted when $P < 0.05$. **Results:** Dopamine behaved as a full agonist in relation to noradrenaline (Emax≈2.0 g); however, the potency for dopamine (pEC_{50} = 4.9±0.1) was approximately 60-fold lower than that of noradrenaline (pEC_{50} = 6.7±0.1). Up to seven consecutive CRCs to noradrenaline with intervals between each CRC ranging from 5 to 30 min showed no tachyphylaxis and presented similar pEC_{50} and E_{max} values. However, there was tachylaphylaxis in the contractions induced by dopamine when the intervals between each CRC was 5, and 10 min, as indicated by a reduction in both the potency (3 to 10-fold loss of potency) and efficacy (up to ≈30% loss in E_{max}). In addition, the treatment of the tissues with dopamine 100 □M/5 min resulted in a 3-fold loss of potency for noradrenaline, whereas the treatment with noradrenaline 10 □M/5 min was unable to affect the potency or efficacy of noradrenaline on a subsequent exposure. **Conclusion:** There is tachyphylaxis in the contractions of the rat vas deferens in response to dopamine, but not in response to noradrenaline, indicating that dopamine desensitizes the alpha-1A adrenoceptors. This differential ability of dopamine to desensitize the alpha-1A adrenoceptors may result from a biased agonism for dopamine favoring receptor uncoupling/internalization and this is currently under investigation. Ethics Committee for the use of experimental animals: Protocol# 896/2016 **Financial support**: Capes

01.016 Evaluation of Mitochondrial Metabolism in Different Pharmacological Models of Amyotrophic Lateral Sclerosis. Brito MD, Rosenstock TR FCMSCSP – Ciências Fisiológicas

Introduction: Amyotrophic Lateral Sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by motor neurons death. Approximately 90% ALS cases are sporadic (with no definite cause), and 10% are due to familial cases. Regarding to that, between 15-20% are related to mutations in the gene that encodes the antioxidant enzyme Cu / Zn superoxide dismutase (SOD1). Due to this fact, ALS is considered a multifactorial disorder, and mitochondrial dysfunction is one of the main neuropathological characteristics. Thus, the aim of this study is to evaluate mitochondrial function, namely modifications in its metabolism, to better understand the role of this organelle in the ALS pathology, using different pharmacological models: astrocytes after the induction of oxidative stress, due to exposure to peroxide (H_2O_2), and glutamatergic excitotoxicity resulting from the treatment with the neurotoxin L-BMAA. **Methods:** For the purpose of this study, astrocytes (obtained from primary culture) from the cortex of C57 / bl6 mice were used. The cells were then exposed for 24 h to L-BMAA (10 mM) or 20 minutes to H_2O_2 (1 mM). Next, mitochondrial function was assessed by spectrofluorimetry after cells were incubated with different fluorescence indicators; the mitochondrial membrane potential (ΔΨm) was evaluated with TMRE (500nM), cytosolic Ca^{2+} with Fluo-4 AM (10μM) and oxidative stress with H2DCFDA (20μM). **Results:** In basal conditions, the mitochondria of the cells exposed to L-BMAA are depolarized in relation to the control group, since they captured less TMRE (cationic indicator) than the cells of the other groups. Interestingly, L-BMAA led to a decrease in cytosolic calcium represented by the decrease in Fluo-4-AM fluorescence, which could indicate a capture of this ion by intracellular organelles such as mitochondria. However, when the mitochondria of the L-BMAA treated cells were analyzed after FCCP exposure, a protonophore agent, they showed a lower mitochondrial calcium concentration when compared to the control group (CTR, without treatment) (CTR: 107.95% ± 17.49; LBMAA: 87.53% ± 5.98). In addition, we observed a depolarization of the mitochondria after treatments with LBMAA and H_2O_2 in the presence of FCCP (CTR: 100% ± 5.28, LBMAA: 76% ± 8.91, H_2O_2: 69% ± 7.53) indicating that these mitochondria were already depolarized. No changes were observed in oxidative stress levels. **Conclusion:** Taken together, we conclude that the drugs used here as pharmacological models of ALS alter mitochondrial function through the induction of mitochondrial membrane depolarization; this, in turn, can trigger a cellular dysfunction that leads to increased death with consequent appearance of the neuropathological features of ALS. FAPESP, CNPq/CAPES CEUA 003/15

01.017 Epinephrine enhances osteoblastic differentiation of mesenchymal stem cells from spontaneously hypertensive rats (SHR) Barreto AEA[1,2], Brito VGB[1,2], Beltran CT[1], Queiroz DP[1,2], Oliveira SHP[1,2] [1]FOA-Unesp – Ciências Básicas, [2]FOA/Unesp/SBFis

