

01. Cellular and Molecular Pharmacology

01.001 Protective effect of (-)- α -Bisabolol in a Model of Ischemia/Reperfusion in renal tubular cells. Sampaio TL¹, Bezerra de Menezes RRPP¹, de Azevedo IEP², Meneses GC¹, da Costa MFB¹, Medrado KA², Martins AMC² ¹UFC – Farmacologia, ²UFC – Análises Clínicas e Toxicológicas

Introduction: Ischemia/reperfusion (IR) is a complex phenomenon that contributes to mortality and morbidity and is a predisposing factor for the establishment of acute kidney injury (AKI). Various strategies and resources are used by cells to prevent or decrease the cellular injury caused by oxidative stress. Thus, it emphasizes the importance of studying using substances such as (-)- α -Bisabolol (Bis), which has an antioxidant potential. This work aims to study the possible protective effects of Bis in AKI in an *in vitro* model of I/R. **Methods:** An *in vitro* model of I/R was performed in a culture of renal tubular cell lineage, LLC-MK2 (*Macaca mulatta*, monkey's kidney normal cells) using an anaerobic chamber and a specific culture media, after this, the cells were treated with Bis in different concentrations. To assess cell viability was performed the MTT reduction assay. It was performed the cellular respiration tests by flow cytometry: evaluation of the production of cytoplasmic reactive oxygen species by DCFH-DA assay and mitochondrial transmembrane potential analysis with the dye Rhodamine 123. For all statistical analysis was considered $p < 0.05$. **Results:** The Bis did not show toxicity in the studied concentration range (1000, 500, 250, 125, 62.5 and 31.25 μM). Furthermore, the process of ischemia was able to reduce the viability to $55\% \pm 1.3$ compared to the control group (100%). However, there was an increase in cell viability in the groups treated with Bis, especially at concentrations of 62.5 μM ($82.06\% \pm 1.4$) and 31.25 μM ($78.35\% \pm 0.99$), the concentration of 250 μM did not show statistical difference to the I/R group ($60.09\% \pm 1.6$). At the DCF-DA assay, the relative fluorescence intensity in the I/R group was in a ratio of 1.5 ± 0.04 when compared with control, indicating an increase in the oxygen-reactive species production. The treatment with Bis reduced the ratio to 0.69 ± 0.01 in the group treated with Bis 250 μM and to 0.62 ± 0.005 in the 62.5 μM concentration, indicating an antioxidant effect potential. At the mitochondrial transmembrane potential analysis, the I/R group presented a reduction in the relative fluorescence intensity to a ratio of 0.27 ± 0.03 in relation to control, indicating that I/R leads to mitochondrial depolarization. The treatment with Bis was capable to increase this ratio in the studied concentrations: 250 μM (0.69 ± 0.04) and 62.5 μM (0.47 ± 0.005), indicating an improvement in cellular respiration. **Conclusion:** The Bis stands out as a molecule with promising activity involving antioxidants mechanisms, which are essential in the pathophysiological development of the lesion by I/R. **Financial Support:** CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico **Acknowledgments:** UFC – Universidade Federal do Ceará

01.002 Membrane cholesterol regulates IL-10 secretion and tumor cell migration. Selos F¹, Fernandes PD¹, Costa ML², Mermelstein C² ¹UFRJ – Farmacologia e Química Medicinal, ²UFRJ – Biologia Celular e Molecular

Introduction: Lipid rafts are cholesterol-enriched membrane microdomains and play critical roles in the regulation of cell adhesion, migration, proliferation and protein sorting during endocytosis and exocytosis. Cholesterol depletion induces the disorganization of lipid rafts and also the dissociation of proteins that are bound to the lipid raft. In the present study we investigated the role of membrane cholesterol in the migration of the breast tumor cell line MDA-MB 231 and its relation to the Wnt signaling pathway. **Methods:** To deplete membrane cholesterol we used methyl-beta-cyclodextrin (MbCD) at a final concentration of 2 mM. Cell migration was evaluated using wound healing assay in which a scraping is made in the cell monolayer and subject to the closing of the shaved area by the migration of the cells for 24 hours. Morphological analysis was carried out by immunofluorescence microscopy using fluorescently labelled-Phalloidin to analyse microfilaments distribution. The concentration of IL-10 in the cell culture media was performed by ELISA. We used Wnt3a and Wnt5a enriched media to analyze the involvement of the Wnt signaling pathway. Statistical analyses were performed by ANOVA with Newman-Keuls- post-test (*p<0.05). **Results:** Our results show that cholesterol depletion by methyl-beta-cyclodextrin (MbCD) reduced cell migration. MbCD induces changes in cell morphology observed when cells were labeled with Phalloidin, which stains filamentous actin. Control cells displayed many ruffled membranes and lamellipodia whereas in cholesterol depleted cells membrane membrane protusions were less prominent and most of the cells displayed a spindle shaped morphology. Cholesterol depletion enhances the levels of IL-10 in the cultured media collected 2, 8 and 24 h after MbCD treatment. Wnt5a enriched media induced a significant increase in IL-10 levels after 2, 8 and 24 h of treatment, while Wnt3a enriched media did not induce IL-10 secretion. **Conclusions:** Our results suggest the involvement of membrane cholesterol in the control of IL-10 secretion that in turns regulates breast tumor cell migration. Our results showing an increase in IL-10 levels after cholesterol depletion and after Wnt5a treatment strengths our hypothesis that the non-canonical Wnt pathway is involved in the inhibition of cell migration observed after MbCD treatment. We can suggest that the inhibition of MDA-MB 231 cell migration induced by cholesterol depletion might be mediated by Wnt5a and subsequent IL-10 secretion. **Financial support:** CAPES, CNPq, FAPERJ, Programa de Oncobiologia/RJ.

