01. **Cellular and Molecular Pharmacology**

01.001 LDT3 and LDT5 prevent prostate contraction and have antiproliferative effect in human prostate cells. Nascimento-Viana JB[1], Carvalho AR[1], Chagas-Silva F[1], Romeiro LAS[2], Souza PAVR[3,4], Nasciutti LE[3], Noël FG[1], Silva CLM[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]UnB – Desenvolvimento de Estratégias Terapêuticas, [3]UFRJ – Biologia Celular e do Desenvolvimento, [4]Hospital Geral do Andaraí RJ – Urologia

Introduction: Previously, a new *N*-phenylpiperazine was synthesized and the pharmacological evaluation unveiled a potent α_1-adrenoceptor (AR) antagonist, being selective for α_{1A} and α_{1D}-AR (Romeiro et al., Eur. J. Med. Chem. 2011). New *N*-phenylpiperazine analogues (LDT series) with substitutions at the main pharmacophoric subunit showed high affinity for $\alpha_{1A/D}$-AR and 5-HT_{1A} receptors acting as a multi-target antagonists. The lower urinary tract symptoms are the major complaint among aging men and they are usually caused by benign prostatic hyperplasia (BPH), a urological disorder which the usual pharmacological treatment is the use of the α_{1A}-AR antagonist tamsulosin. Recently, α_{1D}-AR and 5-HT_{1A} receptors antagonists have also been considered for the treatment of BPH since they have a prostatic anti-proliferative effect. Therefore, the objective of this study was to evaluate *in vitro* the affinity for prostate α_{1A}-AR, the cell antiproliferative effect and the blood pressure effect of LDT3, LDT5 and LDT8.

Methods: All protocols were approved by the Ethics Committee of UFRJ (CAAE-0029.0.197.000-05 and DFBC/ICB011). Functional studies: isometric contraction experiments were performed using rat prostate (α_{1A}-AR). Prostate strips were contracted with phenylephrine (PE) (1-10,000 nM) before and after incubation with LTDs or tamsulosin (10 nM, 1h). The results were analyzed by non-linear regression to calculate the parameters EC_{50} and E_{max}. The K_B value was calculated using Schild equation. Cell proliferation and viability assays: DU-145 (human prostate cell line) or BPH primary stromal cell cultures were established and treated with 50 nM of LDT3, LDT5 and LDT8, or controls (BMY7378, α_{1D}-AR antagonist, or p-MPPF, 5-HT_{1A} receptor antagonist), in the absence or presence of 5-HT (1-3 μM) or PE (3 μM). We performed MTT and Trypan Blue exclusion assays 48 and 72h after treatment. Blood pressure assay: Male Wistar rats were anesthetized with pentobarbital sodium (60 mg/Kg, i.p.). Jugular vein and carotid artery were cannulated for administration of drugs (LDTs or tamsulosin, 100 μg/Kg) or connection of the pressure transducer, respectively. **Results and discussion:** Previous data of our group showed that LDT3 and LDT5 have high affinity for $\alpha_{1A/D}$-AR and 5-HT_{1A}receptors acting as multi-target antagonists with low affinity for others receptors analyzed, and none of the compounds proved to be toxic. Confirming previous data obtained in binding assays, all LDTs showed high affinity for α_{1A}-AR in isometric contraction assay (mean K_B value = 3.9 and 2.11 nM, LDT3 and LDT5 respectively, n = 5) preventing the contraction mediated by PE. In addition, pre-incubation with these compounds prevented cell proliferation induced by PE and 5-HT in both cell lines (n = 5 assays performed in quintuplicate, $P < 0.05$, ANOVA followed by post hoc Dunnet test). Preliminary data showed that LDT3 reduces blood pressure similarly to that observed with tamsulosin. In conclusion, LDT3 and LDT5 are potential candidates as new prototypes for the treatment of BPH. **Acknowledgement:** CNPq, CAPES and FAPERJ.

01.002 Effects of neolignan 2,3-dihydrobenzofuran against *Leishmania amazonensis* **and its cytotoxicity in macrophages and hemolytic activity**. Oliveira LGC[1], Carvalho FAA[2], Amorim LV[3], Sobrinho Júnior EPC[3], Lima DS[1], Vieira MM[4], Sousa-Neto BP[3], Brito LM[3], Carvalho CES[3] [1]NPPM-UFPI, [2]UFPI – Bioquímica, [3]UFPI, [4]UFPI – Biotec

Introduction: The ring system of 2,3-hydrobenzofurano constitutes the main skeleton of a large number of natural neolignan products. Natural that have been identified for a range of biological activity. Leishmaniasis is characterized by infections in which the etiological agent is an intracellular hemoflagellate protozoan of the genus Leishmania, family Trypanosomatidae and order Kinetoplatida, having a scourge and two genomes: nuclear DNA and cinetoplastidea DNA (kDNA). **Objectives:** To evaluate the anti-leishmania, cytotoxic and hemolytic activities of 2,3-dihydrobenzofuran. **Method:** Promastigotes forms of *Leishmania amazonensis* were distributed in 96-well plates with the following concentrations of natural neolignan to evaluate the inhibitory activity of 2,3-dihydrobenzofuran (1600, 800, 400, 200,100, 50, 25 and 12.5 mg/mL); peritoneal macrophages from BALB/c were placed in 96 well with MTT containing RPMI plus the previously mentioned concentrations of the lupeol and read at 550 nm on a plate reader. Hemolytic activity was investigated by incubating 20 µL of lupeol in the concentrations above with 80 µL of a 5% suspension of human blood cells Red (O^+) for 1 hour at 37 °C in test tubes. **RESULTS:** 2,3-dihibrobenzofuran inhibited 71%, 69%, 67%, 65% of the growth of *L. amazonensis* in the form promastigotes respective concentrations of 1600, 800, 400, 200 µg/mL. Hemolysis was 0.27% and 0.14% at the concentration of 1600 µg/mL respectively 800 µg/mL and practically with no hemolysis at the concentrations studied. **CONCLUSION:** The present study shows that 1600 and 800 µg/ml 2,3-dihydrobenzofuran has anti-leishmania activity with discrete cytotoxicity and is not toxic at lower concentrations.

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01.003 **Towards the mechanism involved in telocinobufagin-induced cell death**. Amaral LS, Cunha-Filho GA, Noël FG, Quintas LEM[1] ICB-UFRJ

Introduction: Cardiotonic steroids (cardenolides and bufadienolides), considered specific Na^+/K^+-ATPase inhibitors, also promote the activation of signaling pathways via Na^+/K^+-ATPase. The signaling Na^+/K^+-ATPase interacts with neighboring membrane proteins such as Src, epidermal growth factor receptor (EGFR), and caveolins, leading to activation of multiple signal transduction pathways. We have recently shown that the bufadienolide telocinobufagin (TCB), in sharp contrast to its close congener marinobufagin (MBG), now considered mammalian steroid hormones, promotes cell death probably by apoptosis (high Bax/Bcl-2 ratio). Our aim is to investigate the mechanisms underlying this effect. **Methods:** LLC-PK1 cells (porcine proximal renal tubule cell line) were treated for 48 or 72 h with 1, 10 and 100 nM TCB, with or without the presence of MEK (U0126, 10 μM), Src (SU6656, 10 μM), p38 (SB202190, 10 μM), JNK (SP600125, 1,5 μM) or AKT (LY2940025, 5 μM) inhibitors. The evaluation of cell viability/quantity was measured by MTT analysis or counting the number of Trypan blue-viable LLC-PK1 cells after 72 h. Cell cycle examination of LLC-PK1 cells was determined by flow cytometry after 48 h. We performed lactate dehydrogenase (LDH) release assays for necrosis analysis after 72 h of TCB treatment. Cholesterol depletion was obtained by treating the cells with 10 mM methyl-β-cyclodextrin (MBCD) for 30 min at 37°C and then cells were incubated with or without 100 nM TCB in the presence of 0.5 MBCD for 72 h. **Results:** At 100 nM, TCB reduced the cell number by 73% when compared to untreated cells ($p<0.05$, $n=4$). SU6656 or U0126 did not affect cell death caused by TCB. Inhibition of other pathways (p38, JNK and AKT) also did not prevent TCB effect. Treatment with the caveolar disrupter MBCD partially prevented TCB effect (51%, $p<0.05$, $n=3$). TCB 100 nM treatment increased cells in the S phase (49%, $p<0.05$, $n=3$) of the cell cycle, with a concomitant decrease in the proportion of those in the G2/M phase (60%, $p<0.05$, $n=3$). TCB did not affect LDH release. **Discussion:** Src-evoked signaling cascades, like MEK/ERK (which mediates MBG-induced cell proliferation), and other pathways as PI3K/AKT, JNK and p38 are not related to the apoptotic effect of TCB. Nevertheless, TCB-induced signal transduction seems to be responsible, at least in part, for its cellular effect. Other possible mechanisms (e.g., GSK3) are still being investigated. **Financial support:** CAPES, Faperj, CNPq.

01.004 **New digoxin derivatives**: **Effects on Na^+/K^+-ATPase**. Silva NP, Noël FG, Quintas LEM CCS-ICB-UFRJ

Introduction: Cardiotonic steroids (CTSs) comprise a large group of naturally-originated substances. Their classic mechanism of action involves the inhibition of the Na/K-ATPase (NKA) enzyme activity. However, due to other newly discovered functions of NKA, such as signal transduction through protein-protein interactions, new CTS mechanisms of action are being established resulting, for example, in cellular apoptosis. Therefore, innovative therapeutic uses of NKA ligands have been considered in recent years. After Stenkvist *et al.* unexpected observation on the therapeutic effect of the CTS digitoxin on breast carcinoma in the 1980s, many studies have investigated the antitumor effect of CTSs, indicating a growing interest in the development of CTSs as antitumor agents. Our study intends to evaluate the effects of new digoxin derivatives (DGB1 to DGB7) on NKA activity and cell proliferation. **Methods:** DGBs were obtained through organic synthesis with addition of functional groups to their lactone ring. For the enzymatic experiments, the colorimetric determination of Pi released by ATPasic reaction was carried out using increasing concentrations of each DGB. After incubation of 2 h, the reaction was stopped by addition of ice-cold Fiske solution. Membrane preparations of rat brain (containing digoxin-resistant α1 and also digoxin-sensitive α2 and α3 isoforms) and human kidney (digoxin-sensitive α1) were used. The evaluation of cell proliferation was measured by counting the number of Trypan blue-viable porcine proximal renal tubule LLC-PK1 cells treated with DGBs for 24, 48 and 72 h. **Results:** The standard drug digoxin inhibited in a biphasic manner the different brain isoforms (IC50 = 0.22 ± 0.04 μM, α2/α3, 72 ± 9 μM, α1). We observed that DGB1 and DGB7 did not inhibit the enzyme even at a high concentration (100 μM), DGB2 and DGB4 inhibited the high sensitivity NKA component with a potency similar to digoxin (IC50 = 0.46 ± 0.10 μM and 0.66 ± 0.20 μM, respectively), while DGB3, DGB5 and DGB6 were less potent (IC50 = 18 ± 10 μM, 20 ± 7 μM and 3.9 ± 1.4 μM, respectively). With human kidney only DGB1-4 have been studied so far and we observed an inhibition profile similar to that observed in the brain (DGB1, no effect; DGB2, IC50 = 0.57 ± 0.10 μM; DGB4, IC50 = 0.21 ± 0.07 μM) and digoxin (IC50 = 0.29 ± 0.02 μM). At the moment, cell proliferation studies revealed no DGB1-3 effect at 1, 10 and 100 nM. **Discussion:** The results showed that DGBs present distinctive inhibition patterns which could be of therapeutic interest. The investigation of whether these effects affect cell proliferation and viability is in progress. **Financial Support:** FAPERJ, CNPq, CAPES.