Introduction: Several factors are appointed as potential regulators of bone metabolism, and most of them actively participate by modulating the activity of bone formation and resorption, mediated by osteoblasts and osteoclasts, respectively. Among the systemic regulatory factors, the sympathetic nervous system has been studied due to its possible ability to interfere with bone remodeling through of adrenergic receptors on the surface of the osteoblast. Thus, the present study aimed to evaluate the *in vitro* osteoblastic differentiation of mesenchymal stromal cells from Spontaneously Hypertensive Rats (SHR) stimulated with epinephrine (adrenergic agonist) and treated with carvedilol (nonselective adrenergic antagonist). **Methods:** Study of experimental character using 40 days-old male SHR, which were euthanized for dissection of the femurs and collection of bone marrow, which was placed in cell culture flasks and after reaching sub confluence, were seeded in to 24-well plates, where they received osteogenic inductors (ascorbic acid 50 µg/mL, dexamethasone 10^{-8}M and β-glycerophosphate 10 mM) and the stimulus with epinephrine (1 µM) (non-specific adrenergic agonist). In order to study the activity of adrenergic receptors, they were blocked with a nonselective adrenergic antagonist - carvedilol – at three concentrations (0.1, 1, and 5 µM). The stimulus and the antagonist was added at the same time to the cell culture. We analyzed of cell viability and total protein content on day 0, 7, 14 and 20, by colorimetric assays, gene expression of osteogenic markers on day 14, by qRT-PCR and biological mineralization on day 20 and 28, by Alizarin Red S stain. The protocol was approved by Institutional Animal Care and Use Committees (School of Dentistry of Araçatuba; Process 00686-2016). **Results:** It was observed that cells stimulated with epinephrine had reduced viability in late periods, and carvedilol (5 µM) in the presence of epinephrine showed to be cytotoxic. Epinephrine stimulus lead to an increased osteocalcin and osterix expression on day 14, while carvedilol (1µM) treatment was able to block only osteocalcin expression, but not osterix. Interestingly, carvedilol treatment at 1 µM further reduced the *Runx2*, alkaline phosphatase, osteopontin, osteocalcin and bone sialoprotein expression. In biological mineralization, the group stimulated only with epinephrine showed higher mineral deposition, and it was reduced by carvedilol treatment at 1 µM. **Conclusion:** Our results evidence a potential promoter effect of epinephrine towards osteoblastic differentiation, and since concomitant treatment with an adrenergic antagonist was able to block epinephrine effects we may suggest that epinephrine acts on bone turnover via the adrenergic receptors. **Financial support and acknowledgments:** FAPESP (Grant: #2015/03965-2) and CAPES for financial support.

01.018 Disassemble caveolae structure does not change the proteoglycans syndecan-2 and glypican-1 present on the endothelial glycocalyx. Potje SR[1], Grando MD[1], Antoniali C[2], Bendhack LM[1] [1]FCFRP-USP – Física e Química , [2]FOA-Unesp – Ciências Básicas

Introduction: The endothelial glycocalyx on the apical surface of endothelial cells consists of acidic oligosaccharides and proteoglycans with glycosaminoglycan side chains (heparan sulfate, chondroitin sulfate and hyaluronic acid). It has been described as a structure responsible for mechanotransduction which is a translation of the biomechanical forces in biochemical signaling in endothelial cells, promoting activation of endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) production. Proteoglycans can be divided into three different groups based on their protein backbones (syndecans, glypicans, and perlecans). Syndecans 1-4 are integral membrane proteoglycans with highly conserved transmembrane domains and two constant regions in the cytoplasmic domain, whereas glypican-1, which are tethered to caveolae membrane via glycosylphosphatidylinositol (GPI) anchor. Also, hyaluronic acid is anchored to caveolae by CD44 receptor. We tested the hypothesis that changing the structure of caveolae can compromise the endothelial glycocalyx. Therefore, this study aimed to investigate the protein expression of the enzymes eNOS, syndecan-2 and glypican-1 in human umbilical vein endothelial cells (HUVECs) treated or not with heparinase III or hyaluronidase to degrade glycocalyx components and methyl-beta-ciclodextrin (MCD) to disassemble caveolae. **Methods:** In this study, some enzymes were used as selective pharmacological tools (Heparinase III 15 mU/mL, 2 hours; hyaluronidase 14 µg/mL, 20 minutes) to degrade glycocalyx components and MCD (10 mM, 30 minutes) to deplete membrane cholesterol (caveolae) from the surface of HUVECs. eNOS, syndecan-2 and glypican-1 expression were analyzed by Western Blot. **Results:** Disassemble the caveoale structure by treatment with MCD in HUVECs did not change syndecan-2 or glypican-1 expression. However, it was verified the reduction of eNOS expression (more than 50%). When we treated the HUVECs with heparinase III (which cleaves heparan sulfate), eNOS or glypican expression were not changed, but syndecan-2 expression was decreased by heparinase III. The HUVECs treated with hyaluronidase (which cleaves hyaluronic acid) showed a decrease in glypican-1 and eNOS expression, but no changes were observed in syndecan-2 expression. **Conclusion:** Our previous results suggest that deplete the caveolae structure does not change the glycocalyx's proteoglycans syndecan-2 and glypican-1. Further evaluation of other enzymes as caveolin-1, syndecan-1, 3 and 4 can clarify and improve the findings in this study. **Financial Support:** Fundação de Amparo À Pesquisa do Estado de São Paulo (FAPESP 2016/21239-0 and 2015/17080-2). **Animal Research Ethical Committee:** (School of Dentistry, FOA-UNESP process number 00423-2017).