01.003 Host response of miRNA profile to enteroaggregative. *Escherichia coli* infected mice fed with zinc deficient diet Prata MGP¹, Bolick DT², Kolling GL², Havt A¹, Guerrant RL², Lima AAM¹ ¹IBISAB-UFC – Fisiologia e Farmacologia, ²University of Virginia – Infectious Diseases and International Medicine

Enteroaggregative *Escherichia coli* (EAEC) is a pathogen of great relevance in diarrheal diseases worldwide. This bacterium is also linked mainly with malnutrition children from countries in development. Zinc is an important micronutrient that plays a central role in the immune system. The effects of zinc on these key immunologic mediators are deeply connected with the innumerable roles in basic cellular functions. Besides, the zinc deficient enhances apoptosis and increases susceptibility to a variety of pathogens. The potentiation of EAEC-infection in zinc deficiency causes significant changes in physiological and pathological processes that can change gene expression of microRNA (miRNAs or miR). miRNAs are class of genome-encoded small RNAs that regulates expression posttranscriptionally regulates of mRNA expression in epithelial tissues. We investigated the effect of zinc deficiency in the transcription of miRNA in intestinal epithelium from EAEC infected mice. Mice were fed with a normal diet (dN) or a diet without added zinc (dZD) for 2 weeks. All mice were pretreated with antibiotic cocktail. After 3 days some of them were treated with a single oral challenge of 10⁹CFU/ml EAEC strain 042. The infection challenge lasted for three days followed by the animal euthanasia. In this model, we analyzed body weight curves, fecal myeloperoxidase and lipocalin-2, muc-2 mRNA expression and miRNA expression of ileum (miScript miRNA PCR Arrays). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Virginia (Protocol Number: 3315). The results showed significantly weight loss, high myeloperoxidase and lipocalin-2 levels in fecal samples from EAEC challenged mice fed with zinc deficient diet compared to other groups (P<0.05). To verify mucus production we performed the muc-2 transcription in ileum. The data showed muc-2 mRNA expression was increased in infected animals (P<0.05). In mice fed with dN, EAEC infection challenge increased miR-146a transcription and miR-146a may be acting to control the infection. In contrast, mice fed with dZD and infected with EAEC presented different miRNA profile and higher infection. The EAEC challenged mice fed with zinc deficient diet caused alteration on miRNA profile that interfered on the infection control. Further studies using the microRNA silence gene transcription will be necessary to clarify potential immune response in infection with different diet. **Financial support:** This work was supported by NIAID Contract No. HHSN272201000056C from the National Institutes of Health. I was supported by CNPq Postgraduate Fellowships.

01.004 Capsaicin activates macrophages and adrenocortical cells by TRPV1-dependent mechanism. Ferreira LGB¹, Silva PMR¹, Martins MA¹, Faria RX², Carvalho VF¹ ¹Fiocruz – Inflamação, ²Fiocruz – Toxoplasmosse

Introduction: TRPV1 channels serve primarily as heat sensors, which are activated by temperatures above 43°C. These channels are mostly present on sensory neurons, including dorsal root ganglia and trigeminal neurons; however its receptors may be expressed on non-sensory organs, as lungs, too. Beyond heat, TRPV1 is also activated by capsaicin, an active ingredient in hot chili peppers, and different classes of lipids derived from the metabolism of arachidonic acid, as 12- and 15-HPETE which are produced by 12- and 15-lipoxygenase, respectively. These enzymes are found in several cells and organs, including macrophages and adrenal. ACTH-induced adrenal steroidogenesis involved 15-lipoxygenase pathway. Furthermore, TRPV1 activation increase Ca²⁺ currents and Ca²⁺-induces steroidogenesis in adrenal cells both as dependent and independent of ACTH. In addition, it is know that others alternative routes are able to induces steroidogenesis independently to ACTH by adrenal glucocorticoids, including IL-1, IL-6 and TNF- α produced by immune cells as macrophages. In this study, we investigated if TRPV1 is able to activate macrophages and adrenal cells directly.

Methods: All the procedures used in this study were in accordance with the guidelines of the Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License LW – 33/12. Peritoneal macrophages were obtained from Swiss-Webster mice. Y1 adrenocortical cell line producing corticosterone was maintained in DMEM medium supplemented with 5% FBS and 15% Horse Serum. Expression of TRPV1 was evaluated in Macrophages and Y1 by immunocytochemistry and western blot, respectively. Macrophages and Y1 cells were stimulated with 10 and 100 μ M capsaicin and we measured Ca²⁺ influx and IL-6 production through live cell fluorescent microscopy and ELISA, respectively. Cells were pre-treated with TRPV1 antagonists' ruthenium red or AMG 9810 (50 μ M) 15 min before stimulation with capsaicin. **Results and Discussion:** Both macrophages and Y1 adrenocortical cells expressed TRPV1, in which capsaicin induced Ca²⁺ influx concentration dependent manner. Treatments with ruthenium red or AMG 9810 abolished capsaicin-induced Ca²⁺ influx in macrophages and Y1 cells. Moreover, capsaicin induced IL-6 production by macrophages of 1540 \pm 690.5 pg/mL (mean \pm SD, n =3; P < 0.05), as well as ruthenium red inhibited capsaicin-induced IL-6 release with values of 11.55 \pm 11.50 pg/ml (mean \pm SD, n = 3; P < 0.05). In conclusion, we found that capsaicin activated both macrophages and Y1 cells by TRPV1 dependent way. **Keywords:** Glucocorticoids, HPA axis, TRPV1 **Financial Support:** CNPq, FAPERJ and FIOCRUZ.