01.005 Snakebites envenomation and alternative serotherapy by camelid nanobodies. Prado NDR[1], Pereira SS[1], Moraes MSS[1], Moreira-Dill LS[2], Luiz MB[1], Kayano AM[2], Pereira-da-Silva LH[1], Soares AM[2], Stabeli RG[1], Fernandes CFC[1] [1]Fiocruz Rondônia, [2]UNIR – Estudos de Biomoléculas Aplicado a Saúde

In Brazil, venomous snakes have been classified into *Bothrops, Crotalus, Lachesis* and *Micrurus* genera. The *Bothrops* genus comprises more than 60 species and causes about 73.5% of reported envenoming by snakebites (1). Two myotoxic phospholipases were isolated from *B. jararacussu* venom, bothropstoxin I and II (BthTX-I and BthTX-II). Although both toxins participate in the damage caused by *B. jararacussu* poisoning, as hemorrhage, edema and necrosis, BthTX-I presents no enzymatic activity, while BthTX-II is enzymatically active (2, 3). Heterologous serum produced in horses remains the standard treatment for snake bite envenoming. Despite high efficacy, this treatment is not able to reverse effectively local damage caused by snake envenoming, making necessary to search for new therapies (4, 5). Camelids, in addition to conventional antibodies, produce functional immunoglobulins G devoid of light chains, in which the antigen binding site is formed only by the single domain called VHH or nanobody. Besides small size and neutralization capability, VHHs presents thermal and pH stability, low immunogenic potential, and low cost production. Considering the camelid VHH characteristics, this work aimed to produce and to characterize VHH fragments against BthTX-I and II. For that, a VHH immune library was constructed employing the phage display technology. VHHs regions were isolated by PCR using peripheral lymphocyte cDNA obtained from one *Lama glama* immunized with BThTX-I and II toxins (Animal Ethic Committee: 03/2012). Subsequently, amplicons were cloned into PHEN-1 phagemid using TG1 *E. coli* strain, and the primary library infected with M13K07 helper phage to display VHH fused to phage coat protein (PIII). After selection step performed on immobilized BThTX antigens, 26 and 6 clones recognized BThTX-I and BthTX-II, respectively, by ELISA. Only the clone KC329718 was able to recognize both toxins. To characterize the clones *in silico*, the complementarity determining regions (CDRs), frameworks (FRs) and the known camelid VHH hallmark of ten distinct amino acid sequence profiles were determined. Clone KC329718 was transformed in a non-supressor *E.coli* strain HB2151 and purified by affinity chromatography using Ni-NTA column. Affinity between VHH and BthTX-I and II was demonstrated by surface plasmon resonance analysis. Selected VHHs could be a powerful strategy to improve the treatment of snake poisoning. Further experiments have to be performed to verify the neutralization capability of identified VHHs. **References:** 1. BRASIL. Ministério da Saúde. Acidentes por Animais Peçonhentos, 2009. 2. Homsi-Brandeburgo, et al., Toxicon, v.26, p.615-627, 1988. 3. Kashima, et al., Biochimie, v. 86, p. 211–219, 2004. 4. Espino-Solis, et al., Journal of Proteomics, v. 72, p. 183–199, 2009. 5. Cardoso, et al., Animais peçonhentos no Brasil. São Paulo, 2° edição, Sarvier, 2003. **Financial Agencies**: FINEP, CNPq **Acknowledgments**: CAPES

01.006 The *N*-phenylpiperazine LDT66 is a competitive antagonist of alpha$_{1A}$-adrenoceptors and an inhibitor of human prostate cell proliferation. Chagas-Silva F[1], Nascimento Viana JB[1], Romeiro LAS[2], Souza PAVR[3,4], Nasciutti LE[3], Noël F[1], Silva CLM[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]UnB – Desenvolvimento de Estratégias Terapêuticas, [3]UFRJ – Biologia Celular e do Desenvolvimento, [4]Hospital Geral do Andaraí RJ – Urologia

Introduction: Benign prostatic hyperplasia (BPH) is a prevalent disorder in elderly and involves age-related proliferation of prostate stromal and glandular cells, where both alpha$_{1D}$-adrenoceptors (AR) and 5-HT$_{1A}$ receptors are implicated. Previous binding data showed that LDT66, an *N*-phenylpiperazine derivative, is a high affinity antagonist of alpha$_{1A/D}$-AR and 5-HT$_{1A}$ receptors. Tamsulosin, a selective alpha$_{1A}$-AR, is approved for treatment of BPH due to prostate relaxation and relief of the urinary symptoms, but it does not prevent cell proliferation. Present study addressed: i) determination of the affinity of LDT66 for prostate alpha$_{1A}$-AR, ii) LDT66´s effect on blood pressure and iii) evaluation of the potential anti-proliferative effect in cultured human prostate cells. **Methods:** Functional studies and human cell cultures protocols were approved by the ethics committee of UFRJ (DFBC-ICB011 and CAAE-0029.0.197.000-05, respectively). **Isometric contraction experiments:** strips of rat ventral prostates (alpha$_{1A}$-AR; *Nanda et al. Eur.J. Pharmacol. 607:213, 2009*) were contracted with phenylephrine (PE) (0.01 to 10000 µM), in the presence of 0.1 µM propranolol, before and after incubation with LDT66 (10 - 100 nM; 37ºC). Data were individually analyzed by nonlinear regression (GraphPad Prism 5.0) to calculate the EC_{50} ratio (CR) values obtained before and after treatment with LDT66. The K_B values were obtained by the Schild regression**.** Mean arterial pressure (MAP): The effects of LDT66, prazosin (pzs, a non-selective alpha$_1$-AR antagonist) and tamsulosin (100 µg/kg, i.v.) were evaluated **on the MAP of adult rats using an invasive pressure transducer placed in the carotid artery.** Cell viability and proliferation assays: **Th**e evaluation of cell viability and proliferation were measured by MTT and Trypan blue exclusion assays, respectively. Human prostate cancer cell line (DU-145) and BPH stromal cells were treated with LDT66 50 nM, in the absence or presence of 5-HT (1-3 µM) or 3 µM PE for 48h. Specific antagonists of each receptor studied (BMY 7378 for alpha$_{1D}$-AR and *p*-MPPF for 5-HT$_{1A}$ receptor) were used as positive controls. **Results and discussion:** In rat prostate, LTD66 caused a parallel concentration-dependent shift of the log-concentration curve induced by PE to the right. Schild regression resulted in a mean K_B value of 3.4 nM, and a slope close to unity (n=7-8), suggesting a competitive antagonism. These data are in agreement with the affinity estimated by binding assays. Preliminary results on blood pressure showed that LDT66 was less prone to reduce the MAP in anesthetized rats than pzs and tamsulosin at the same dose (n=2). Cell-based assays showed that LDT66 prevented DU-145 and BPH cell proliferation induced either by PE or 5-HT, suggesting an inhibition of signaling pathways of alpha$_{1D}$-AR and 5-HT$_{1A}$ receptors (n=8 replicates using two different cultures, $P< 0.05$). Thus, our results suggest that LDT66 could be considered as a new hit compound for BPH treatment through prostate relaxation and an anti-proliferative cell effect. **Supported by:** FAPERJ, CNPq and CAPES

01.007 Study of P2X7 antagonist treatment on wound healing process in rats. Castro AB, Magalhães JF, Souza BB, Raimundo JM, Bonavita AG UFRJ-Macaé

Introduction: Wound healing is crucial to maintain the anatomy and function of tissues. Some studies suggest that extracellular ATP released by cell in the injured tissue could have a paper in the wound healing process and tissue regeneration. The effects of extracellular ATP are mediated by the purinergic receptor being the P2X7 the most noteworthy. However the role of the P2X7 receptor in the wound healing process is not yet elucidated. Then this study evaluated the effect of BBG, a P2X7 receptor antagonist, treatment on the wound healing in rats. **Methods:** Female Wistar rats weighing 250-300g were anesthetized and the dorsal region was depilated and sterilized with a 70% alcohol solution. A 1 cm^2 excision of the skin was surgically made in each animal. The animals were separated in two groups. The control group was treated with saline (0,9%) and other group was treated for five consecutive days with BBG (100mg/kg) injected in the peritoneal cavity. The wound healing was evaluated by photos taken of the dorsal area for a period of 14 days and the images were analyzed using ImageJ software and represented as percentage of open wound. **Results and Discussion:** Our data showed that the treatment of animals with BBG did not affect the healing process of the wound when compared with control group. The percentage of open wound was of 57.5 ± 6,7% and 55.45 ± 6,3% to saline and BBG treated groups respectively, and both groups have the same total number of days to complete re-epithelization. These results suggest that P2X7 receptor would not role in the wound healing process, however new experiments are being performed to confirm these finding. **Financial Support**: FAPERJ, UFRJ

01.008 Characterization of new *N*-phenylpiperazine derivatives designed by homologation of the antipsychotic lead compound LASSBio-579. Pompeu TET[1], Moura BC[1], Alves FRS[2], Figueiredo CDM[1], Antonio CB[3], Herzfeldt V[3], Rates SMK[3], Barreiro EJ[2], Fraga CAM[2], Noël FG[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]LASSBio-UFRJ, [3]UFRGS – Ciências Farmacêuticas

Introduction: As part of a drug development project, we previously described a series of *N*-phenylpiperazine derivatives in the search for new atypical antipsychotics (Neves et al., *Bioorg. Med. Chem.*18:1925, 2010) with emphasis on LASSBio-579 whose pharmacological profile has been extensively characterized in vitro and *in vivo* (Neves et al., *Behav. Brain Res*. 237:86, 2013). LASSBio-579 is a dual D_2-like/5-HT_{1A} ligand with *in vivo* activity reassembling more clozapine than haloperidol but with a relatively low affinity for the 5-HT_{2A} receptor, classically considered as an important target for the atypical antipsychotics. The objective of present work was to characterize the binding profile of five new *N*-phenylpiperazine derivatives, designed using the homologation strategy for increasing the affinity at the 5-HT_{2A} receptor, and to select one compound for testing in two mice models of schizophrenia positive symptoms. **Methods:** Five new derivatives were obtained through the extension of the methylene spacer between the 4-chloro-*N*-phenylpyrazolyl and *N*-phenylpiperazine subunits of LASSBio-579. The binding profile of the compounds was evaluated using classical competition assays with [^{3}H]radioligands and membrane preparations either from rat brain synaptosomes or cells transfected with human receptors. The intrinsic activity at these receptors was evaluated using the GTP-shift assay. For the *in vivo* assays, the test compound was administered to mice (i.p.) 30 min before 4 mg/kg apomorphine (apomorphine-induced climbing assay) or 10 mg/kg ketamine (ketamine-induced hyperlocomotion assay). **Results and discussion**: Increasing the length of the space between the functional groups of LASSBio-579 proved to be appropriated since the affinity of these compounds increased 3 to 10-fold for the 5-HT_{2A} receptor, with no relevant change in the affinity for the D_2-like and 5-HT_{1A} receptors. The most promising derivative (1-(4-(1-(4-chlorophenyl)-1H-pyrazol-4-yl) butyl)-4-phenylpiperazine; LASSBio-1635) had ten-fold higher affinity (K_i = 0.65 μM) than its parent compound (LASSBio-579) for the 5-HT_{2A} receptor. A GTP-shift assay also indicated that it has the expected efficacy at the 5-HT_{2A} receptors, acting as an antagonist. The affinity of this compound for the alpha$_2$ receptors (K_i = 0.61 μM) was also increased being similar to the affinity for the D_2-like and 5-HT_{2A} receptors (K_i = 0.25- 0.80 μM) an attribute that could be beneficial for an antipsychotic since the GTP-shift showed that it has the right (null) intrinsic activity. Intraperitoneal administration of LASSBio-1635 prevented the apomorphine-induced climbing behavior in a dose dependent way (0.1 – 10 mg/kg i.p.) and prevented the ketamine-induced hyperlocomotion in mice at doses with no effect on the mice locomotor activity (1 and 3.0 mg/kg i.p.). Together, these results show that LASSBio-1635 could be considered as a new antipsychotic lead compound. **Acknowledgement: CNPq, CAPES and FAPERJ.**

01.009 **Melatonin inhibits $P2Y_1$ receptor-mediated leukocyte adhesion to endothelial cell**. Cardoso TC, Noël FG, Silva CLM UFRJ – Farmacologia Bioquímica e Molecular

Introduction: The activation of endothelial cells in an inflammatory process changes their phenotype increasing the expression of adhesion molecules that promote the adhesion and migration of immune cells (Carlos and Harlan, Blood 87:2068, 1994). Several biological molecules modulate this process. Melatonin reduces *in vivo* and *in vitro* the adhesion and migration of leukocytes to the endothelium (Lotufo *et al*., *Eur J Pharmacol*. 430:351 , 2001; Lotufo *et al*. *Eur J Pharmacol*. 534:258, 2006). The activation of purinergic receptor $P2Y_1$ present on endothelial cells by ADP increases the rolling of leukocytes to the endothelium, indicating the relevance of this receptor in the process of endothelial leukocyte adhesion (Zerr *et al*., Circulation 123:2404, 2013) . The objective of this study was to evaluate *in vitro* the putative effect of melatonin on leukocyte adhesion to endothelial cells induced by a $P2Y_1$ receptor ($P2Y_1R$) agonist. **Methods:** All protocols were approved by the ethics committee of the UFRJ (DFBCICB011). Male Wistar rats were anesthetized and euthanized. The mesenteric microvessels were isolated, minced and in a 24-wells plate. Tissues were maintained in DMEM supplemented with 20% fetal bovine serum, gentamicin, and incubated at 37 °C and 5% CO_2. After 48 hours the tissue was removed and the culture medium replaced every 48 hours until the cells reach confluence. Then, they were treated with pancreatin and transferred to a 96-wells plate (10^4 cells/well). After 48 hours, the medium was changed, and endothelial cells were incubated for 4 hours with vehicle (basal), melatonin (30 nM), the selective agonist of the $P2Y_1R$ 2MeSATP (60 μM) or melatonin (30 nM) plus 2MeSATP (60 μM). Rat mononuclear cells were isolated from blood obtained by cardiac puncture and purified by Ficoll-Paque Plus gradient (Oliveira *et al*., Plos One 6:e23547, 2011). At the end of the fourth hour, 10^4 mononuclear cells/well were added and incubated for 30 minutes. After this period the non-adherent mononuclear cells were removed by washing with PBS and four fields per well were randomly chosen and analyzed. The number of adherent mononuclear cells per field was determined by microcopy (Olympus IX71 microscope, 400X magnification). Data were expressed as mean and SEM. The differences between groups were analyzed by Analysis of Variance (one-way ANOVA) followed by Newman-Keuls test, considering $P < 0.05$. **Results and Discussion:** The treatment of endothelial cells with 60 μM 2MeSATP increased significantly the adhesion of mononuclear cells (33.13 ± 2.5, n = 33 fields, $P < 0.001$) compared with baseline (15.06 ± 1.1, n = 33 fields using two different cultures). The addition of 30 nM melatonin did not change basal values (20.76 ± 1.8, n = 21 fields), but prevented the effect induced by 2MeSATP 60 μM (19.6 mononuclear ± 1.8, n = 22 fields, $p < 0.001$). These preliminary results indicate that melatonin has an inhibitory effect on the stimulation of endothelial leukocyte adhesion promoted by the $P2Y_1$ receptor. The cellular signaling responsible for this effect is under investigation. **Acknowledgement:** CAPES, CNPq.