01.019 Modulation of oxidative stress in vascular muscle cells from renal hypertensive rats by co-culture with endothelial cells. Paulo M[1,2], Grando MD[2], Vercesi JA[2], Minshall RD[1], Bendhack LM[2] [1]University of Illinois at Chicago, [2]FCFRP-USP – Física e Química

Introduction: Interactions between endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) play a pivotal role in maintaining the vascular structure and function. Communications between ECs and VSMCs occur through synthesis and release of mediators or through direct cell-to-cell contact. Vascular relaxation molecules, such as nitric oxide (NO) and prostacyclin produced by ECs, are known to have a growth-inhibitory action on cultured VSMCs. On the other hand, vasoconstrictor molecules such as endothelin-1 have a stimulatory effect on VSMC growth in culture. In *vitro studies* are generally based on one type of cell culture, which, unfortunately, excludes important interactions between the two cell types. This study aimed to evaluate the modulation between the ECs and VSMCs in the production of NO and oxidative stress in renal hypertensive (2K-1C) and normotensive (2K) rat aortas. **Methods:** Using a co-culture system we tested the role of the interaction between ECs and VSMC from 2K-1C in producing oxidative stress. VSMC production of NO and reactive oxygen species (ROS) was assessed by fluorimetric assay by using fluorescence-based probes, DAF-2DA and DHE, respectively. The data were considered statistically different when $P<0.05$. Data are expressed as media± SEM of n=5. **Results:** In VSMC from 2K-1C, the NO production was lower (936.00 ± 11.0 FI, $P<0.05$) than in VSMC from control 2K rats (1,234.08 ± 20.0 FI). On the other hand, ROS production was higher in VSMC from 2K-1C (1,072.6 ± 17.0 FI, $P<0.05$) than in VSMC from control 2K rats (958.02 ± 27.1 FI). The generated intracellular O_2^- was significantly lower in co-culture for 72 hours of VSMCs and ECs from 2K-1C aortas as compared to VSMCs in monoculture 2K-1C (monoculture: 1,072.66 ± 17.3 FI; co-culture: 938.0 ± 12.0 FI, $P<0.05$). On the other hand, the NO levels were greater in VSMCs co-cultured with ECs than in monoculture (monoculture: 929.66 ± 13 FI; co-culture: 1,096.0 ± 29.0 FI, $P<0.05$). **Conclusion:** In VSMC from 2K-1C rats the oxidative stress is higher than in VSMC from 2K control rats. ECs negatively modulate the production of ROS in VSMC from 2K-1C rat aortas. Supported by Capes, CNPq, and Fapesp. Ethics Committee approval: 15.1.1293.60.1

01.020 Functional new world monkey oxytocin forms elicit an altered signaling profile and promote parental care in rats. Parreiras-e-Silva LT[1], Vargas-Pinilla P[2], Duarte DA[1], Longo D[2], Espinoza-Pardo GV[2], Finkler AD[2], Paixão-Côrtes VR[3], Paré P[2], Rovaris D[2], Oliveira EB[1], Caceres RA[4], Gonçalves G[2], Bouvier M[5], Salzano FM[2], Lucion AB[2], Costa-Neto CM[1], Bortolini MC[2] [1]FMRP-USP – Biochemistry and Immunology, [2]UFRGS, [3]UFBA, [4]UFCSPA, [5]University of Montreal