01.005 Preeclampsia prevents plasticity in alpha1-adrenoceptors from rat abdominal aortae: Contribution to the pathophysiology. Silva KP, Caldeira-Dias M, Possomato-Vieira JS, Golçalves-Rizzi VH, Sandrim VC, Dias-Junior CA, Pupo AS IBB-Unesp-Botucatu – Farmacologia

Introduction: Preeclampsia (PE) is a gestational hypertensive syndrome and preeclamptic women present an inadequate increase in sensitivity to various vasoconstrictors, such as endothelin (Verdonk, K., Hypertension, 65, 1316, 2015) and norepinephrine (NE) (Lampinen, K., J. Hum. Hypertens., 269, 2014). It is known that NE participates in blood pressure regulation through the activation of alpha₁-adrenoceptors (alpha₁-ARs) and that there is an increased reactivity of arterial alpha₁-ARs in animal models of hypertension (Gisbert, R., Br.J.Pharmacol, 135, 206, 2002). However, the involvement of alpha₁-ARs in PE pathophysiology remains unknown. **Methods:** Female Wistar rats (90-120 days old) were divided into Control (virgins rats), Sham (pregnant rats) and RUPP (pregnant rats that underwent a surgical procedure to reduce uterine perfusion pressure leading to hypertension) groups. Blood pressure was monitored and rings (0.5 cm length) of abdominal aortae were isolated to record in vitro contractions to NE or A61603 (alpha_{1A}-AR selective agonist). The alpha₁-AR subtypes mediating contractions to NE were identified by Schild analysis of the antagonism displayed by subtype selective antagonists. Antagonist affinities (pK_B) or potencies (pA₂) were evaluated. Alpha_{1A}-AR, Alpha_{1B}-AR and Alpha_{1D}-AR gene expression was analyzed by RT-qPCR. **Results:** Mean arterial pressure was 110±1 mmHg, 96±2 mmHg and 120±1 mmHg in Control, Sham and RUPP respectively (n= 18-21). Contractions of the abdominal aortae of all groups to NE were competitively antagonized by prazosin (Control: pK_B=9.22±0.07; Sham: pK_B=9.2±0.1; RUPP: pK_B=9.2±0.1; n=4-7). Alpha_{1D}-AR selective antagonist BMY 7378 displayed classical competitive antagonism against NE-induced contractions (Control Schild slope=0.83±0.15; pK_B=8.22±0.07; Sham Schild slope=0.9±0.1; pK_B=8.5±0.1 and RUPP Schild slope=0.9±0.1; pK_B=8.4±0.1; n=4-7). Alpha_{1A}-AR selective antagonist RS100329 displayed high potency, but insurmountable antagonism against NE-induced contractions in Control and RUPP, but did not show this behavior in Sham group (pA₂=8.4±0.1; n=3). Contractions of abdominal aortae of Control and Sham rats to A61603 were antagonized by RS100329 (Control: pA₂=9.48±0.04; Sham: pA₂=9.3±0.1; n=3-7) but not by BMY7378. Nonetheless, both RS100329 and BMY 7378 shifted to the right as well as decreased maximum response in abdominal aortae of RUPP group. Alpha_{1B}-AR selective antagonist L765,314 displayed low potencies in abdominal aortae all groups (Control pA₂=6.3±0.3; Sham pA₂=7.5±0.1; RUPP pA₂=7.5±0.1; n=3-4). The expression of alpha_{1A}-AR and alpha_{1B}-AR mRNA was not different in the three experimental groups, but mRNA encoding alpha_{1D}-AR was significantly upregulated in aortae from RUPP rats. **Conclusion:** Heterogeneous functional population consisting of alpha_{1A}-ARs and alpha_{1D}-ARs coexists in abdominal aortae from female rats. Pregnancy induces plasticity of alpha₁-ARs and PE prevented this physiological change, pointing alpha₁-ARs function as a potential key mechanism in the pathophysiology of PE. **Financial support:** CAPES and FAPESP. **Local Ethics Committee for the Use of Experimental Animals: Process #634**

01.006 Anti-Apis serum failed to antagonize cytotoxicity induced by *Apis mellifera* venom *in vitro*. Jhonatha-Cruz JM¹, Tavares-Henriques MS¹, Strauch MA², Barraviera B³, Ferreira-Junior RS³, Quintas LEM¹, Melo PA¹ ¹UFRJ – Farmacologia, ²IVB – Diretoria Científica, ³Unesp – Venenos e Animais Peçonhentos

Introduction: The actions of *Apis mellifera* venom components are responsible for local and systemic damage. So far, there is no specific treatment for poisoning of bee stings, which makes interesting the development of a specific antivenom. We have previously demonstrated a newly-developed anti-apis serum that reduces some of the poison activities of *A. mellifera* venom. **Objective:** Our goal was to evaluate the efficacy of anti-Apis serum on *in vitro* cytotoxicity in tubular renal cells induced by *A. mellifera* venom. **Methods:** Cell culture of proximal tubule strain of swine kidney (LLC-PK1) was used. Cytotoxicity was assessed by the release of lactate dehydrogenase (LDH) after exposure of LLC-PK1 cells to different concentrations of poison (5-25 µg/mL) for 15, 30 and 60 min. The highest concentration (25 µg/mL) was evaluated with different doses of anti-Apis serum (1-4 µL/µg venom). Data are expressed as mean ± standard error, with ANOVA followed by Bonferroni post-test for statistical analysis; p<0.05 was considered statistically different. **Results:** *Apis mellifera* poison enhanced LDH release in a concentration-dependent fashion, which was steady between 15 and 60 min (at 15 min in U/L: control: 24 ± 2; 5 µg/mL: *134 ± 22; 10 µg/mL: *336 ± 38; 25 µg/mL: *505 ± 43, n=6, *p<0.05). At the maximum concentration, even the highest serum amount could not block the cytotoxic effect (25 µg/mL: 400 ± 26; 25 µg/mL + 4 µL/µg venom: 317 ± 3, n=6). **Conclusion:** We observed that the anti-Apis serum did not reduce cytotoxicity in pig renal cells caused by *Apis mellifera* venom. **Financial support:** CAPES; CNPQ; FAPERJ; CEVAP; IVB Research approval by Animal Research Ethical Committee - Protocol DFBCICB 026