01.010 ***In vitro*** **effects of relaxin in early and late steps of rat spermatogenesis**. Pimenta MT, Francisco RAR, Porto CS, Lazari MFM Unifesp – Pharmacology

Introduction: Spermatogenesis is controlled by FSH, testosterone and paracrine factors produced by Sertoli cells. Our laboratory works with the hypothesis that the insulin-related peptide relaxin (RLN) plays an autocrine/paracrine in test is based on two main evidences: 1) RLN and its receptor RXFP1 are expressed in rat Sertoli cells, and RLN stimulates Sertoli cell proliferation (Cardoso et al. Reproduction 139:185, 2010; Nascimento et al., *Eur J Pharmacol*. 691:283, 2012); 2) The knockout of *Rln* gene in mice blunts spermatogenesis (Samuel CS et al. Lab Invest. 83:1055, 2003). RLN could affect spermatogenesis either by an indirect effect on Sertoli cells or a direct effect on germ cells. Our aim was to explore the role of RLN in spermatogenesis, using a co-culture of rat Sertoli and germ cells. **Methods:** All experimental procedures were approved by the Ethical Committee from UNIFESP (CEP 0937/10). Testis from 7-day old Wistar rats were enzymatically dispersed and cultured for 2, 5 or 8 days, in the absence or presence of 100 ng/mL RLN during the initial 48 h, on 60 mm dishes covered with a thin layer of Matrigel pre-incubated for 20 h with serum-free medium to remove diffusible components. Cell number and differentiation were analyzed by: 1) flow cytometry, after staining with propidium iodide to determine ploidy, anti-vimentin antibody, to distinguish somatic from germ cells, and antibodies against other germ cell markers, to identify different stages of spermatogenesis; 2) real time PCR for *Rln*, relaxin receptors and genes related to different stages of germ cell differentiation; 3) immunocytochemistry and immunofluorescence with markers for undifferentiated (promyelocytic leukaemia zinc finger protein, PLZF) and differentiated (c-KIT) pre-meiotic germ cells, meiotic germ cells (synaptonemal complex protein 3, SYCP3), and post-meiotic germ cells (Acrosin and Odf2). **Results:** Pre-meiotic, meiotic and post-meiotic germ cells were detected after 5 days in culture, even in the absence of RLN. Based on DNA content, flow cytometry identified four populations (HC, 1C, 2C and 4C). The ratio between germ and somatic cells fell drastically from culture days 2 to 5, and this fall was prevented by RLN. RLN especially preserved diploid germ cell population, and flow cytometry showed that RLN increased the number of PLZF and c-KIT positive germ cells after 5 days in culture. Gene expression, corrected by germ cell proportion, revealed that RLN concomitantly increased mRNA levels of *c-Kit* and *Odf2*. In addition, RLN seemed to favor organization of cells in tubular-like structures. **Discussion:** These findings suggest that RLN plays a role in spermatogenesis by increasing the number of pre-meiotic germ cells, increasing the expression of differentiation genes, and affecting testicular architecture. **Financial support:** FAPESP and CNPq.

01.011 G-protein coupled estrogen receptor-1 traffics between plasma membrane and nucleus and activates MEK-ERK-CREB signaling in primary cortical culture. Lopes DCF, Novaes LS, Santos NB, Duque EA, Wiezel G, Scavone C, Munhoz CD USP – Farmacologia

Introduction: GPER-1 (G-protein coupled estrogen receptor 1) has been suggested to mediate the rapid effects induced by estrogens in several tissues, including the central nervous system. In a physiological context, we aimed to investigate the cellular mechanisms that support the protection mediated by the rapid estrogen signaling pathway via GPER-1.

Methods: Primary cortical cultures were obtained from newborn female rats (P1-P4) as described previously (Ahlemeyer, *J Neurosci Methods*, 149:110, 2005). Mixed cultures were maintained in DMEM High Glucose media supplied with 10% Fetal Bovine Serum, 10% Horse serum, 2 mM L-glutamine and 0,1% penicillin/streptomycin. Enriched neuronal cultures were maintained in DMEM High Glucose media supplemented with 2% B27 and 0,1% penicillin/streptomycin. For immunofluorescence assays, cells were cultured in cover slips, fixed with 4% formaldehyde, and stained using a plasma membrane specific marker (pan-cadherine) and an anti-GPER-1 antibody as described previously (Piccioli, J Neurosci Res, 66:1064, 2001). Thus, primary cultures were treated at 8th day in vitro with vehicle (DMSO 0.001% v/v), 17β-estradiol (10 nM) or G1 (10 nM) in the presence or absence of G15 (50 nM). **Results:** Mixed cultures were composed by 15% to 20% neurons, 20% to 30% microglia, and 60% to 70% astrocytes. The phenotype of our enriched neuronal culture was 60% to 70% neurons, 10% to 20% microglia, and 20% to 30% astrocytes. Cortical cells in cultures expressed GPER-1 and this receptor was dispersed both in the cytoplasm and the perinuclear region of cells in the absence of the ligand (E2). At 10 minutes of E2 exposure, the perinuclear localization of GPER-1 was diminished and we observed an increase of GPER-1 in the cytosol. At 15 minutes, the receptor tended to be throughout the cytoplasm and in plasma membrane, suggesting that the perinuclear compartment is likely not the final destination of GPER-1, and at 30 minutes after E2, the GPER-1 was disperse again in cytosol, perinuclear and nuclear regions. When these cultures were stimulated with G1, GPER-1 trafficking occurred earlier than the one induced by E2 exposure (5 minutes). Regarding the intracellular pathways, we observed, in mixed cultures, an evident time course of MEK-ERK activation. The GPER-1 agonist G1 induced MEK activation on 5, 15, and 30 minutes while, downstream to MEK, ERK activation was in 10 minutes. Although we had observed MEK-ERK activation, we did not observe modulation of the transcription factor CREB in this culture phenotype. In enriched neuronal cultures both MEK and ERK were activated up to 5 minutes. Moreover, our preliminary findings suggested that GPER-1 participates of CREB rapid modulation induced by G1 in enriched neuronal cultures. **Conclusion:** Taken together, these findings suggest that the GPER-1 trafficking from the plasma membrane could be constitutive (occurring even in the absence of its ligand), and show the existence of rapid estrogen effects through GPER-1 in the brain, which is cell type-, and time-dependent. **Financial Support:** FAPESP **Animal Ethics Committee Number 50, Sheet 87, Book 02/2010.**

01.012 **Bufalin promotes epithelial to mesenchymal transition in LLC-PK1 cells**. Martins-Ferreira J, Ferreira LLB, Cunha-Filho GA, Quintas LEM, Noël FG ICB-UFRJ

Endothelial to mesechymal transition (EMT) is a phenomenon that happens at least in three situations: 1- during embryo development, 2- fibrosis and 3- cancer. It has been showed that Na^+/K^+ pump plays an important role in the mechanism (Rajasekaran, 2010). Cardiotonic steroids (CS), which includes cardenolides and bufadienolides, inhibit Na^+/K^+ pump and increase it's endocytosis. Also, it has been showed that use of CS triggers intracellular signaling pathways as MAP kinases. Bufalin (BFL) promotes Na^+/K^+ pump endocytosis in NT-2 cells. Another bufadenolide, marinobufagenin, promotes EMT in LLC-PK1 cells. Based on these information we aim to describe Bufalin´s effect in LLC-PK1 E-cadherin (E-cad) surface expression, signaling pathway activation and cell phenotype. **Methods**: LLC-PK1 cells (porcine proximal renal tubule) cultured in DMEM with 10% SBF were starved for 24 hours and treated, or not (control), with 1- 75 nM bufalin (BFL) for additional 24 hours and photographed in bright field. Retraction in cell membrane indicated a decrease in adherent proteins surface expression in BFL treated cells. We split control and treated cultures to new plates and accompanied the kinetics of cells adherence trough pictures taken 1, 2, 3, 12 and 30 hours after the seeding. In LLC-PK1 cells treated with 20nM BFL and control cells was performed biotinylation of surface protein followed by western blot to E-cad or cells were lysate with 2% SDS and used for western blot to measure expression of β-catenin and GSK-3 activity, which was determined by the ratio of phosphorilated/total GSK3 blot quantification. Immunofluorescence was performed in control and 20nM BFL treated LLC-PK1 stained with primary antibodies directed to E-cad (1:500) and β-catenin (1:300) followed by Alexa Fluor® 546 secondary antibodies (1:1.500).

Results and Discussion: LLC-PK1 cells treated with 20nM Bufalin for 24 hours displayed a fibroblastic morphology. Immunofluorescence detection of E-cad and β-catenin showed a redistribution of these proteins from membrane area to intracellular regions, this result suggests a decrease in surface expression for E-cad. Biotinylation of membrane surface proteins confirmed the decrease observed with immunofluorescence. Surface/total E-cad ratio decreased from 1.541 ± 0.0080 in control to 0.8788 ± 0.027 in 20nM BFL treated cells (n=3). β-catenin is required for E-cad membrane stabilization, we found 21 ± 4.36 % (n=3) diminution in its expression, that could contribute to E-cad decrease in membrane. β-catenin is phosphorylated by GSK3 and directed to degradation. We found a suggestive increase in GSK3 activation by 20nM BFL treatment. Besides, 10mM LiCl, a known GSK3 inhibitor, prevents the transformation of morphology of LLC-PK1 epithelial cells in fibroblastic morphology cells. We conclude that BFL triggers the mechanism of EMT in LLC-PK1 cells in culture in a way that depends on GSK3 pathway activation, β-catenin degradation and E-cadherin membrane destabilization and internalization. **Financial Support**: CAPEs; CNPq; FAPERJ.

01.013 Relaxin and Follicle-Stimulating Hormone (FSH) differentially affect signaling pathways and cell cycle gene expression in prepuberal rat sertoli cells. Nascimento AR, Lucas TFG, Porto CS, Lazari MFM Unifesp – Farmacologia

Introduction: Sertoli cells provide support and nutrition to germ cells and are essential for spermatogenesis. Each Sertoli cell supports a limited number of germ cells therefore it is important that the correct number of Sertoli cells is formed. Only immature Sertoli cells proliferate, and although thyroid hormone, retinoic acid and testosterone can induce cell differentiation *in vitro*, the mechanisms that determine the end of proliferation and the start of differentiation remain unclear. The insulin-related peptide relaxin is expressed in prepuberal rat Sertoli cells, and stimulates cell proliferation (Cardoso et al. Reproduction 139:185, 2010; Nascimento et al., *Eur J Pharmacol*. 691:283, 2012). Relaxin effects are mediated by RXFP1, a GPCR that belongs to the subfamily of leucine-rich repeat containing GPCRs (LGRs), which also includes the receptor for FSH, a major mitogen of Sertoli cells. Our aim was to investigate a possible interplay of relaxin and FSH to control Sertoli cell proliferation and differentiation.