Introduction: The neurohormone oxytocin (OXT), which acts through a GPCR, OXTR, is a key player in the modulation of reproductive and social behavioral traits, such as parental care. Recently, a correlation between different forms of oxytocin and behavioral phenotypes has been described in the New World Monkeys (NWMs). **Methods**: The Pro8OXT and Val3Pro8OXT variants were synthesized by solid-phase peptide synthesis and purified by HPLC. The efficiency of synthesis was assessed by amino acid composition analysis. HEK293T cells were cultured in supplemented DMEM and transfected with OXTR- or AVPR1a-encoding plasmids using polyethylenimine. After transfection, cells were transferred to specific plates for development of different cell-based functional assays. *In vivo* Tests. Forty-two pairs of *Rattus norvegicus* were mated randomly, and pregnancy was confirmed on the day after mating by the presence of sperm in vaginal smears. Pairs were assigned to four experimental groups and received intranasal treatment with saline or the peptides. A total of 14 behaviors related to female–pup, male–pup, or male–female interactions were scored. Maternal and paternal motivations were evaluated in the pup-retrieval test. Results were analyzed by a pairwise comparison (Dunn's test). Ethics. All experimental procedures were approved by the Ethics Committee on Animal Use of the Universidade Federal do Rio Grande do Sul (Approval no. 28542) and are in accordance with Brazilian Law 11.794/2008, which regulates these procedures in Brazil. Results and Discussion Here, we demonstrate that the Cebidae Pro^8OXT and *Saguinus* Val^3Pro^8OXT taxon-specific variants act as efficacious agonists for the G_q-dependent pathways but are worse agonists for the β-arrestin dependent pathways, when activating the oxytocin receptor (OXTR). Intranasal treatment with either of the variants increased maternal behavior as well as promoted unusual paternal care in rats, as measured by pup retrieval tests. We therefore suggest that Val^3Pro^8OXT and Pro^8OXT are functional variants, which might have been evolutionarily co-opted as an essential part of the adaptive genetic repertoire that allowed the emergence of taxon-specific complex social behaviors, such as intense parental care in the Cebidae and the genus *Saguinus*. Financial Support: FAPESP, CAPES, CNPq and FAEPA.

01.021 Is there a differential expression pattern of the β-defensin SPAG11C in hippocampus and cortex during aging? Machado IN[1], Arantes G[1], Viel TA[2], Buch HS[3], Avellar MCW[1] [1]Unifesp-EPM, [2]EACH-USP, [3]FCMSCSP

Introduction: Aging is associated with the establishment of a state of systemic inflammatory activation. The main mediators of inflammatory responses in the central nervous system (CNS) are the microglia and the astrocytes, dynamic cells that play pivotal roles in plasticity and immune surveillance. β-defensins (DEFBs) are a highly conserved family of cationic antimicrobial and immunomodulatory peptides expressed primarily in epithelial cells from different mammalian tissues. Recently, our research group observed that two DEFBs, DEFB1 and sperm-associated antigen 11C (SPAG11C), are expressed in hippocampal and cortical neurons, but not in glia cells, from adult mouse. DEFBs have been found in brain areas of patients with Alzheimer' disease and others neurodegenerative disorders[1,2] suggesting a role in the regulation of immune responses. Nevertheless, whether certain DEFB play a crucial role in the onset or promotion of central inflammation over the course of aging is currently unknown. **Aim:** Here we tested if changes in DEFB expression pattern in microglia, astrocytes and neurons may contribute to the regulation of neuronal cell injury and death observed in the mouse brain with aging. **Methods:** C57BL/6J mice at 3, and 23 months old (adult, middle aged and elder group, respectively) were perfused and brains were isolated and included in cryopreserving solution. Criosections (20 µm) were obtained for immunofluorescence studies that were performed using primary antibodies against the DEFB SPAG11C and the molecular markers NEUN, IBA1, GFAP, and MAP1B (microtubule-associated protein 1B). Negative controls were performed in the absence of the primary antibodies. **Results:** In all ages, SPAG11C expression was detected in neurons (co-localization with NEUN and MAP1B molecular makers), but not in glia cells (immunostained with the molecular markers IBA1 for microglia and GFAP for astrocytes) in the cortex and hippocampus. In neurons, SPAG11C immuno-localization was mostly nuclear. SPAG11C-positive immunostaining was also detected in disperse neuronal processes (neurite) in the cortex and hippocampus from adult mice, and seemed to diminish in number with age. **Conclusion:** Aging is not changing the cell-type expression profile of SPAG11C in the cortex and hippocampus from control adult mice. Ongoing studies are now investigating the immunolocalization of this DEFB in these brain areas collected from aged mice exposed or not to an *In vivo* inflammatory challenge. Our results contribute to a better understanding of the role and mechanisms by which DEFB may modulate neuroinflammation in the CNS. **Financial Support:** FAPESP (#2017/05257-0; #2014/19378-6) e CAPES. UNIFESP-EPM Ethics Committee approval CEUA#7130190517/2017. References: 1.Kountouras, J. *et al. Med. Hypotheses* **82,** 368–373 (2014). 2.Williams, W. M. *et al*. *J. Neuroinflammation* **10,** 127 (2013).