01.007 Characterization of the serotonin receptors mediating contraction of the rat distal cauda epididymis. Mueller A^{1,2}, Kiguti LR³, Silva EJ¹, Pupo AS¹ ¹IBB-Unesp-Botucatu – Farmacologia, ²UFMT – Ciências da Saúde, ³FCM-Unicamp – Farmacologia

Introduction: Serotonin (5-hydroxytryptamine, 5-HT) receptor activation contracts smooth muscle from male reproductive organs such as vas deferens (Jurkiewicz, Acta Physiol Pharmacol Ther Latinoam, v.49, p.210, 1999) and prostate (Killam, Eur J Pharmacol, v.273, p.7, 1995). However, the roles of 5-HT receptors in the distal cauda epididymis (CE) smooth muscle are still unknown. Thus, the present study characterizes the contraction of the rat CE duct to 5-HT and the receptors involved. **Methods:** Adult male Wistar rats (120-150 days-old) were killed by decapitation and both epididymides were isolated. The CE duct was uncoiled and 1.0 cm length segments freed from intraluminal content were mounted in 10mL organ baths to record isometric tension. Cumulative concentration-response curves to noradrenaline (NA) and 5-HT were obtained in the absence and presence of prazosin (alpha₁-adrenoceptor antagonist), ketanserin (5-HT₂ receptor antagonist), WAY 100635 and buspirone (5-HT_{1A}-selective antagonists) and fluoxetine (an inhibitor of serotonin reuptake transporter with high affinity for 5-HT_{2C}). All concentration-response curves were obtained in the presence of a cocktail of inhibitors to allow experimental conditions to evaluate antagonist affinities (pK_B) and/or potencies (pA₂). **Results:** 5-HT induced concentration-dependent contractions of distal CE duct that amounted to approximately 50% of maximal response (E_{max}) induced by NA (pD₂ 5-HT=6.45±0.14, E_{max}=45.4±1.9% of NA maximal; n=7). Contractions to 5-HT were non-competitively antagonized by prazosin (pA₂=9.30±0.30, n=3) with a potency similar to that observed for prazosin against NA (pA₂=9.61±0.03, n=5) suggesting involvement of alpha₁-adrenoceptors in the *in vitro* contractions of distal CE duct to 5-HT. Therefore, serotonergic receptors involved in the 5-HT-induced contractions were further characterized in presence of 100 nM prazosin to avoid alpha₁-adrenoceptor activation. Under these conditions, the contractions to 5-HT were antagonized by ketanserin with high potency (pA₂=9.35±0.27, n=3) in an unsurmountably manner demonstrating the involvement of 5-HT₂ receptors and further suggesting heterogeneity of receptors mediating 5-HT-induced CE contractions. The CE 5-HT₂ receptor subtype mediating contractions to 5-HT seems to be the 5-HT_{2C} because fluoxetine inhibited these contractions with high potency (pA₂=7.75±0.12, n=7). The two 5-HT_{1A}-selective ligands WAY 100635 and buspirone inhibited the 5-HT-induced contractions with potencies consistent with interaction with 5-HT_{1A} receptors (pA₂ WAY 100635=9.47±0.02, n=3; pA₂ buspirone=7.19±0.14, n=4). **Conclusion:** Our results demonstrated that the contractions of the CE duct to 5-HT *in vitro* involves multiple receptors comprised at least by alpha₁-adrenoceptors, 5-HT_{2C} and 5-HT_{1A} receptors. This study contributes to the understanding of the roles of biogenic amines in the contractile function of the epididymal smooth muscle. **Financial support:** Fapesp and CNPq. **Process number of Local Ethics Committee for the Use of Experimental Animals:** 749-CEUA.

01.008 β_2 -adrenoceptor agonists modulate neuromuscular transmission through the extracellular cyclic AMP-adenosine pathway. Duarte T, Pacini ESA, Godinho RO Unifesp-EPM – Farmacologia

Introduction: We have shown that activation of skeletal muscle β_2 -adrenoceptors with clenbuterol increases the generation of intracellular cyclic AMP (cAMP) that is followed by the cyclic nucleotide efflux. Outside the muscle cell, cAMP is sequentially degraded by ectoenzymes into AMP and adenosine, which in turn is able to stimulate postsynaptic A_1 adenosine receptors (A_1R) inducing a negative inotropic effect (Duarte T, *et al.*, J Pharm Exp Ther.: 341:820-8, 2012). Considering that activation of pre-synaptic A_{2A} receptor subtypes has been associated with enhanced Ach release from motor neuron (Ribeiro J.A, *et al.*, Prog Brain Res.: 109:231-241, 1996), we investigated the possible influence of postsynaptic β_2 -adrenoceptor-induced cAMP efflux on the regulation of skeletal neuromuscular transmission. **Aim:** The aim of the present study was to evaluate the possible influence of postsynaptic β_2 -adrenoceptor-induced cAMP efflux on the regulation of skeletal neuromuscular transmission. **Methods:** Mouse phrenic nerve stimulation was used to assess neuromuscular transmission when hexamethonium, a pre-synaptic nicotinic acetylcholine receptor (nAChR) antagonist, was given to block neuromuscular transmission. We analyzed the effects of drugs on the train-of-four (TOF) ratio, which is the quotient between twitch tension of mouse diaphragm muscle produced by the 4th (T4) and the 1st (T1) stimulus within 4 consecutive 2 Hz stimuli of phrenic nerve (n=3-4). The effect of 100 nM clenbuterol (β_2 -adrenoceptor agonist) was evaluated 1.5 mM hexamethonium-induced partial neuromuscular block. In order to evaluate the possible involvement of cAMP efflux and extracellular cyclic AMP-adenosine pathway on the β_2 -adrenoceptor agonist effects, phrenic nerve-diaphragm preparation was incubated with the adenosine receptor antagonist CGS15943 (100 μ M), the ecto-5'-nucleotidase inhibitor AMP-CP (100 nM) and the organic anion transporter inhibitor Probenecid (300 nM), prior to the addition of clenbuterol.