Methods: Experimental procedures were approved by the Ethical Committee from UNIFESP (CEP1311/11). Sertoli cells were removed from 15-day old Wistar rats, and were cultured for 5 days. Cells were treated with FSH (100 ng/mL) or relaxin (50 ng/mL) or a combination of both, for different periods. Cyclic AMP was measured by enzyme immunoassay (GE Healthcare), ERK1/2 and AKT phosphorylation by Western blot and gene expression with the cell cycle PCR array from Qiagen, followed by validation with quantitative PCR. Only genes with at least a 2-fold increase or decrease in expression were selected for further analysis. **Results:** FSH markedly increased cAMP production (control: 13.37 ± 1.09 vs. FSH: 2,179.00 ± 494.30, N=5), whereas relaxin decreased cAMP production (control: 13.37 ± 1.09 vs. relaxin: 8.75 ± 1.84 fmol/well; N=4), after 30 min incubation. Relaxin causes a rapid and transient increase on ERK1/2 and AKT phosphorylation (Nascimento et al., *Eur J Pharmacol*. 691:283, 2012), whereas FSH progressively inhibited ERK1/2 (7-fold decrease for ERK1 and 4-fold decrease for ERK2) and AKT (3-fold decrease) phosphorylation. Pretreatment with FSH completely blunted the relaxin-induced ERK1/2 and AKT phosphorylation. Treatment with FSH for 4 hours markedly affected expression of genes involved either in cell proliferation and survival or in cell differentiation. The most affected genes were the antiapoptotic *Bcl2* (5-fold decrease), the protein phosphatase 2, regulatory subunit B", alpha, implicated in the negative control of cell growth and division (*Ppp2r3a*, 3-fold decrease), and the differentiation marker inhibin-A (*InhA*, 6-fold increase). All these changes were confirmed by quantitative PCR. On the other hand, relaxin did not affect most of the genes and only significantly, but marginally, decreased the expression of *Ppp2r3a* (1.9-fold). **Discussion:** At this particular stage of Sertoli cell development, relaxin stimulates while FSH inhibits signaling pathways that favor cell proliferation. We propose that the interplay between relaxin and FSH signaling in Sertoli cells may contribute to determine the end of cell proliferation and the beginning of cell differentiation.

Financial support: FAPESP and CNPq.

01.014 Effect of hydrogen peroxide in the metabolic activity and viability of adults stem cells. Machado AK[1], Cadoná FC[2], Homrich SG[3], Treichel TLE[3], Aramburú Jr JS[3], Pippi NL[3], Rodrigues CCR[3], Duarte MMMF[3], Saldanha JRP[3], Duarte T[3], Cruz IBM[1,2,3] [1]UFSM – Pharmacology, [2]UFSM – Toxicology Biochemistry, [3]UFSM – Biogenomics

Introduction: The stem cells have the capacity to differentiate in different cell lines (ANISIMOV et al, 2007). This feature makes these cells are used as treatment of various diseases such as diabetes and neurodegenerative disorders (HELMY et al, 2010). The hydrogen peroxide (H_2O_2) began to be used to induce cellular proliferation and differentiation of stem cells (ZHANG et al, 2012). However, whether this response is a universal condition is an open question. Thus, the aim of this research was to compare the effect of H_2O_2 in the metabolic activity and viability between mesenchymal stem cells (MSCs) derived from human and rabbit fat tissue and rabbit dental pulp. **Methods:** First the project was approved by the research ethics committee of the Federal University of Santa Maria, with the number 23081.015838/2011-10. So, MSCs were collected and isolated from human lipoaspirates fat tissue and from rabbit dental pulp and fat tissue obtained by surgery intervention. The MSCs were maintained culture medium at 37^{o}, 5% of CO_2. Samples of these cells were exposed to H_2O_2 at 1, 3, 10, 30, 100, 200, 300 and 1000 µM concentrations during 6 hours. The metabolic activity was evaluated by MTT assay and the cellular viability was determined by free DNA presented in culture medium measured by fluorimetry using PicoGreen® dye that present higher binding-specific to double-strand DNA molecules. **Results:** The cultures showed high cell growth, demonstrating the success of the cell isolation procedure. All MScs exposed to H_2O_2 treatment showed a positive dose-concentration effect of cell metabolic activity from > 100 µM when compared to untreated cells. On the other hand, despite the short time of H2O2 exposition a decreasing of cellular viability was obtained from > 300 µM in all cultures. **Discussions:** The results suggest that the H_2O_2 effect can be broadly shared by MScs cells from different source (tissues and species) and can be related to role of these cells when occur body injury that triggered inflammatory process that consequently increase the oxidative conditions in the lesion local. However, as the preconditioning H2O2 exposition is generally performed at 200 µM complementary studies need to be performed to evaluate if this concentration is not genotoxic. **References:** Anisimov, S. V. et al. *BMC Gen* v. 8, p. 1, 2007. Helmy, K. Y. et al. *Therap. Deliv***.** v. 1, p. 693, 2010. Zhang, J. et al. Chin. Med. J**.** v. 125, p. 3472-3478, 2012. **Financial Agencies:** CAPES, CNPq and FAPERGS. **Acknowledgments:** UFSM, CAPES, CNPq and FAPERGS.

01.015 New surgical procedure using permissive hypoxia in reperfusion decreases inflammation and edema from ischemia-reperfusion syndrome in hind limb of sheep. Neves DQ[1], Massucati-Negri M[1], Grees MAK[2], Castro DS[2], Ascoli FO[1], Marostica E[1] [1]UFF – Physiology and Pharmacology, [2]UFF – Veterinary Medicine

Introduction: In clinical practice are common situations with blood withdrawals of organs or limbs in the case of surgical procedures that require clamping of vessels over prolonged periods. The restoration of blood flow in ischemic tissues is essential for preventing irreversible damage. However, reperfusion itself results in an increase of free radicals resulting from sudden excess of oxygen, accompanied by local and systemic inflammatory response, increasing tissue injury that leads to ischemia-reperfusion syndrome (Li *et al*., Am J Physiol, 282 : C227, 2002). The arm of this work is to evaluate the impact of a direct method of permissive hypoxia on the ischemia-reperfusion syndrome in sheep. **Methods:** Adult Santa Ines sheep were divided in two groups (n=6/group): control (CG) and experimental (EG). After inhalation general anesthesia, ischemia was induced by related femoral artery occlusion in hind limb of the sheep during three and a half hours, followed by reperfusion. In CG, high PO2 was maintained during reperfusion, as in clinical practice, while in EG PO2 was maintained at around 60 mmHg, gradually returning to normal levels within 30 minutes (CEPA 19/2010). In addition to arterial gasometry and blood samples for biochemical analyzes, biopsies were harvested from skeletal muscle and artery after reperfusion for pathological analysis, morphometric studies and immunostaining assay for activated NF-kB. The values are mean ± SEM; Student "*t*" test, $P<0.05$. **Results:** Our preliminary data showed a decrease in the number of inflammatory cells in histopathological artery ischemic limb, as well as less immunostaining of NF-kB in local skeletal muscle, suggesting less activation of inflammatory mechanisms. In addition, morphometric studies showed less edema in GE (subjected to hypoxia), when compared to GC (skeletal muscle cell area: CG=1267.44 ± 159.54 um^2; EG=920.57 ± 92.11 um^2). **Discussion:** These results suggest that hypoxia permissive may diminish the impact of ischemia and reperfusion tissue. This study can contribute to the establishment of a new protective strategy that minimizes syndrome or ischemia-reperfusion, reducing the complications and amputations in clinical practice. **Financial Support:** FAPERJ, CNPq, PROPPi/UFF.

01.016 Effect of multifactorial malnutrition in rat vas deferens: **Ca^{2+} Modulators of alfa1-adrenergic signaling**. Bezerra CGP[1], Muzi-Filho H[2], Silva AMS[3], Zapata-Sudo G[3], Sudo RT[3], Einicker-Lamas M[2], Vieyra A[2], Lara LS[1], Cunha VMN[1] [1]UFRJ – Farmacologia e Inflamação, [2]IBCCF-UFRJ, [3]UFRJ – Pesquisa e Desenvolvimento de Fármacos

Introduction: It is generally accepted that multifactorial malnutrition leads to impairment of reproductive capacity in adult male rats through different mechanisms. The loss of reproductive capacity is probably associated with the atrophy of prostatic vas deferens and with adaptive changes of Ca2+ homeostasis, important to the ejaculatory function. According to our previous works changes of Ca2+ homeostasis seems to be related to modifications of oxidative status and *alfa*1- adrenergic receptor signalling at least in two different malnutrition models. The aims of the present study were: 1-evaluate the expression of Ca^{2+} regulatory proteins (Calmodulin (CaM) and calmodulin kinase II (CaM-KII) and 2- evaluate the contractile activity of the vas deferens after addition of increasing concentrations of an *alfa*1- adrenergic agonist.

Methods: Pregnant rats (Wistar) were fed with the regional basic diet (RDB) of the Brazilian Northeast, and their offspring were fed with a regular diet (RDB-IU); or male rats immediately after weaning were fed with RDB (RDB-CR) until 13 weeks of age. After this period, controls and malnourished male rats, were sacrificed (CEUA DFCBICB007), the pair of vas deferens (VD) was removed and an ultracentifuged homogenate (108000 x g) was obtained for biochemical assays. For contraction assays, the epididymal portion of RVD was dissected and placed within experimental vats filled with the nourishing solution (modified Tyrode). One of the extremities of the muscle was attached to a force transducer and the other one to a fixed rod. The signals generated by the force transducers were digitized and processed on a computer for later analysis using LabChart software. **Results:** Western Blot assays showed no change in the protein expression of CaM and CaMKII in RDB-CR and RDB-IU groups compared to the controls. The values of densitometric analysis were 14.5 (n=4); 13.6 (n=3); 13.8 (n=5) % for CaM and 11.8 (n=6) ; 12.0 (n=4); 12.6 (n=5) for CaMKII in RDB-CR; RDB-IU and control groups, respectively. The contractile response promoted by phenilephrine in epididimal vas deferens exhibited EC50 values of 1.2; 60.5 and 2.1 μM for RDB-CR; RDB-IU and control groups, respectively. **Discussion:** Our group has shown previously that the expression of alfa1-adrenoceptor is increased only in RDB-IU, despite of the increment of the activity of Ca2+ATPase observed in both groups of rats (RDB-IU and RDB-CR), indicating that some other component of alfa1-adrenergic signaling could be modified. However, as the present data shows that neither CaM nor CaMKII expressions are changed in vas deferens, we can postulate that the activity of both modulators could be altered. Changes of expression/activity of another modulator of Ca2+ should be also considered. Futhermore, it seems that the sensitivity to adrenergic agonist is decreased in RDB-IU rats. **Support:** FAPERJ *APQ1*; FAPERJ Primeiros Projetos

01.017 Daily variation of microRNAs expression in endothelial progenitor cells. Marçola M[1], Ramos CML[2], Parmigiani RB[2], Camargo AA[2], Markus RP[1] [1]IB-USP – Physiology, [2]IEP-HSL – Molecular Oncology
Introduction: Melatonin, the nocturnal hormone produced by pineal gland and by activated leukocytes in a non-rhythmic way, modulates endothelial cell activity. Recently, we have shown that plasma melatonin primes cultured endothelial cells, which has different phenotypes according to plasma melatonin at the hour of euthanasia. The activation of nuclear factor kappa B (NF-kB), expression of adhesion molecules and iNOS in endothelial cells in culture for 21 days are inversely correlated with plasma melatonin (Tamura, Plos One, 5-e13958, 2010; Marçola, J Pineal Res, 54:162, 2013). Here we evaluated whether melatonin could prime endothelial cells epigenetically, interfering on the expression of microRNAs (miRNAs), which are non-coding RNAs that regulate cell processes by inhibiting mRNA transduction. **Methods:** Cultured cells were obtained from cremaster muscle of rats maintained at 12h/12h light/dark cycle and killed during daytime (12h00) or nighttime (24h00). The muscle was cut into small pieces and cultured for 48h when tissue was removed and migrated cells maintained till confluence. Cell phenotype was determined by RT-PCR and imunofluorescence and miRNAs expression by whole-microRNA sequencing (Solid EZ Bead). The analysis of miRNAs differential expression was done by EdgeR and the predictable target genes were evaluated by TargetScan and Metacore software. All animal procedures were performed under the ethical conditions of our institute (license 124/2011). **Results:** Cells present high expression of CD133 (Ct mean=27.15 ± 0.12), low expression of CD34 (Ct mean = 34.2 ± 0.54) and von Willebrand factor (Ct mean=32.9 ± 0.34) and no expression of CD31 (Ct mean>35). This data was confirmed by immunofluorescence and suggests typical progenitor cells phenotype. In addition, nighttime cells present lower CD133 expression than daytime cell ($p<0.05$). The expression of 448 miRNAs was detected and nighttime cells express much more miRNAs amounts than daytime cells. Among seven miRNAs that are differently expressed (FDR<0.1), miR-96 (FDR=0.08) and miR-182 (FDR=0.009) are more expressed in nighttime cells and have fibroblast growth factor receptor substrate 2 gene (*Frs2*) as a predictable target, which is closely related to stemness. In addition, miR-146, known to inhibit inflammatory response, is also more expressed in nighttime cells, suggesting that NF-kB signaling is mediated by epigenetic control. **Discussion:** Our data strongly suggest that the method here employed favors the cultivation of endothelial progenitor cells, and that the plasma level of melatonin at the hour of death of the donor animal impose an epigenetic phenotype for the future culture. miRNAs expressed at nighttime confer a more quiescent state regarding to inflammatory response and development cell fate. Our findings contribute to the better understanding of progenitor cell biology and insert melatonin in the cell therapy field. **Financial support:** FAPESP (2011/01304-8), CAPES, CNPq.