01.022 Microparticles derived from obese adipose tissue elicit a pro-inflammatory phenotype of CD16+, CCR5+ and TLR8+ monocytes. Renovato-Martins M[1], Andrade IR[2], Matheus E[3], Moraes JA[3], Silva SV[2], Souza AAP[4], Silva CC[4], Bouskela E[5], Barja-Fidalgo C[2] [1]UFRJ – Química Biológica, [2]UERJ – Biologia Celular e Molecular, [3]UFRJ – Farmacologia, [4]UFRJ – Cirurgia, [5]UERJ – Ciências Fisiológicas

Obesity, considered today as an ongoing worldwide epidemic, is a low-grade inflammatory sub-acute state characterized by increased concentrations of several cytokines, chemokines and acute phase proteins. In this context the macrophage infiltration into adipose tissue (AT) is a hallmark of the chronic inflammatory response. Furthermore, this response is supported by an intense monocyte migration to AT. The obese AT secretes inflammatory factors such as cytokines, adipokines and free fatty acids into the circulation. It also releases microparticles, such as from adipocytes, which can act on macrophages. Although it has been detected an increased proportion of circulating $CD16^+$ monocyte subsets in obesity , the mechanisms underlying this effect and the contribution of these cells to the inflamed profile of obese AT were still poorly understood. We investigated whether factors secreted by human obese AT could polarize monocytes to a $CD16^+$ enriched phenotype, and how these changes could modify their migratory capacity towards adipose tissue itself. We show that explants of human obese AT, obtained during bariatric surgery, released higher levels of MIP1-α and TNFα, leptin and also VEGF, together with increasing amounts of microparticles, when compared to AT explants from lean subjects. A higher content of circulating microparticles derived from pre-adipocytes and leucocytes were also detected in plasma of obese patients. The incubation of $CD14^+CD16^-$ monocytes from lean subjects with the conditioned media or microparticles released from obese AT increased CD16 and CCR5 expression in these cells, and augmented their migratory capacity towards conditioned media of obese AT. This effect was inhibited by neutralizing MIP1-α. Additionally, we demonstrate that obese AT-derived microparticles carry and transfer TLR8 to $CD14^+CD16^-$ monocytes, triggering an increase in CD16 expression in these cells. Furthermore, we observed an increased frequency of CD16 positive macrophages in the adipose tissue from obese subjects, supporting the hypothesis that CD16 monocytes migrates towards obese adipose tissue and then preferentially differentiate in M1 macrophages. Altogether, our data suggest a positive feedback loop between blood monocytes and obese AT, which releases chemotactic mediators and TLR8-enriched microparticles inducing an up-regulation of $CD16^+$ monocytes, favoring their infiltration in the obese AT. All experiments were approved in accordance with the Committee for Ethics of Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro, RJ, Brazil (UERJ/CAAE 36880914.0.0000.5259).

01.023 Role of the fructose 1,6-bisfosfato on osteoclastogenesis and bone resorption in vitro. Wilches-Buitrago L[1], Fukada SY[2] [1]USP – Farmacologia, [2]USP – Física e Química

Bone remodeling is a coordinated metabolic process actively orchestrated by osteoblasts and osteoclasts. Alterations in the balanced function of these cells type may cause a change in the bone mineral density, a condition observed in certain inflammatory diseases such as osteoporosis, rheumatoid arthritis and periodontitis. Recently, there is a growing interest in assessing the role of the glycolysis on the cell proliferation, survival, and differentiation. In particular, it has been demonstrated the protective effect of the Fructose 1,6-bisphosphate (FBP), a high-energy glycolytic intermediate, in several cell types, although there is no evidence in the literature that associate FBP with the function of osteoclasts. This work aimed to evaluate the role of FBP in osteoclastogenesis and bone loss. To this end, murine bone marrow derived pre-osteoclasts were differentiated into osteoclasts by M-CSF (30ng/mL) and RANKL (10ng/mL). The results showed that FBP (100 and 300 μM) inhibits the differentiation of osteoclasts since the number of TRAP stained osteoclasts was reduced in a dose dependent manner, without affecting the cell viability (MTT assay). The treatment with the FBP decreases the marker genes expression evaluated by Real Time PCR and protein level of *Nfatc1*, *Trap* and *Cathepsin K* ($p < 0.01$) evaluated by Western Blot. Osteoclast resorption function was assessed on a osteassay plate and the resorption area of FBP-treated osteoclasts was reduced. Together, these data denote the important regulatory role of the FBP on osteoclastogenesis and function, proving to be a potential agent for the treatment of osteolitic diseases.