Results: Incubation of phrenic-diaphragm preparation with 1.5 mM hexamethonium caused a 25% reduction in the TOF ratio (TOF fade). On the other hand, pre-incubation of nerve-muscle preparation with 100 nM clenbuterol increased by 15% the amplitude of diaphragm contraction and prevented the tetanic fade caused by hexamethonium. Inhibition of a) the efflux of cAMP with 300 nM probenecid, b) the degradation of cAMP into adenosine with 100 nM AMP-CP or c) adenosine receptors with 100 μ M CGS15943 prevented the effect of clenbuterol on hexamethonium-induced tetanic fade. **Conclusion:** Our results show that muscular cAMP efflux induced by activation of β_2 -adrenoceptor agonist is able to modulate neuromuscular transmission via extracellular generation of adenosine and activation of presynaptic adenosine receptors. **Financial Support:** Capes, CNPq and FAPESP. **Animal Ethics Committee:** CEP n. 1389121115

01.009 Antioxidant supplementation with tempol attenuates gain in the physical performance of trained rats. Maia IC¹, Brum PC², Angelis K³, Marostica E¹, Soares PPS¹
¹UFF – Physiology and Pharmacology, ²USP – Biodynamic of the Movement of the Human Body, ³Uninove – Laboratory of Translational Physiology

Introduction: Antioxidant supplementation is widely used by most of the population that cares about health and those who aiming to increase physical capacity. However, recent studies have shown conflicting results on the association of antioxidant supplementation and aerobic training in experimental models and humans (Am J Clin Nutr 87; 142-9, 2008). **OBJECTIVE:** The purpose of this study was to investigate the effect of antioxidant treatment with tempol, a scavenger of free radical, superoxide dismutase (SOD) mimetic, in trained and sedentary animals. **Methods:** (CEUA-UFF 698/15) Wistar rats 60 days old were adapted treadmill and after maximal exercise test (TE_{max}), were divided into four groups sedentary control (CS; n=12); training control (CT; n=10); sedentary tempol (TS; n=13) and training tempol (TT; n=9). The training consisted of treadmill running for ten weeks (50-70% of maximum speed, incline of 6%, 1 h/day, 5 days/week), with adjustable load at the 5th after a new TE_{max} . Treatment with tempol was done in the last ten days of the training period (30 mg.kg⁻¹.day⁻¹, ip). Another TE_{max} was performed at the end of training. After 48 hours the animals were anesthetized (thiopental, 80 mg.kg⁻¹). Blood sample was collected for serum assays, and the heart and the soleus muscle was dissected, frozen in liquid N₂ and stored at -80 °C, the first two for assay of biomarkers of oxidative stress (antioxidant enzyme activity, including SOD, catalase e glutathione peroxidase; total antioxidant capacity, lipid peroxidation and oxidation protein), and the last to qRT PCR assay of genes related to mitochondrial biogenesis (PGC-1 α and Nrf1) and antioxidant pathway (Nrf2). **Results** are means \pm SDs, analyzed by ANOVA two-way and post hoc Bonferroni's. For the real time RT-PCR, values were analyzed by Mann Whitney test ($P<0,05$). **Results:** At the end of the training, we observed an increase in physical performance of the animals in groups CT and TT (3.2 \pm 0.5; 2.6 \pm 0.3 km.h⁻¹; respectively) compared to CS and TS (1.4 \pm 0.3; 1.9 \pm 0.4 km.h⁻¹, respectively), but antioxidant supplementation with tempol reduced the gain on the physical capacity of the trained animals TT compared to CT. The TS group, that received tempol supplementation, did not increase performance as compared to CS. Although no change in the levels of lipid peroxidation, protein oxidation and antioxidant activity in serum and heart occurred, similar reduction in cardiac O₂⁻ levels were found in the groups CT, TS and TT (61,16 \pm 15,53*; 60,76 \pm 21,37*; 50,57 \pm 10,33* mmol.mg⁻¹protein respectively), compared to CS (90,11 \pm 22,81 mmol.mg⁻¹protein). In addition, the trained groups (CT and TT) increased gene expression of PGC-1 α in the soleus similarly. However, only TT increased the gene expression of Nrf2, while Nrf1 was not affected by any intervention (n=4-6) **Conclusion:** Thus, our data showed that supplementation with tempol decreased the gain in physical performance of aerobically trained rats. However, the molecular mechanisms that may explain these adaptations apparently are not directly related to mitochondrial biogenesis or just with oxidative stress. **Financial Support:** CAPES, FAPERJ, CNPq.