01.018 Activation of the Kinin B1 receptor modulates pathways involved in protein metabolism and skeletal muscle mass control. Parreiras-e-Silva LT[1], Reis RI[1], dos Santos GA[1], Pires-Oliveira M[2], Pesquero JB[3], Gomes MD[1], Godinho RO[2], Costa-Neto CM[1] [1]FMRP-USP – Bioquímica e Imunologia, [2]Unifesp – Farmacologia, [3]Unifesp – Biofísica

Introduction: Regulation of muscle mass depends on the balance between synthesis and degradation of proteins, which is under control of different signaling pathways regulated by hormonal, neural and nutritional stimuli. Such stimuli are altered in several pathologies such as chronic obstructive pulmonary disease, diabetes, AIDS and cancer (cachexia), as well as in some conditions such as immobilization and aging (sarcopenia), leading to muscle atrophy, which represents a significant contribution to patient morbidity. The kallikrein-kinin system (KKS) is composed of the enzymes kallikreins, which generate active peptides called kinins that activate two G protein-coupled receptors, namely B_1 and B_2, expressed in a variety of tissues. The local modulation of the KKS may account for its described participation in different diseases, such as those of cardiovascular, renal and central nervous systems, cancer, and many inflammatory processes, including pain. Due to such pleiotropic actions of the KKS by local modulatory events and the probable fine-tuning of associated signaling cascades involved in skeletal muscle catabolic disorders (e.g. NFκB and PI3K/Akt pathways), we hypothesized that KKS could contribute to modulation of intracellular responses in atrophing skeletal muscle.

Methods: In the present study, *in vitro* and *in vivo* approaches were performed. C2C12 myotubes were treated with the B_1 receptor agonist des-Arg^9-bradykinin (DABK) and submitted to measurement of the myotubes diameters, immunocytochemistry and total RNA and proteins isolation. Balb-C mice, treated or non-treated with the B_1 receptor antagonist R-715 (0.5 mg/kg/day, i.p.) were submitted to gonadectomization for induction of levator ani (LA) muscle atrophy. After the desired periods of castration, the LA muscles were collected, weighed and submitted to total RNA and proteins isolation. C57BL/6 B_1 receptor knockout mice were also submitted to the same surgical procedures. Total RNA from myotubes and muscles were used for cDNA production and real-time PCR analysis of target genes and total proteins were used for Western Blot. Experiments with animals were approved by the local Ethics Committee, protocol number: FMRP-046/2006. **Results and Discussion:** Our data showed that the kinin B_1 receptor activation induced a decrease in C2C12 myotubes diameters and IGF-1 mRNA levels, increase in atrogin-1 and MuRF-1 mRNAs levels, translocation of NFκB to the nuclei and decrease in Akt phosphorylation. Castrated Balb-C mice showed an increase of B_1 receptor mRNA expression in LA muscle, which was paralleled by an increase in MuRF-1 nad atrogin-1 mRNAs. Treatment with the B_1 receptor antagonist, R-715, impaired the expression of MuRF-1 and atrogin-1 proteins in LA muscle of castrated mice. Moreover, *knockout* of B_1 receptor in mice led to an impairment of MuRF-1 mRNA expression after induction of LA muscle atrophy. We have obtained pharmacological and genetics evidence that the KKS plays a significant role in regulation of muscle proteolysis. Financial Support: FAPESP, CAPES, CNPq and FAEPA.

01.019 Effect of antipsychotic drugs on GSK-3β signaling in SH-SY5Y human neuroblastoma cells. Pompeu TET, Liquori DMS, Noël FG ICB-UFRJ

Introduction: Recently, some authors have shown that the dopamine D2 receptors can signal by activating AMPc-independent mechanisms involving β-arrestin 2 and activate different signaling cascades, as the Akt/GSK-3β pathway (Beaulieu et al., *Front. Mol. Neurosc.* 4(38):1, 2011). Activation of the D2 receptor (D2R) by dopamine promotes the formation of a signaling complex composed by Akt/β-arrestin 2 and PP2A (serine/threonine protein phosphatase 2A) which inactives Akt and activates GSK-3β (BEAULIEU et al., *Cell* 122:261, 2005). Several antipsychotics can activate Akt and thus inhibit (phosphorylate) GSK-3β in rodent brain and cell cultures (Sutton et al., *Neurosc.* 199:116, 2011; Park et al., *Neuropharmacol*. 61:761, 2011). GSK-3β is associated to numerous functions like differentiation and cellular development, apoptosis and gene transcription. Besides, decreases in phosphorylated GSK-3β protein levels have been reported in the brain of schizophrenic patients (EMAMIAN et al., *Nature Genetics* 36(2):131, 2004). The aim of this study was to evaluate the different effects of clozapine, haloperidol, lithium and the *N*-phenylpiperazine antipsychotic lead compound LASSBio-579 on GSK-3β in SH-SY5Y cells. **Methods**: In the acute protocol, we used SH-SY5Y cells differentiated or not into dopaminergic neurons by a 6-days treatment with retinoic acid and TPA (phorbol ester) (Presgraves et al., *Neurot. Res.* 5:579, 2004). These cells were treated with clozapine (10 μM - 15 min or 1h), LASSBio-579 (10 μM - 1h) or lithium (10 mM - 3h). In the prolonged protocol, undifferentiated SH-SY5Y cells were treated for 96h with clozapine (10 μM), haloperidol (5μM), LASSBio-579 (5 μM) and lithium (1 mM). Following the treatment, the cells were lysated and 20 μg of protein were resolved on a polyacrylamide gel. The membranes (PVDF) were incubated with antibodies against phospho-GSK-3β, total GSK-3β, anti-D2DR and the proteins quantified by densitometry. **Results and discussion:** The differentiation protocol was successful since our western blot analysis indicated a 100% increase of the D2DR density. In these cells, the acute treatment with the antipsychotics had no effect since the levels of phosphorylated and total GSK-3β were similar (n=4-6). On the other hand, lithium increased by 15.5% the level of the phospho-GSK-3β ($p<0.05$, n=6). Preliminary results with the prolonged protocol indicated that clozapine and lithium could increase the phosphorylation of GSK-3β without changing the level of the total GSK-3β whereas LASSBio-579 and haloperidol had apparently no effect. Financial Support: CNPq, CAPES, INCT-INOFAR, FAPERJ.

01.020 Nanobodies of camelid assets against crotoxin, a neurotoxin of the snake *Crotalus durissus terrificus*. Luiz MB[1], Prado NDR[1], Pereira SS[1], Moreira-Dill LS[2], Kayano AM[2], Soares AM[2], Stabeli RG[1], Fernandes CFC[1] [1]Fiocruz-RO – Tecnologia de Anticorpos/Genética, [2]CEbio-Fiocruz

In Brazil, more than 20,000 cases of snakebite envenoming occur each year. From these, about 9.2% of the injuries are caused by snakes of the genus Crotalus, which originate a mortality rate of 1.87% (1). Neurotoxic, nephrotoxic and myotoxic effects of *Crotalus* envenoming are mainly related to the crotoxin (CTX) (2), a dimer formed, via noncovalent interactions, between the phospholipase A2 (CB), basic and enzymatically active, and crotapotin (CA), acid and enzymatically inactive (3). Treatment in case of poisoning is performed by administering immunobiologics derived from hyperimmunized horses (4). Besides high cost, due to the need for specialized animal care, and nonlinearity between production batches, immunoglobulins of non-human origin can cause hypersensitivity reactions. Thus, the search for alternative methods that can minimize the disadvantages of conventional serum therapy has becomming relevant. Camelids produce functional antibodies devoid of light chains, in which the antigen recognition region is formed by the single domain called VHH or nanobody. In addition to thermal and pH stability, important for field treatment, nanobodies are one tenth the size of conventional antibodies, cause low immunogenicity, is capable to neutralize animal toxins and can be produced in microorganisms. Exploring these advantages, this work aimed to produce VHH fragments of *Lama glama* that specifically recognize the crotoxin. For this, the phage display technology was employed. After monitoring the immune response of a *Lama glama* immunized with monomers CA and CB and CTX by ELISA (Animal Ethic Committee: 03/2012), VHHs fragments were amplified by RT-PCR using cDNA synthetized after RNA extraction from peripheral lymphocytes of the animal. The amplified product was inserted into the phagemid (PHEN1) and a VHH library with a titer of 3.6 $X10^{12}$ was constructed using *E.coli* TG1. After infection of primary library with helper phage M13K07, VHHs expressed on the surface of the bacteriophage were selected using immunotubes previously adsorbed with crotoxin, CA and CB. Two clones recognized CA, while 76 and 58 clones recognized CTX and CB by ELISA, respectively. Clones that showed greater reactivity against the selected targets were sequenced and characterized *in silico.* Further experiments aiming to measure the affinity of the clones (Surface Plasmon Ressonance) and to verify the toxin neutralization ability will be performed. According to preliminary results, VHHs anti-CTX could be safe and cost-effective tools to contribute in the treatment of envenoming by Crotalus snakes. **References:** 1. Brasil, Manual de Diag e Tratam de Acid por Animais Peçonhentos, 7, 131, 2001. 2. Sampaio, Toxicon, 55, 1045, 2010. 3. Chippaux, J. P. Toxicon, 36, 1998, 823. 4. Faure, J. Molec. Biology, 412, 176, 2011. 5. Wesolowski, J. Med Microbiol Immunol, 198, 2009, 157. **Financial Agencies**: CNPq **Acknowledgments**: CAPES

01.021 Inhibition of NAD(P)H oxidase reverts the effects of chronic ethanol consumption on the contraction induced by Endothelin-1 in rat corpus cavernosum. Muniz JJ[1], Leite LN[2], Lacchini R[2], Tanus-Santos JE[2], Tirapelli CR[1] [1]EERP-USP, [2]FMRP-USP – Farmacologia

Introduction: Endothelin 1 (ET-1) is a peptide with vasoconstrictor action that controls the tonus of cavernosal muscle contributing to maintain the flaccid state of penis. In addition to its effects in vasoconstriction, the ET-1 actives other pathways, such as mitogen-activated protein kinase (MAPK), metalloproteinases (MMPs) and increases the production of reactive oxygen species (ROS). The increase of ET-1 has been associated with erectile dysfunction (ED) of different etiologies, including the ED caused by chronic ethanol consumption. **Aim:** To assess the effects of chronic ethanol consumption on the endothelinergic system and intracellular pathways activated by ET-1 in cavernosal tissue. **Methods:** All protocols were approved by the local Ethics Committee (12.1.317.53.9). Male Wistar rats were divided in two groups: ethanol group– treated with ethanol (20% vol/vol) for 6 weeks; control group– water for 6 weeks. Cumulative concentration-response curves for ET-1 were performed on isolated cavernosal tissues on the presence or absence of apocynin (APO), an inhibitor of NAD(P)H oxidase. Antioxidant activity and MMP-9 and MMP-2 levels were measured in plasma. mRNA levels of p38MAPK, SAPK/JNK, ERK1/2, AKT, MMP-9 and MMP-2 were assessed by RQ-PCR in cavernosal tissue. **Results:** ET-1-induced contraction was higher in ethanol-treated rats (36.1 ± 2.7% KCl 120mM; n=5) compared to control group (20.7 ± 0.9% KCl 120mM; n=5) ($P<0.05$, Student´s T test). In ethanol group, the contraction induced by ET-1 was significantly reduced in the presence of APO (25.2 ± 2.2% KCl 120mM; n=3) ($P<0.05$, Student´s T test). The antioxidant activity and MMP-9 and MMP-2 plasma levels were increased in ethanol group when compared to control group ($P<0.05$, Student´s T test). It was not found alteration in mRNA level of MAPKs, MMPs and AKT when control was compared to ethanol group. **Conclusion:** These results show that the chronic ethanol consumption increases the ET-1-induced contraction and APO reverts this response. Furthermore, the chronic ethanol consumption increases the antioxidant activity and plasma levels of MMP-9 and MMP-2. Therefore, the chronic ethanol consumption affects intracellular pathways activated by ET-1 in cavernosal tissue, which can lead to ED. **Financial Support:** FAPESP