01.024 Human organotypic skin explant for *Mycobacterium leprae* **artificial cultivation** . Paula NA[1,2], Leite MN[2], Rosa PS[3], Das PK, Frade MAC[2] [1]FMRP-USP – Biologia Celular e Molecular, [2]FMRP-USP – Clínica Médica, [3]ILSL

Introduction: *Mycobacterium leprae* (*M. leprae*) was the first bacterial pathogen identified and was considered the cause of an infectious human disease, Leprosy. Since its discovery, numerous unsuccessful attempts were made to cultivate this *Mycobacterium in vitro* using various culture media. *In vivo*, murine experimental model has shown important advancement in *M. leprae* studies, but due to the limiting nature of this model along with the inefficiency of *M. leprae* culture *in vitro,* it is impossible to have a clear understanding of the basic aspects involved in the transmission, genetics and immunological factors that regulate resistance/susceptibility and therapeutics. Hence, in this study we purpose a new culture method an ex vivo model for *M. leprae*, using organotypic skin culture. **Methods**: *M. leprae* was inoculated intradermally in human skin fragments maintained in culture medium. The maintenance, growth and morphology of the bacilli in the explants were evaluated for histopathological techniques using specific staining and immunohistochemical analysis. After the culture periods, bacilli were recovered from explants and implanted into the paw of a nude mouse, to confer that the bacilli were alive and infective. **Results**: After 28 days in culture many bacilli were observed in the Fite-Faraco staining, where vast majority (more of 90%) were integral bacilli. On the 60th day many bacilli became fragmented, however 63.8 % integrate bacilli were visualized. Bacillus RNA was detected by RT-PCR in cultured samples up to 60th day. **Conclusion**: Our preliminary results show that it is possible to maintain viable *M. leprae* in an *ex vivo* skin culture. **Financial Support**: FAEPA, FAPESP. Human Ethical Committee: 1.744.888 Animal Ethical Committee: 026/2015-1

01.025 Changes of cardiorenal Na^+/K^+-ATPase triggered by chronic ouabain administration in rats are pressure-dependent. Feijó PRO[1], França-Neto A[2], Rossoni LV[2], Noël F[1], Quintas LEM[1] [1]ICB-UFRJ – Farmacologia Bioquímica e Molecular, [2]ICB-USP – Farmacologia

Introduction: Na^+/K^+-ATPase (NKA) is an integral membrane protein that carries Na^+ and K^+ against their electrochemical gradients through the hydrolysis of ATP and is involved in several physiological functions, such as Na^+ and water reabsorption, important for regulation of blood pressure. Ouabain, a cardiotonic steroid (CTS) that inhibits NKA activity, induces arterial hypertension in rats after chronic administration and modulates NKA in organs involved in controlling blood pressure, but this is still poorly understood. Moreover, the separate roles of ouabain and high blood pressure on NKA regulation is unknown. Our goal was to evaluate the effect of chronic treatment with ouabain on activity and expression cardiac, renal and cerebral NKA and Na^+/Ca^{2+}-exchanger (NCX1) and to assess these effects when blood pressure is under control. **Methods:** Male Wistar rats (6 weeks) were administered with ouabain (OUA, 8 µg/day s.c.), ouabain and antihypertensives (OUA+HH, hydrochlorothiazide 9.4 mg/kg + hydralazine 44 mg/kg), vehicle (VH) or vehicle plus antihypertensives (VH+HH) for 5 weeks. Kidneys, brain and both heart ventricles were dissected, weighed and stored at -80ºC. Then, half was homogenized and the pellets from ultracentrifugation were resuspended and used for enzyme activity experiments (NKA), and protein expression determined by Western blot technique. The other half was used to histologically evaluate the organs staining with hematoxylin-eosin and trichrome Masson. Values are expressed as mean ± SEM and statistical analysis was performed by One-way ANOVA followed by Dunnett posthoc test ($p<0.05$ was considered statistically significant). **Results:** There was a significant increase in systolic blood pressure, without cardiac and renal hypertrophy or histological alterations, in OUA group compared to VH (135.5 ± 0.6 vs. 118.3 ± 0.7 mm Hg, respectively; n=6, $p<0.05$), which was blocked in the OUA+HH group (116.1 ± 0.9 mm Hg). Only renal NKA activity increased in OUA group (VH: 9.1 ± 0.5 vs OUA: 11.8 ± 1.1 µmol Pi/mg/h, n=6, $p<0.05$), similar to what was observed for the expression of renal NKA α1 isoform (increase of 28%, $p<0.05$, n=5-6). We also detected an increase of 65% of α2 expression in the left ventricle and reduction of 61% of α3 in the brain in OUA groups compared to VH groups. More importantly, such effects have been completely abolished when antihypertensive drugs were administered and blood pressure normalized. We did not observe changes in the expression of NKA β1 isoform and NCX1 in these organs. **Conclusions:** Our work shows for the first time that in ouabain-induced hypertension model blood pressure is more important than ouabain per se for the modulation of cardiorenal NKA. The increased activity and expression of the renal NKA may explain, in part, the pressure effect of ouabain, since it increases sodium reabsorption and blood pressure. The increase in the cardiac NKA α2 isoform may be adaptive mechanism to reduce Ca^{2+} overload. **Financial support:** CAPES, FAPERJ, FAPESP and CNPQ. Approved by the Ethics Committee on Animal Experiments of ICB/USP (protocol: 034/2012).