01.010 Verapamil modulates skeletal muscle contraction via activation of adenylyl cyclase/cAMP/PKA signaling pathway. Silveira SS¹, Duarte T¹, Paredes-Gamero EJ², Godinho RO¹ ¹Unifesp-EPM – Farmacologia, ²Unifesp-EPM – Bioquímica

Introduction: Skeletal muscle contraction is triggered by sequential activation of postsynaptic nicotinic acetylcholine receptors, depolarization of the sarcoplasm and release of Ca²⁺ from sarcoplasmic reticulum. The extracellular calcium is not required for skeletal muscle contraction, but it was recently shown that L-type calcium channel blockers have transient positive inotropic effect on mice skeletal muscle linked to increased intracellular cAMP levels (Menezes-Rodrigues et al., Eur J Pharmacol., 720:326, 2013). **Aim:** The aim of the present study was to evaluate the involvement of adenylyl cyclase/cAMP/PKA signaling pathway over the effect of verapamil on skeletal muscle contraction. **Methods:** The effect of 30 µM verapamil on isometric contraction was studied in mice diaphragm muscle, under direct electrical stimulus (0.1 Hz frequency, 2 ms duration, supramaximal voltage). The involvement of AC and PKA activation on the positive inotropic effect of verapamil was evaluated by pretreating diaphragm preparations with inhibitors of AC (NKY80, 300 µM, n=3) or PKA (Rp-cAMPS, 80 µM, n=3). The possible influence of verapamil on intracellular Ca²⁺ concentration was evaluated by laser confocal scanning microscopy in L6 myogenic cells using Fluo-4 probe. Data were normalized to maximal intensity obtained with 1 mM ionomycin and analyzed. **Results:** 30 µM verapamil induced a sustained positive inotropic effect (~30%) that persisted throughout at least 40 min. The verapamil-induced increase in contraction force was abolished by inhibition of AC or PKA with NKY80 and Rp-cAMPS, respectively. Incubation of muscle preparation with AC or PKA inhibitors had negligible effects on basal muscle contraction. Interestingly, verapamil increased (~60%) intracellular concentration of Ca²⁺ evaluated in L6 cells loaded with Fluo-4AM. **Conclusion:** Our results show that verapamil enhances skeletal muscle contraction and increases intracellular Ca²⁺ concentration. The inhibition of verapamil-induced positive inotropic effect by AC and PKA inhibitors indicate that by limiting calcium influx via L-type channels, verapamil allows the activation of Ca²⁺-sensitive AC and PKA, which in turn increases skeletal muscle contraction. **Financial Support:** Capes, CNPq and FAPESP. **Animal Ethics Committee:** CEP n. 022/12 – 9943030216.

01.011 A new functional role of extracellular cyclic AMP in the contraction of vascular, non-vascular and airway smooth muscle. Pacini ES, Moro RP, Godinho RO Unifesp-EPM – Farmacologia

Introduction: cyclic AMP (cAMP) is a universal intracellular second messenger, which is involved in a large number of biological processes such as cell proliferation, gene transcription and muscle contraction. In many tissues, in addition to its classical intracellular second messenger function, cAMP may also have an extracellular third messenger activity, which is secondary to its efflux and the sequential conversion into AMP and adenosine by ecto-phosphodiesterase and ecto-5'-nucleotidase, respectively (Godinho RO, et al., *Front Pharmacol.*: 6:58, 2015). Nevertheless, the existence and relevance of the so-called "extracellular cAMP-adenosine pathway" in the smooth muscle contraction of aorta, vas deferens and trachea is unknown. In the present study, we evaluated the contractile effects of extracellular cAMP in the rat isolated aorta, vas deferens and trachea. **Methods:** The aorta, epididymal portion of the vas deferens and trachea obtained from adult male Wistar rats were isolated and mounted in an organ bath containing carbogenated physiological salt solutions at a constant temperature, under optimal resting tension. After a 60 min stabilization period, the tissues were subjected to different protocols, and isometric contractions were measured. **Experimental Protocol:** the aorta, vas deferens and trachea were pre-contracted, respectively, with 100 nM noradrenaline, 30 mM KCl and EC₃₀ carbachol. After the stabilization of the pre-contractile responses, the effects of a) 300-1000 µM cAMP alone or in the presence of CGS-15943 or b) 100 µM 8-Br-cAMP were evaluated. The isometric contraction forces were expressed as mean ± S.E.M. All values were normalized and presented as percentage of the response induced by the pre-contractile agent. **Results:** 300-1000 µM cAMP induced a phasic contraction in pre-contracted rat vas deferens and trachea with amplitude values of 33 ± 13% (n=4) and 31 ± 3.5% (n=5), respectively. In contrast, incubation of rat aorta with 1 mM cAMP evoked relaxations that reached 35 ± 4.2% of the noradrenaline contraction (n=4). The pre-incubation of aorta, vas deferens and trachea with CGS-15943, a non-selective adenosine receptor antagonist, significantly reduced (p<0.05, Student's t- test) the contractile effect of cAMP (n=5-4). 8-Br-cAMP, a cell membrane-permeable analog of cAMP, induced a relaxation response in pre-contracted rat aorta (66 ± 8.3%, n=3), vas deferens (32 ± 6.1%, n=4) and trachea (46 ± 2.7%, n=4). **Conclusions:** These results suggest that the "extracellular cAMP-adenosine pathway" regulates smooth muscle contraction in rat aorta, vas deferens and trachea. Importantly, we provided experimental data that supports a new functional role of cAMP in addition to the classical intracellular second messenger pathway. **Financial Support:** CAPES, CNPq and Fapesp. Animal Ethics Committee: CEUA #9987150714 and #8359060315

01.012 Co-expression of Olfr287 in a CD36-positive subpopulation of olfactory sensory neurons Xavier AM, Ludwig RG, Nagai MH, Almeida TJ, Watanabe HM, Hirata MY, Rosenstock TR, Papes F, Malnic B, Glezer I. Unifesp-EPM