01.022 Effect of atorvastatin on oxidative stress induced by lysophosphatidylcholine in human endothelial cells. Fernandes VA, Navia-Pelaez JM, Diniz TF, Cortes SF, Lemos VS, Capettini LSA UFMG – Fisiologia e Farmacologia

Introduction: Lysophosphatidylcholine (LPC) is formed during phospholipid oxidation of low density lipoproteins (LDL) vesicles and it has an important role in atherogenesis. LPC effects on the two main pathways of endothelial synthesis of nitric oxide (NO): eNOS and nNOS are not known. Recent studies suggest that atorvastatin has a direct vasodilator effect on the rat aorta and microvessels. However, the role of atorvastatin on endothelial dysfunction induced by LPC is not known. So, the aim of this work is to evaluate the mechanisms involved in endothelial dysfunction induced by LPC and to evaluate the role of atorvastatin on these alterations at cellular level. **Methods:** Were used human endothelial cells derived from HUVEC cells (EAhy.926) treated with different concentrations of LPC (0.5, 1 and 5 uM) for 6 hours. Protein expression (total and phosphorylated proteins) was evaluated by immunofluorescence and NO production was measured using the fluorimetric method of 2,3-diaminonaphthalene (DAN). Superoxide production was measured by the using of the fluorescent probe dihidroetidium (DHE) by fluorescence microscopy and flow cytometry. Production of hydrogen peroxide (H_2O_2) was assessed by flow cytometry. All results were analyzed using the ImageJ software. **Results:** Exposure of EAhy.926 cells to LPC led to an increased production of superoxide radicals (CTR: $4{,}27x10^5 \pm 2{,}74x10^3$ and LPC: $1{,}738x10^7 \pm 1{,}59x10^6$; flow cytometry; $n=4x10^4$), H_2O_2 (CTR: $7{,}6x10^5 \pm 2{,}65x10^5$ and LPC: $2{,}29x10^6 \pm 2{,}54x10^5$; flow cytometry; $n=4x10^4$) and NO, as measured indirectly by DAN (CTR: 59,7 ± 0,41 and LPC:67,58 ± 1,11; n=5). Parallel to superoxide increased, NO and H_2O_2 levels, LPC reduced eNOS (CTR: $1{,}004x10^6 \pm 5{,}87x10^3$ and LPC: $8{,}74x10^5 \pm 6{,}85x10^3$; n=21) and nNOS expression (CTR: $1{,}25x10^6 \pm 13.39\ 10^3$ and LPC: $9{,}02x10^6 \pm 7{,}63x10^3$; n=14). By the other hand, LPC increased levels of $eNOS^{Ser1177}$ phosphorylation (CTR: 1,19 ± 0,015 and LPC: 1,365 ± 0,017; n=10) and reduced $eNOS^{Thr495}$ phosphorylation (CTR: 2,36 ± 0,24 and LPC: 1,48 ± 0,01; n=21), but did not alter the levels of $nNOS^{Ser852}$ phosphorylation (CTR: 0,79 ± 0,013 and LPC: 1,47 ± 0,015; n=14). After incubation with atorvastatin (1µM) and LPC simultaneously, we found a significant reduction of the superoxide (CTR: $4{,}27x10^5 \pm 2{,}74x104$, LPC: $1{,}738x10^7 \pm 1{,}59x10^6$ and ATORVASTATIN+LPC: $1{,}107x10^7 \pm 2{,}59x10^{5;}$ n=20) and NO production (CTR: 43,03 ± 0,81 and LPC: 50,83 ± 0,81 and ATORVASTATIN+LPC: 44,43 ± 2,32; n=20), suggesting a reduction in cellular oxidative stress. Atorvastatin increased levels of eNOS expression (CTR: $1{,}66x10^6 \pm 9{,}84x10^3$ and LPC: $8{,}7x10^5 \pm 6{,}8x10^3$ and ATORVASTATIN+LPC: $1{,}39x10^6 \pm 1{,}43x10^4$) and reduced overall levels of $eNOS^{Thr495}$ phosphorylation (CTR: 1,07 ± 0,008 and LPC: 1,48 ± 0,01 and ATORVASTATIN+LPC: 0,911 ± 0,007) in cells stimulated with LPC, suggesting improvement in eNOS activity. Moreover, atorvastatin increased total nNOS expression (CTR: $9{,}23x10^5 \pm 5{,}48\ 10^3$ and LPC: $6{,}9x10^5 \pm 7{,}6x10^3$ and ATORVASTATIN+LPC: $1{,}26x10^6 \pm 18{,}58x10^3$) and reduced levels of $nNOS^{Ser852}$ phosphorylation (CTR: 0,79 ± 0,013 and LPC: 1,47 ± 0,01 and Atorvastatin + LPC: 0,982 ± 0,013), suggesting activation of this pathway. **Conclusion:** Our results suggest that atorvastatin can act reversing the possible uncoupling of eNOS and nNOS induced by LPC in human endothelial cells. **Financial support:** CNPq, CAPES; PRPq-UFMG.

01.023 Modulatory effects of atorvastatin on nitric oxide pathway in oxidative stress and inflammatory response induced by oxidized LDL in human endothelial cells. Navia-Pelaez JM[1], Diniz TF[2], Capettini LSA[1], Lemos VS[2], Cortes SF[1] [1]UFMG – Farmacologia, [2]UFMG – Fisiologia e Biofísica

Introduction: Maintenance of physiological NO production and reactive oxygen species (ROS) is considered a key factor in the control of oxidative and inflammatory modifications induced by dislipidemia. Although some studies demonstrate the participation of oxidized LDL (oxLDL) in endothelial dysfunction, the mechanisms underlying this phenomenon is unclear as well as the effect provoked by oxLDL on the principal pathways Involved in NO synthesis. Recent studies suggest that atorvastatin, has vascular and anti-inflammatory effects besides from its hypolipidemic effects. All in all, the role of atorvastatin in the endothelial dysfunction and inflammation induced by oxLDL is not known. **Objective:** Evaluate the mechanisms involved in oxLDL induced inflammatory response and oxidative stress, as well as the protective effect of atorvastatin in these alterations. **Methods:** Human endothelial cells (EAhy.926) were treated with different concentrations of oxLDL (6, 12.5 and 25 ug/ml) for 6 hours. Protein expression was evaluated by immunofluorescence and by Western blot. Nitrite production was measured by fluorometric 2,3-diaminonaphthalene (DAN) assay. Flow cytometry was used to measure NO, O_2^- and H_2O_2 production. O_2^- production was also evaluated by fluorescence microscopy. Cytokine production was evaluated by ELISA in supernatant. All results were expressed as mean ± SD. Fluorescence Intensity measurements were analyzed by ImageJ software. **Results and Discussion**: nNOS presented a reduced expression (from $3.7x10^6$ to $1.4x10^6$) and activity (from 1.3 ± 0.001 to 0.8 ± 0.001) after oxLDL incubation. NO production was increased significantly in a dose dependent manner (from basal 59.7 ± 0.7 to 73.15 ± 3.4 uM) partly in consequence of significant increase of eNOS (from $5.5x10^6$ to $9.1x10^6$) and iNOS (from $1x10^6$ to $1.3x10^6$). This result indicates inflammation and oxidative stress, confirmed by ROS and inflammatory markers. O_2^- and H_2O_2 produced, showed a 4,41 and 2,88 fold increased respectively in comparison with un-stimulated cells. Pro-inflammatory cytokines as IL-6, TNF-a and MCP-1 were significantly increased under oxLDL stimuli (10.5 ± 3.5, 54.8 ± 2.1 and 31679 ± 8030 pg/ml respectively) while TGF-b and IL-10 levels were reduced (from 305.5 ± 11.2 to 206.5 ± 23.1 and from 20.5 ± 2 to 12.4 ± 1.7 respectively). Atorvastatin induced an increase on nNOS expression ($3.4x10^6$ compared to $2.8x10^6$) and nNOS activity (0.3 ± 0.001 compared to 0.8 ± 0.0007) and a significant reduction on iNOS expression ($1.2x10^6$ compared to $1x10^6$), suggesting that atorvastatin can modulate NO synthesis. When incubated with oxLDL stimuli, atorvastatin prevented the super production of NO (61.2 ± 0.2 uM) and the elevated O_2^-levels ($1.2x10^6$ reduced to 852951), while it did not presented significant effect on H_2O_2. Atorvastatin showed an anti-pro-inflammatory effect by significantly preventing elevation of pro-inflammatory IL-6, TNF-a and MCP-1 (5.757 ± 0.38, 48.07 ± 0.81 and 19636 ± 5550 pg/ml respectively) and elevating anti-inflammatory TGF-b and IL-10 (18,1 ± 0,7 and 289,5 ± 19 respectively). Together these results show that atorvastatin is able to mediate regulation of NO and ROS production, regulating NOS expression and reducing inflammatory oxLDL induced responses. **Support**: CAPES, FAPEMIG e CNPq.

01.024 Synthesis and functional analysis of novel AT1 receptor ligands with potential biased agonistic properties. Duarte DA, Prando EC, Oliveira EB, Costa-Neto CM FMRP-USP – Biochemistry and Immunology

Introduction: G protein-coupled receptors (GPCRs) are integral membrane proteins characterized by having seven transmembrane (TM) α-helices and for that are also called 7TM receptors. This receptor superfamily can mediate the triggering of cellular responses after binding of a plethora of molecules such as lipids, neurotransmitters, vasoactive peptides, and many others. Currently GPCRs are targets for approximately 50% of all drugs in the market. The renin-angiotensin system (RAS) is known to participate in pathophysiological processes such as blood pressure control and hydro-electrolytic balance. The angiotensin II type 1 (AT1) receptor is the major player of this system and is classically activated by the octapeptide angiotensin II (AngII). Recent reports have described various agonists for distinct GPCRs, including the AT1 receptor, that can selectively (or at least preferentially) activate signaling pathways either dependent of G Protein or of beta-arrestin coupling, therefore termed biased agonism. **Methods:** In the present work we performed rational design for AngII-analogs aiming at to produce novel ligands with distinct biased pharmacological properties. Four analogs were synthesized by solid phase synthesis using Boc and Fmoc strategies. After cleavage from the resin, peptides analogs were purified by HPLC and the efficiency of synthesis was evaluated after acid hydrolysis by amino acid composition analysis. To investigate binding affinity of the analogs, binding assays were performed using the CHO cell line (Chinese hamster ovary) stably expressing the AT1 receptor, with subsequent calculation of IC_{50} values. We also performed functional analysis of ERK1/2 phosphorylation and Ca^{+2} mobilization in HEK293T cells (Human embryonic kidney) transiently transfected with the AT1 receptor. **Results and Discussion:** We obtained values ranging from high (~10^{-11} M) to low (~10^{-7} M) affinities. The functional analysis showed that all analogs were able to activate Ca^{+2} mobilization and MAPK cascade leading to ERK1/2 phosphorylation, although with different extents of potencies, what suggests distinct coupling to downstream signaling proteins. Other functional analyses are currently being performed to comprehensively address the analogs' signaling profiles. We believe that the design of novel biased agonists may lead to development of a new generation of receptor-targeted drugs, i.e. selective for activating not only a receptor subtype but also a specific signaling pathway. **Financial support**: FAPESP, CAPES, CNPq, FAEPA.