01.026 Paracrine control of skeletal neuromuscular transmission by the extracellular cyclic AMP-adenosine pathway. Sanders-Silveira S, Duarte T, Pacini ES, Godinho RO Unifesp-EPM – Farmacologia

Introduction: In skeletal neuromuscular system, cyclic AMP (cAMP) represents a key source of extracellular adenosine. We have shown that activation of skeletal muscle receptors coupled to Gs protein increases the generation of intracellular cAMP which is followed by the cyclic nucleotide efflux (Godinho & Costa-Jr, Br J Pharmacol, 138:995, 2003). Outside the cell, cAMP is sequentially metabolized by ecto-PDEs and ecto-nucleotidases into AMP and adenosine (Chiavegatti T, *et al.*, Br J Pharmacol, 153:1331, 2008). Considering that adenosine is able to stimulate pre-synaptic A_{2A} receptors linked to enhanced ACh release from motor neuron (Ribeiro J.A, *et al.*, Prog Brain Res, 109:231, 1996), we investigated the possible influence of e*xtracellular cAMP-adenosine pathway* on the regulation of skeletal neuromuscular transmission. **Methods:** Mouse phrenic nerve stimulation was used to assess neuromuscular transmission when hexamethonium, a presynaptic nicotinic ACh receptor antagonist, was given to block neuromuscular transmission. We analyzed the effects of drugs on the train-of-four (TOF) ratio, which is the quotient between twitch tension of diaphragm muscle produced by the 4^{th} and the 1^{st} stimulus (T4/T1) within 4 consecutives 2 Hz stimuli of phrenic nerve (n=3-4). The effect of β_2-adrenoceptor agonist 100 nM formoterol and 100 µM cAMP was evaluated on 1.5 mM hexamethonium-induced partial neuromuscular block. The involvement of cAMP efflux and extracellular cAMP-adenosine pathway on the neuromuscular transmission was assessed by incubating nerve-muscle preparation with adenosine receptor (AR) antagonist CGS15943 (100 µM), ecto-5'-nucleotidase inhibitor AMP-CP (100 nM) and the organic anion transporter inhibitor Probenecid (300 nM), prior to the addition of formoterol or cAMP. The effect of formoterol on intra- and extracellular cAMP was quantified by time resolving-FRET assay. **Results:** Incubation of phrenic-diaphragm preparation with hexamethonium promoted a 25% reduction in the TOF ratio. On the other hand, pre-incubation of nerve-muscle preparation with formoterol increased by 15% the amplitude of muscle contraction and prevented tetanic fade promoted by hexamethonium, which was respectively associated with increment of intra- and extracellular cAMP levels. The hexamethonium effect was also prevented by cAMP pretreatment. Inhibition of either cAMP efflux, with probenecid, or cAMP degradation into adenosine with AMP-CP, prevented the effect of formoterol on hexamethonium-induced tetanic fade. The AR antagonist CGS15943 also prevented the effect of either formoterol or cAMP on hexamethonium-induced tetanic fade. **Conclusion:** β_2 adrenoceptor agonists are able to increase the extracellular cAMP levels, which culminates in the prevention of hexamethonium-induced neuromuscular blockade. This effect depends on the cAMP efflux, its extracellular degradation and the activation of presynaptic ARs and probably involves increment in ACh release. The identification of the paracrine action of *the extracellular cAMP-adenosine pathway* may allow us to explore this signaling system as a pharmacological target for development of drugs designed to improve neuromuscular transmission. **Financial Support:** Capes, CNPq and FAPESP **Animal Ethics Committee:** CEUA 1389121115

01.027 Integrative meta-analysis identifies candidate drug targets based in MicroRNA-regulated networks in cancer-cachexia. Freire PP[1], Cury SS[1], Oliveira G[1], Moraes LN[1], Moraes D[1], Marques DVP[1], Lopes LO[1], Reis PP[2], Dal-Pai-Silva M[1], Carvalho RF[1] [1]IBB-Unesp – Morfologia, [2]FCMBB – Cirurgia e Ortopedia