The sensory neurons in the olfactory epithelium (OSNs) are equipped with a large repertoire of olfactory receptors and the associated signal transduction machinery. In addition to the canonical OSNs, which express odorant receptors (ORs), the epithelium contains specialized subpopulations of sensory neurons that can detect specific information from environmental cues and relay it to relevant neuronal circuitries. Here we describe a subpopulation of mature OSNs in the main olfactory epithelium (MOE) which expresses CD36, a multifunctional receptor involved in a series of biological processes, including sensory perception of lipid ligands. We performed Real Time PCR to compare the expression of Cd36 transcript in OE and other tissues. Additionally, we conduct in situ hybridization (ISH) and immunofluorescence (IF) experiments in order to characterize Cd36 transcript and protein expression in the MOE. We next analyzed whether olfactory guided behaviors and the general olfactory capacity are perturbed in CD36-deficient mice. In order to assess these skills, we conducted the risk assessment behavior test and buried food olfactory assay, respectively. In addition, we performed an innate odor preference test in order to evaluate the response to specific odorants. The Cd36 expressing neurons coexpress markers of mature OSNs and are dispersed throughout the MOE. Unlike several ORs analyzed in our study, we found frequent coexpression of the OR Olfr287 in these neurons, suggesting that only a specific set of ORs may be coexpressed with CD36 in OSNs. We also show that CD36 is expressed in the cilia of OSNs, indicating a possible role in odorant detection. CD36-deficient mice display no signs of gross changes in the organization of the olfactory epithelium, but show impaired preference for a lipid mixture odor. Our results show that CD36-expressing neurons represent a distinct population of OSNs, which may have specific functions in olfaction. Approval of Animal Care Committees (CEUA–UNIFESP – 2011/1427). Funding Support: FAPESP – 2007/53732-8, 2013/07937-8; CNPq - 140873/2013-9

01.013 Bioactivity and cytotoxicity evaluation of Botulinum toxin Type A. Xavier B, Silva FS, Remuzzi GL, Silveira AR, Dalmora SL UFSM – Farmácia Industrial

Introduction: Botulinum neurotoxins are primarily produced by the gram-positive, anaerobic spore-forming bacterium, *Clostridium botulinum*, and are among the most potent biological toxins for humans. Botulinum toxin A (BoNTA) is naturally expressed as an inactive single peptide chain with 1296 amino-acids and a 150 kDa relative molecular mass neurotoxin, and was the first to receive attention clinically as a potent muscular paralytic agent. Therapeutic applications have increased for the treatment of strabismus and blepharospasm, and as cosmetic. The aim of this study was to evaluate the bioactivity and the cytotoxicity of the intact molecule and degraded forms of biopharmaceutical products analysed by reversed-phase liquid chromatography (RP-LC). **Methods:** The RP-LC method was performed on a Zorbax 300 SB C₁₈ column (150mm × 4.6 mm) maintained at 45 °C. The mobile phase consisted of 0.05 M sodium phosphate buffer, pH 2.8 and acetonitrile (80:20, v/v), run at flow rate of 0.3 mL/min with PDA detection at 214nm. Samples of BoNTA were subjected to degradation by exposing in a photostability chamber to 200 Wh/m² of near ultraviolet light for 4 h and 30 min and analysed by RP-LC. The *in vitro* cytotoxicity test was performed by the neutral red uptake cytotoxicity assay based on the exposure of the NCTC 929 cells to the samples on the 96 microplate maintained at 37 °C in a CO₂ incubator for 24 h. The neutral red released was evaluated by the addition of an extracting solution and the absorbance was measured at 540 nm using a microplate reader. The bioactivity was assessed by the T47D cell bioassay based on the dose-dependent cytotoxicity curve of the cells, evaluating the response with MTT. The absorbances were determined at 570 nm using a microplate reader. **Results:** The bioactivity and the cytotoxicity tests were performed on degraded forms versus the intact molecule in order to detect possible effects resulting from the instability of the samples during storage. Photodegradation showed non-significant changes of the bioactivity compared to the intact molecule, as evaluated by the T47D cell bioassay, and calculated by the Student's *t* test ($p > 0.05$). Besides, the bioassay applied to the evaluation of biological products, showed potencies between 91.50 and 108.40%, related to the values claimed by the manufacturers. The cytotoxicity test showed mean values of IC₅₀ = 1.37 ± 0.02 U/mL and IC₅₀ = 0.87 ± 0.03 U/mL for the sample and for the photodegraded molecule, respectively, with significant differences ($p < 0.05$). **Conclusion:** The results demonstrated the capability of the *in vitro* bioassay for the potency assessment of BoNTA, and the application of the cytotoxicity test to detect possible undesirable effects of degraded forms. Thus the bioassays contribute to the establishment of alternatives, which improve the potency evaluation and assuring the safe use of the biological product. **References:** BANDALA, C. et al. *Asian Pac J Cancer Prev*, v. 14, p. 891, 2013. FREITAS, G.W. et al. *Anal Methods*, v. 8, p. 587, 2016. WILDER-KOFIE, T. et al. *Comp Med*, v. 61, p. 235, 2011. **Acknowledgments:** CNPq and FATEC for the financial support.

01.014 Friedelin regulates migration and extracellular matrix synthesis in fibroblasts. Carmo JOS¹, Ferro JNS¹, Conserva LM², Barreto E¹, Correia ACC^{1,3} ¹UFAL- Biologia Celular, ²UFAL- Química de Produtos Naturais, ³UFAL- Nutrição

Introduction: Wound healing plays an important role in protecting the human body from external infection. Cell migration and proliferation of fibroblasts are essential for proper wound healing. In a recent study, we demonstrated that friedelin accelerated a healing of skin wounds in diabetic animals (Correia et al, 47^o SBFTE, 2015). Here, we assessed the effect of friedelin on activation of selected genes and proteins related to wound healing in fibroblasts *in vitro*.