01.025 Involvement of channels in the vasorelaxant effect of AAL 195. Silva JCG[1], Costa CDF[2], Herculano EA[3], Ferreira AKB[1], Araújo-Júnior JX[3], SILVA DL[3], Ribeiro EAN[1] [1]ESENFAR-UFAL, [2]RENORBIO-UFAL, [3]IQB-UFAL

Introduction: The phosphodiesterase (PDE) is an enzyme which plays an important role in intracellular signaling by hydrolyzing cAMP/cGMP. Is possible find it in several tissues and that is found in the cardiovascular system. In vascular smooth muscle it promotes relaxation by increasing intracellular levels of second messengers. Currently, the PDE inhibitors are being used to treat many diseases, such as asthma, depression, erectile dysfunction and pulmonary arterial hypertension. The AAL 195, the object of our study, is an inhibitor of PDE 4 family, which uses cAMP as a specific substrate. The main aim of this study was to evaluate the in vitro vasorelaxant effects of AAL 195 on superior mesenteric artery rings of male Wistar rats. **Methods:** Superior mesenteric artery rings of male Wistar rats (2 - 4 mm) mounted vertically between two stirrups in organ chambers filled with 10 ml Tyrode's solution maintained at 37 ºC, constantly bubbled with 95%O_2 – 5%CO_2. One stirrup was connected to a force transducer for recording of isometric tension under a constant tension of 0.5g. We studied the concentration-dependent relaxant effect of AAL 195 on endothelium-intact and endothelium-denuded mesenteric rings that were pre-contracted with phenylephrine (Phe)(10^{-6}M) or 80 mM KCl. During the tonic phase of the contraction, The AAL 195 ($3x10^{-8}$ - 10^{-5}) was added cumulatively to the organ bath. The results were expressed as the percentage reduction of contraction induced by Phe (10^{-6}M) or KCl 80 mM. All values represent mean S.E.M. Data were analized using student's t-test. Probability values<0.05 were considered statistically significant. The pD_2 values were obtained by nonlinear regression. Protocol approved by the ethics committee for animal experimentation: 006/2013. **Results and Discussion:** AAL 195 ($3x10^{-8}$ - 10^{-5}) evoked a concentration-dependent relaxation of endothelium-intact mesenteric rings, pre-contracted with Phe (E_{max} = 112.79 ± 1.99 and pD_2= -6.18 ± 0.03%). In endothelium-denuded rings, the relaxant effect of AAL 195 was not changed (E_{max} = 97.31 ± 4.01% and pD_2 = -6.21 ± 0.04), suggesting that the vasorelaxant effect of the extract is mediated by endothelium independent mechanisms. To check the participation of Ca^+ channels in the mechanism of relaxation of this substance, the mesenteric rings were contracted with KCl 80 mM and cumulative relaxation responses were obtained by adding AAL 195. AAL 195 only partially inhibited the K^+-induced contraction, in both intact (Emax = 56.4 ± 7.03%) and endothelium-denuded (Emax = 48.68 ± 2.05%) mesenteric rings. These results confirm that the presence of endothelium is not essential for the relaxant effect of AAL 195 and suggest a calcium channel blocking partial effect. Therefore, we can conclude that phosphodiesterase inhibitor AAL 195 promotes endothelium-independent vasorelaxation, with the probable involvement of channels. Financial Agencies and **Acknowledgments**: UFAL, CNPq and FAPEAL.

01.026

01.026 ***Tityus serrulatus* venom and its toxins Ts1 and Ts5 increase cytosolic Ca^{2+} concentration in isolated vascular smooth muscle cells**. Neto Filho MA[1], Vasconcelos F[2], Bendhack LM[3], Arantes EC[3]
[1]UNINGA – Pharmacology, [2]UFPA – Toxicology, [3]FCFRP-USP – Physics and Chemistry

Introduction: Voltage-gated Na^+ channel (Na_v channel) scorpion toxins are the most important components of the scorpion venom. Two types of toxins (α and β) that are active on the Na_v channels. In this context, the toxins Ts5 (α-neurotoxin) and Ts1 (β-neurotoxin) from *Tityus serrulatus* venom (TsV) interact with Na_v channels, increasing Na^+ influx and the depolarization of the cell membrane, which induces the opening of the voltage-activated Ca^{2+} channel, leading to increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_C$). These toxins positively modulate cardiac inotropism and the vascular smooth muscle contraction via activators of Na^+ channels. **Objective:** this study aimed to investigate the effect of TsV (Ts1 and Ts5) on the $[Ca^{2+}]_C$ in rat aortic smooth muscle cells. **Methods:** toxins were isolated by ion exchange chromatography (Ts1) followed by RP-HPLC (Ts5). The rat aortic smooth muscle cells were isolated in Hanks buffer pH 7.4 and loaded with 5 mmol/L of Fura2/AM (45 minutes at 37°C), in order to measure $[Ca^{2+}]_C$ by fluorescence of Fura-2/AM (ratio 340/380 nm) (CEUA-USP 05.257.53.9). The fluorescence was measured in one single cell (excitation: 340 and 380 nm; emission: 510 nm). **Results:** the effects of TsV, Ts1 and Ts5 on the $[Ca^{+2}]$c in isolated rat aorta smooth muscle cells were evaluated and compared to the depolarizing effect of KCl 60 mmol/L (positive control). TsV (100 and 500 mg/mL) increased the $[Ca^{2+}]$c in 49,6% ± 2.6% and 103.7% ± 5.2%, respectively, when compared with the positive control (100%). When the cells were incubated with TTX (1 mmol/L), the increase in $[Ca^{2+}]$c elicited by TsV (500 mg/mL) was reduced to 15.7% ± 3.1% ($p<0.001$). However, when the cells were incubated with verapamil (1 mmol/L), the increase in $[Ca^{+2}]$c elicited by TsV (500 mg/mL) was reduced to 47% ± 5.8% ($p<0.01$). Ts1, 50 mg/mL and 100 mg/mL, increased the $[Ca^{+2}]$c in 43.9% ± 3.1% and 121.8% ± 8.9%, respectively. The effect of the higher Ts1 concentration was different from that of lower concentration ($p<0.001$; $n=3$). Ts5 exhibited a similar concentration-dependent effect. The concentrations of 50 mg/mL and 100 mg/mL increased the $[Ca^{+2}]$c in 52.6% ± 8.3% and 79.5% ± 6.1%, respectively. However, the effect of Ts1 (100 mg/mL) was greater than that of Ts5 at the same concentration. **Conclusion:** TsV and its toxins induce a concentration-dependent increase in $[Ca^{2+}]_C$, that probably occurs through interaction of these toxins with Na_v channels, inducing depolarization and consequent Ca^{2+} influx in rat aortic smooth muscle cells. This assumption is based on the fact that this effect is greatly inhibited by tetrodotoxin (a Na^+ channel blocker) and partly inhibited by verapamil. These results confirm that there is a direct action of TsV toxins (via Ts5 α-neurotoxin and Ts1 β-neurotoxin) on the Ca^{+2} influx in aorta smooth muscle cells. **Financial support:** FAPESP and CNPq

01.027 Quercetin induces autophagy, apoptosis and cell cycle arrest in human tumor xenograft model. Maso V[1], Calgarotto AK[1], Franchi Jr GC[2], Nowill AE[2], Vasallo J[3], Latuf Filho P[3], Saad STO[1]
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Introduction: The molecular heterogeneity of myelodysplastic syndrome make therapies using single-target drugs are ineffective. An orchestrated interplay between three important processes: apoptotic, autophagy and cell cycle has been implicated to new anti-cancer therapies. In this concern, natural compounds like quercetin, the main component of propolis extracts are considered new chemicals for the development of drugs against various molecular targets. Accordingly, the goal of this work was to identify the quercetin effects using P39 cells in xenografted mice. **Material and Methods:** The myeloid cell line P39, derived from a patient with MDS-chronic myelomonocytic leukemia (CMML), was kindly provided by Eva Hellstrom-Lindberg, Karolinska Institute, Stockholm, Sweden. And was submitted, in our lab, to karyotyping which showed 46,XY,+del(6),-9,-16,-17,+2mar, indicating that this cell line is not contaminated with HL-60.The model was performed in immunedeficient mice (NOD.CB17-Prkdc^{scid}/J lineage (Jackson Lab. USA), (n= 6), Number of Animal Ethics Committees: 1760-1. Mice were inoculated, subcutaneously, in the dorsal region, with 1x10^{7}cells/mice. Every 7 days the tumor volume was evaluated according to the following formula: tumor volume (mm3) = (length x width2)/2. The quercetin treatment started after tumors reached 100 to 200 mm3; it was given once every four days by intraperitoneal (i.p) injection at 120mg/Kg body weight (diluent: 40% of PEG400 in PBS solution). Control group received equal amounts of vehicle solution. After 21 days, the mice were sacrificed, tumors were removed, minced and homogenized in protein extraction buffer or fixed in formalin immediately for immunohistochemistry. Then detection of apoptosis, autophagy and cell cycle process were performed. **Results and Discussion:** After 21 days with quercetin treatment, we could observe the reduction of 31,6% of tumor volume compared to control. The main proteins related to the autophagy process were analyzed and we found increased expression of beclin-1 and class III PI3K (p=0.0082 and p=0.02, respectively), ATG5-ATG12 (p=0.0058) and ATG7 (p=0.04). LC3I/II staining was higher (41,6% ± 4,1) in mice treated with quercetin compared to control using immunohistochemistry analysis. We could also observe decrease in Bcl2 and McL-1 expression (p<0.0001 and p=0.0002, respectively) increase in Bax (p<0.0001), and any modulation of BcL-xL levels (=0,41). Caspase 3 staining was active in quercetin mice treatment (39,68% ± 4,7). Quercetin induces cell cycle arrest in G1 phase, with reduction of cyclin D and E expression (p=0.0028 and p= 0.0057, respectively) and phosphorilation of Rb (p<0.0001). The p21 staining was increased, 54,5% (± 8,10) compared to control. We could not observe any significant difference in cyclin A (p=0,21), Cdk2, 4 and 6 (p=0.3, 0.25 and 0.3, respectively).The quercetin treatment in xenotransplant model reduces the tumor development, with pronounced activation of apoptotic, autophagic process and arrest cell cycle rather in G1 phase. **Financial Support:** FAPESP

01.028 Structural insight on Angiotensin II Type 1 and Type 2 receptors in the light of the CXCR4 structure. Martin RP, Rodrigues ES, Silva RF, Shimuta SI Unifesp – Biophysics

Introduction: Angiotensin II (AngII) is the main agent of the renin-angiotensin system to regulate blood pressure and hydro-electrolytic homeostasis. Its physiological actions are mainly mediated by the type1 (AT1) and type2 (AT2) receptors that belong to the G protein coupled receptors family (GPCR), bearing seven alpha helices connected by 3 extracellular and 3 intracellular loops and an extracellular N-terminal and an intracellular C-terminal segments. Both receptors present almost always an opposite response in their signaling pathway, where the activation of AT1 receptor is commonly associated with a raise in arterial blood pressure whereas AT2 receptor with a reduction of blood pressure. Several receptor-agonist interaction models have been proposed, and it is noteworthy that several AngII residues are involved in the peptide interaction, such as, Asp1, Arg2, Tyr4 and Phe4 beyond the N and C terminal segments. To predict the structures of both receptors, the solved CXCR4 chemokine receptor structure could be used as a template, and a model with all those previously highlighted interaction could be presented and submitted to a molecular dynamics reaction. **Methods**: It was developed a practical study protocol, which was based on the binding affinity study using several AngII analogs in which were tested in cells expressing either AT1 or AT2 receptor. Moreover, a theoretical study was developed with receptor models generated by the Nest routine of Jackal package based in the alignment of GPCRs using the CXCR4 model as a template. AngII was docked with both types of receptor and the models were submitted to about 20ns molecular dynamics. **Results**: It is noteworthy that in the practical study, the substitution of Tyr4 to a non aromatic residue Ala provoked an 100 times lower affinity, while the use of Phe caused almost no difference in the AT1 receptor binding. On the other hand, the substitution of Tyr4 for both Phe and Ala, in the AT2 receptor, it was found no change in the binding affinity. **Discussion**: Our findings are in accordance with the literature data, since the Asn111 AT1 receptor residue seems to be a switch that could control the changing of a non-activate state R, to an achievable state R', and this state is essential to the correct docking. Nevertheless, the AT2 receptor seems to be on the R' state. Thus, AT2 receptor has a more flexible interaction site than AT1, ergo changes in AngII is more critical in the AT1 binding. To provide a better understanding on the difference of these sites, our molecular dynamics results show a better accommodation of AngII molecule in AT2 receptor, when compared with AT1 receptor. It is noteworthy that the agonist side chains are more stable due to the AT2 receptor conformation, which could be associated to R' conformation, whereas the AT1 receptor model presents a more variable structure which may be associated to the changing between R and R' conformation. This situation could disturb the binding of the analogs to the AT1 receptor. Our findings could lead us to a better understanding in the differences between the two AngII receptors aiming for future drug design. Supported by CAPES, CNPq and FAPESP.