Introduction Cancer cachexia is a distressing, multifactorial and frequently irreversible syndrome that affects around 50–80% of cancer patients, depending on the tumor type, and that leads to significant weight loss. Since cachexia affects up to 20% of cancer deaths, understanding the underlying molecular mechanisms is essential. Unfortunately, besides the efforts to identify the key mechanisms of cachexia-induced muscle atrophy, there is no effective therapy to stop or reverse the cachectic process. Therefore, a critical need remains to identify novel molecular mechanisms of skeletal muscle atrophy in cancer cachexia. Currently, microRNAs (miRNAs) have been shown as gene expression regulators with an important role and new therapeutic potential in several muscular diseases. The purpose of this investigation was to identify potential drug targets in consistent miRNA-mRNA expression networks associated with cancer-cachexia. **Methods** We performed a meta-analysis of previously published gene expression datasets including 59 cancer-cachexia samples. Deregulated genes (validated) were further used to identify miRNAs as potential regulators of gene expression in cancer-cachexia. Data were integrated using bioinformatics methods, and genes were mapped in proteins, which were then used to construct protein-protein interaction networks (PPI). Moreover, using Drug-Gene Interaction Database, we performed an identification of potential compound-target interactions that comprise existing curated databases as well as novel compound-target interactions that are supported by literature evidence. **Results** The deregulated genes identified play roles in regulation of apoptotic process, negative regulation of muscle hypertrophy, regulation of catabolic process, and positive regulation of cell death. Regulatory networks identified 96 validated miRNAs, including the miRNAs miR-17, miR-26a, miR106b, miR-139, miR-20a, miR-223, miR-362, and miR-93; these miRNAs showed the higher number of interactions with deregulated genes, suggesting that they may have a key role in the molecular mechanisms of the syndrome. We also identified potential target agents for myostatin and calcium/calmodulin-dependent protein kinase II that may be useful for the development of novel therapeutic strategies for patients with cancer-cachexia. **Conclusion** MicroRNA-regulated networks may play a role in the development and progression of cancer cachexia and reveal potential target drugs for the development of novel therapeutic strategies for the syndrome. **Financial Support** This study was supported by CNPq process 141919/2016-7.

01.028 Biochemical and pharmacological characterization of Angiotensin II AT1 receptors containing mutations associated. Simoes SC[1], Silva ALB[1], Parreiras-e-Silva LT[1], Costa-Neto CM[1] [1]FMRP-USP – Biochemistry and Immunology

Introduction: G protein-coupled receptors (GPCRs) are integral membrane proteins characterized by seven transmembrane alpha-helices. These receptors are important targets of biomedical studies and approximately 40% of the currently marketed drugs act on these receptors. The type 1 angiotensin II receptor (AT_1) is a GPCR that recognizes the octapeptide Angiotensin II (AngII), being considered the main mediator of the Renin-Angiotensin System. Recently, the mutations A244S and I103T-A244S in the AT_1 receptor have been identified in a group of male patients with atrial fibrillation which could be related to higher predisposition to the occurrence of atrial fibrilation. Objective: To produce the above mentioned AT_1 receptor mutants through site-directed mutagenesis and characterize them biochemically and pharmacologically. **Methods:** In this study, site-directed mutations were performed in the coding sequence of AT_1 receptor to generate 3 mutants: AT1-I103T, AT1-A244S and AT1-I103T-A244S. After heterologous expression of the different receptors on HEK293T cells and activation with AngII, the activation profile of these receptors was compared to that obtained for the wild-type AT1 receptor on competition binding assays with radioactive ligand, mobilization of intracellular calcium using FLIPR™, activation of Gq and Gi proteins and recruitment of β-arrestins 1 and 2 by BRET, and ERK phosphorylation by Western blotting. **Results:** Mutants showed significant differences in potency and efficacy for the different pathways when compared to the wild-type receptor. For the I103T mutant, AngII showed similar affinity to that of the wild-type receptor (pIC50 = 8.52 ± 0.10, and 9.018 ± 0.14 respectively), but less efficacy for calcium mobilization (Emax = 66%) and recruitment of β-arrestin 1 (Emax = 36%). For ERK phosphorylation, this mutant was 3-fold more efficient than the wild-type receptor. The mutants A244S and I130T-A244S presented reduced recruitment efficacy of β-arrestin 1 (Emax = 36% and 32% respectively) and β-arrestin 2 (Emax = 67% and 48.5%, respectively), and similar efficacy to the wild-type receptor for ERK phosphorylation. Discussion and **Conclusion:** The contribution of β-arrestins in cardiac protection has been described in the literature. Thus, our results suggest that the low capacity of mutants AT1-I103T, AT1-A244S and AT1-I103T-A244S in recruiting β-arrestins may be one of the factors involved in a greater predisposition to the development of atrial fibrillation in individuals who carry these mutations. Further studies are ongoing to a better understanding of the effect of these signal transduction mechanisms and their possible correlation with the development of atrial fibrillation. **Financial Support**: FAPESP, CAPES, FAEPA.