01.029 ERK serves as a converging point in attenuation of skeletal muscle proteolysis induced by Gs and Gi-coupled adenosine receptors. Figueiredo LB, Duarte T, Godinho RO Unifesp – Pharmacology

Introduction: Increased protein breakdown is the major determinant of skeletal muscle atrophy associated with pathological conditions. Conversely, receptor-dependent activation of Gs protein/ adenylyl cyclase (AC)/ cAMP signaling cascade have been implicated in the maintenance of muscle mass as result of attenuation of protein breakdown. Thus, GsPCR represent reasonable targets for developing therapeutic interventions to prevent/reverse muscle atrophy. Based on the protective role of adenosine (ADO) in mammalian skeletal muscle, herein we evaluate the effects of ADO on muscle proteolysis. Considering that muscle fiber expresses all 4 ADO receptor subtypes, coupled to Gs (A_{2A}/A_{2B}) or Gi (A_1/A_3) proteins with opposite effects on intracellular cAMP levels, we hypothesized that ADO would exerts distinct effects on muscle proteolysis, by acting on A_{2A}/A_{2B} or A_1/A_3 receptors. **Methods:** The overall proteolysis was evaluated by measuring the dynamic tyrosine release from lumbrical muscles (n=4-8) of adult Wistar rats. All procedures were approved by the research ethical committee from UNIFESP (0034/12). **Results and Discussion:** ADO (1-100 μM) reduced by up to 44% the basal muscle proteolysis (0.16 ± 0.01 nmol tyr/mg/h), for at least 4h. Pre-incubation of muscles with selective antagonists of A_1 (DPCPX, 50 nM), A_{2A}/A_{2B} (DMPX, 10 μM) or A_3 (MRS1191, 100 nM) receptors reduced by 50-64% the ADO effect, which was mimicked by selective agonists for A_1 (CCPA, 30 nM), A_{2A}/A_{2B} (CV-1808, 30 μM) and A_3 (IB-MECA, 10 nM) receptors. The AC inhibitor SQ 22,536 (100 μM) abolished the effect of CV-1808, indicating that anticatabolic action of A_{2A}/A_{2B} agonists depends on cAMP signaling. Interestingly, the antiproteolytic effect of A_1/A_3 agonists was completely inhibited by pertussis toxin, but resistant to SQ 22,536 treatment, indicating that it involves activation of Gi protein but does not depend on inhibition of AC. Assuming that ADO is able to stimulate MEK/ERK1/2 via Gβγ (Germack and Dickenson, *Br J Pharmacol,* 141:329, 2004), we evaluate the effect of MEK inhibitor PD98059 on the anticatabolic effects of A_{2A}/A_{2B} (CV-1808) and A_3 (IB-MECA) agonists. In both cases, PD98059 abolished the effects of ADO receptor agonists, indicating that anticatabolic effects triggered by A_1/A_3 and A_{2A}/A_{2B} receptors converge on the MEK/ERK1/2 cascade. The preferential coupling of A_1/A_3 to Gi and A_{2A}/A_{2B} to Gs protein was endorsed by analysis of lumbrical isometric twitch contraction elicited by transmural electrical stimulation, (0.1 Hz; 2 ms; supramaximal voltage), which showed a positive inotropic effect of A_2 agonist CV-1808 (3 μM) and a negative inotropic effect of IB-MECA (1 nM). Taken together, our results show that a) activation of AC mediates the antiproteolytic effect of Gs-linked A_{2A}/A_{2B} receptors; b) anticatabolic effect of Gi-linked A_1/A_3 receptors does not involve the Gai-dependent inhibition of AC but rather Gβγ downstream signaling pathways; and c) ERK1/2 serves as a converging point in attenuation of skeletal muscle proteolysis induced by Gs- and Gi-coupled ADO receptors. These mechanistic insights should be taken into consideration in designing therapeutic strategies that use adenosine signaling as target to develop new anticatabolic drugs. **Financial support:** CNPq and Fapesp. **Keywords:** GPCR, ERK1/2, adenosine, skeletal muscle, proteolysis, contraction.

01.030 **Role of arginine1 residue of bradykinin in the activation of kinin B2 receptors**. Silva RF, Martin RP, Rodrigues ES, Oliveira L, Shimuta SI Unifesp – Biofísica

Introduction: Kinins are important mediators of cardiovascular homeostasis, nociception and inflammation and cause contraction and relaxation of several vascular and nonvascular smooth muscles. The major kinin effects are ascribed to bradykinin (BK, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), which exerts its actions activating the B_2 receptor, a GPCR. Once activated, the B_2 receptor causes an increase in IP3 and intracellular Ca^{2+} concentration. Our aim was to reevaluate the structure-activity relationship for activation of the B_2receptor by BK. Studies were performed with four BK analogs and two receptor mutants, and the biological activity and binding affinity were determined. **Methods:** The four analogs analyzed with substitution of Arg at position 1 were: Ala^1BK, Gly^1BK, Lys^1BK and Glu^1BK. Two B_2 receptor mutants were generated, wherein alanine was used to replace the glutamic acid at position 196 (B2-E196A), and the aspartic acid at position 268 (B2-D269A). Wild type (WT) B_2 receptor, B2-E196A mutant and B2-D268A mutant were transfected into CHO cells. The potency (EC_{50}) and efficacy (Emax) of BK and its analogs were determinated in these cells by measuring the intracellular Ca^{2+} (iCa^{2+}), and the binding parameter (IC_{50}) was evaluated by competition binding assays using fluorimetric method, with Europium labeled BK (Eu-BK). All experiments were performed in agreement with Ethics and Research Committee (CEUA nº 192697) and with Internal Biosecurity Commission (CIBio nº 01/2013). **Results:** Considering the WT receptor, BK and its analogs yield the following results for the values of Emax and EC_{50}, for iCa^{2+} determination, and IC_{50} (Eu-BK), for binding, respectively, 495,0ΔAFU, 11,2nM and 10,8nM, for BK; 219,3ΔAFU, 686,9nM and 676,4nM, for Ala^1BK; 91,5ΔAFU, 71,6nM and 70,0nM, for Gly^1BK; 341,5ΔAFU, 263,1nM and 272,5nM, for Lys^1BK; Glu^1BK (no detectable). For the mutant B2-E196A the values were: 502,7ΔAFU, 23,0nM and 24,4nM, for BK; of 247,8ΔAFU, 41,4nM and 45,5nM, for Ala^1BK; of 282,6ΔAFU, 109,3nM and 114,8nM, for Gly^1BK; of 302,6ΔAFU, 48,3nM and 42,6nM, for Lys^1BK; Glu^1BK (no detectable). The B2-D268A mutant was shown to be activated by BK whereas all analogs were unable to activate this mutant. For BK, the values were: Emax (iCa^{2+}), 388,8ΔAFU, EC_{50} (iCa^{2+}), 156,0nM and IC_{50}, 159,6nM. **Discussion:** The lack of activity of Glu^1BK and the markedly reduced values of the analyzed parameters of the others analogues studied suggest a major role for the positive charge and principally the guanidinium group of Arg^1 of BK in the binding and activation of WT B_2 receptor. The attenuated decrease in the potency of the analogues, when compared with BK, in the B2-E196A mutant receptor suggests that, in this mutant, the C-Terminal region of the agonist makes a loose binding with the receptor, what allows a greater mobility of the N-Terminal region, facilitating the binding of the agonist with other residues than arginine. The drastic reduction of BK potency in the B2-D268A mutant receptor, and the lack of activity of the others agonists in this mutant, indicate that the replacement of Asp^{268} for Ala causes a conformational change in the receptor, reallocating the important residue Asp^{286} in an inappropriate position for the correct binding to BK. **Supported by** FAPESP and CNPq.

01.031 Label-free quantitative proteomic analysis revealed the molecular profiling of Imatinib Mesylate and 5-Azacytidine treatment in lung cancer. Sousa JCC, Abdelhay E, Pizzatti L CEMO-INCa

Introduction: Lung cancer is a major cause of deaths worldwide. Since most cases are diagnosed in advanced clinical stage, the treatment of choice is chemotherapy, despite its high toxicity and low impact on the survival rate of patients (1-3). New treatments strategies are focusing on less toxic and more effective drugs, among them, target-specific drugs are being investigated for possible future use in treatment of lung cancer such as imatinib mesylate (Novartis) and 5-Azacytidine (5-Az) (3-5). Label-free MS^E proteomic analysis is one of the most powerful strategies used in the identification of novel pathways and mechanisms in drug development pipeline (6). **Objective:** In this work, we used a high-resolution proteomic approach to identify differential protein expression and altered pathways, in lung cancer cell line A549, after combinatorial treatment with imatinib mesylate and 5-azacytidine. **Methodology:** The label-free proteomic analysis was performed in treated cells after proliferation and viability assays using imatinib mesylate (IM) and 5-Azacytidine (5-Az) in several concentrations and incubation time. The total protein extracts were obtained following and proteomic analysis was conducted following. (7,8) System biology data were obtained using MetaCore Gene Go software. Western blot and Real Time RT-PCR were performed as validated approaches. **Results:** 48h IC50 was obtained in concentrations of 0.8 mM 5-Az and 1.0 mM IM. The proteomic analysis identified more than 2000 proteins in each treated sample. We identified 1027 differential expressed proteins between two conditions (IM and 5-Az). After bioinformatics and system biology analysis we were able to identified EGFR, Shh, Slit-Robo, IGF, HER and WNT signaling pathways altered after drug treatment. The analysis of EGFR, HER2 and beta-catenin expression showed a reduction of protein phosphorylation during combined treatment with 5 IM 1.0 mM 0.5 mM 5-Az, when compared to cells untreated or treated with IM only 0.8 mM, or 5-Az 0.5 mM, at 24 and 48 hours. **Conclusions:** Moreover our result indicates that several signalling pathways extremely important for lung cancer biology were affected after combinatorial treatment with imatinib mesylate and 5-Aza treatment. Thus, our data shed new light in the lung cancer biology and treatment strategies. **References:** 1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J. Clin. 2012; 2. Cagle PT, TC Allen, Dacic S, et al. Revolution in lung cancer: new challenges for the surgical pathologist. Arch Pathol Lab Med 2011; 3. Cagle PT, Dacic S. Lung cancer and the future of pathology. Arch Pathol Lab Med 2013; 4. Saintigny P, Burger JA. Recent advances in non-small cell lung cancer biology and clinical management. Discov Med 2012; 5. Bonomi M, Pilotto S, Milella M, et al. Adjuvant chemotherapy for resected non-small-cell lung cancer: future perspectives for clinical research. J Exp Clin Cancer Res 2011; 6. Russell C, Rahman A, Mohammed AR. Application of genomics, proteomics and metabolomics in drug discovery, development and clinic. Ther Deliv. 2013; 7.Pizzatti L, Binato R, Cofre J, Gomes BE, Dobbin J, Haussmann ME, D'Azambuja D,Bouzas LF, Abdelhay E. SUZ12 is a candidate target of the non-canonical WNT pathway in the progression of chronic myeloid leukemia. Genes Chromosomes Cancer.2010 Feb; 8. Pizzatti L, Panis C, Lemos G, Rocha M, Cecchini R, Souza GH, Abdelhay E. Label-free MSE proteomic analysis of chronic myeloid leukemia bone marrow plasma:disclosing new insights from therapy resistance. Proteomics. 2012; **Acknowledgements:** Ministério da Saúde (MS) e Instituto Nacional de Ciências e Tecnologia (INCT);

01.032 **Kinin B1 function on insulin resistance and control of weight gain**. Sales VM[1], Gonçalves-Zillo T[1], Batista C[1], Silva ED[1], Barros CC[2], Mori MAS[1], Pesquero JB[1] [1]Unifesp – Biofísica, [2]UFPEL – Nutrição

The kinin B1 receptor (B1R) is known to be involved in the inflammatory process and pain. Our group has demonstrated its role in metabolism control. We have shown that the kinin B1 knockout mice (B1-/-) are more sensitive to insulin and leptin, with lower plasma levels of this hormones and they are resistant to diet induced obesity. In addition, we generated a transgenic mice model expressing the B1R exclusively in the adipose tissue (aP2-B1/B1-/-). The expression of the B1R in the adipose tissue promotes weight gain, glucose intolerance and normalization of the blood levels of insulin, which were blunted in the B1-/- model. We have transplanted a mass of 50% of total body fat of visceral adipose tissue from the wild type mouse (B1+/+) into the subcutaneous region of the B1-/- mouse (B1+/+→B1-/-) and respective controls (B1+/+→B1+/+; B1-/-→B1-/-). At the day 0 of high fat-diet (HFD), the B1+/+→B1+/+ presented higher cholesterol levels than B1-/- mouse receptors, despite lower triglycerides plasma levels, with no difference in weight. After 16 weeks of HFD the B1-/-→B1-/- was protected from diet induced obesity as the B1-/- mouse, but the B1+/+→B1-/- mouse gained as much weight as the B1+/+→B1+/+ mouse indicating the importance of the adipose tissue B1R for the weight gain. We have observed that the weight gain was mainly due to increase in the endogenous adipose tissue depots from the receptor mice and a tendency to hepatomegaly. This weight gain was not accompanied by changes in energy intake. The B1+/+→B1+/+ mice became glucose intolerant and insulin resistant when compared to the other groups. The plasma cholesterol and triglycerides increased in all groups in a comparable way. The plasma leptin levels was lower in the B1-/-→B1-/-, as expected. The VEGFα level was lower in the B1-/-→B1-/- and rescued by the fat transplantation in the B1+/+→B1-/-. The balance between the amount of subcutaneous fat vs. the visceral fat seems to be important for the phenotype observed in the B1+/+→B1-/-. i.e. weight gain without insulin resistance or glucose intolerance and probably changes in adipose cytokine production, as VEGFα. In conclusion, the presence of the B1R in the adipose tissue is important for the development of insulin resistance and the control of the adipose tissue mass. **Financial support**: FAPESP/CNPq Protocol of Animal Use Ethic Committee from Unifesp: CEP 1403/11.