Methods: For these experiments, 3T3 fibroblast were cultured in DMEM medium containing 10% fetal calf serum, 2 mM glutamine, 1% gentamicin and incubated at 5% CO₂ at 37°C. The viability of fibroblast treated with friedelin (1, 10 or 100 µM) for 24 h was determined by the MTT assay. Scratch wound healing assay was used to determine cell migration. For this purpose, after mechanical injury to the adherent cells the treatment with friedelin for 12h and 24 h was performed. Untreated cells were used as control. The extent of cell migration was photographed and measured using image analyzing software. In another set of experiments, production of fibronectin laminin and transforming growth factors (TGF-β1) was assessed using immunofluorescence staining, while mRNA expressions to matrix metalloproteinases 9 (MMP-9), tissue inhibitor of metalloproteinases (TIMP-1), collagen type 1, collagen type 3 and TGF-β1 were analyzed by RT-PCR. All assays were performed in three independent controlled experiments. **Results:** Statistical analysis, using two-way ANOVA with post hoc Bonferroni test, revealed that friedelin at 100 µM increased the migration of fibroblast by 12% (12h) and 24% (24h), respectively. Immunofluorescence analysis revealed a significant increase in the amount of fibronectin (p<0.05), laminin (p<0.001) and TGF-β1 (p<0.001) in fibroblast after treatment with 100 µM friedelin. Meanwhile, friedelin induced a decrease in the messenger RNA levels of TIMP-1 (p<0.01), without affecting the expression of collagen type 1, collagen type 3 or TGF-β1. Despite a rise in levels of mRNA, the expression of MMP-9 in friedelin-treated cells no exhibited statistical difference compared with control group. Friedelin alone did not affect cell viability at all doses tested. **Conclusions:** On the basis of these results, we suggest that friedelin may exert a stimulating effect on fibroblasts by enhancing their migration by modulating genes related to wound healing. **Financial Support and Acknowledgments:** CNPq and CAPES.

01.015 Fenofibrate promotes weight loss in mice via miR-103. Rocha KC¹, Frias FT¹, Sousa E¹, Cruz MM², Rodrigues AC¹ ¹USP – Farmacologia, ²Unifesp-Diadema – Ciências Biológicas

Introduction: Obesity is characterized as an expansion due to increase in adipocyte number (hyperplasia) and/or size (hypertrophy), thus inducing dysregulation of glucose and lipid metabolism¹. Energy metabolism related genes are regulated by peroxisome proliferator-activated receptor alpha (PPAR α)². Evidence shows that microRNAs can regulate adipocyte differentiation by regulating PPAR α expression³. To evaluate metabolic changes via PPAR α activation and its consequences we treated obese mice with PPAR α agonist fenofibrate.

Methods: Male C57BL/6 mice at 8 weeks' old (n=26) were randomly assigned to receive a control (CD) or a high-fat diet (HFD) for 6 weeks. After that, they were subdivided into four groups: C group (fed with CD, untreated), CF group (fed with CD and treated with fenofibrate), H group (fed with HFD, untreated) and HF group (fed with HFD and treated with fenofibrate). Mice were administered by oral gavage with fenofibrate at 50 mg/Kg/day for 2 weeks, and then they were euthanized. Insulin Tolerance Test (ITT) was performed on the last week of protocol. Samples of blood were collected for biochemical measurements and mesenteric, epididymal, and retroperitoneal fat depots were weighted for adipose index calculation. Epididymal adipose tissue was immediately flash-frozen in liquid nitrogen for total RNA extraction and retroperitoneal adipose tissue was used for lipolysis assay. Expression of microRNAs miR-30d, miR-103 and miR-150 and mRNAs (Cebpa, Ppara, Npyr1, Pparg, Ppargc1a, Cpt1b, and Ucp1) were measured by Real-time PCR assay in epididymal adipose tissue, using sno-202 and 234 or Hprt1 for normalization, respectively. Statistical analysis was performed using Two-way ANOVA, followed by Bonferroni post-test. Differences at $P < 0.05$ were considered significant.

Results: Mice fed with HFD showed augment of body weight gain, weights of adipose depots and fasting leptin levels, however it reduced constant of glucose disappearance (Kitt) ($p < 0.05$). Treatment with fenofibrate didn't change the food intake, but attenuated the weight gain in both CF and HF groups, contributing to lower the adipose tissue storage. Leptin levels were restored to control mice serum levels, but insulin resistance measured by ITT was not different from H group ($p > 0.05$). In epididymal adipose tissue expression of miR-103 and miR-150 were upregulated and Ppargc1a, Ppara and Npyr1 gene expression were downregulated in obese mice, but treatment with fenofibrate only restored miR-103 levels to those found in C group. Interestingly, fenofibrate upregulated Pparg and Cebpa gene expression, suggesting an increased adipocyte differentiation in adipose tissue. Compared with C group, the adipocytes diameter and basal lipolysis were both higher in the HFD group, however the fenofibrate treatment was not able to normalize it.

Conclusion: Fenofibrate induced weight loss in mice caused by HFD feeding probably by a mechanism involving miR-103. References: 1. Ferreira AV. *Metabolism* 63(4):456; 2014. 2. Martinelli R. *Obesity* 18(11):2170; 2010. 3. Son YH. *Endocrinol Metab* 29(2):122; 2014. **Financial Support:** CNPq and FAPESP. Animal Research Ethical Committee Process Number: 165/2011.

01.016 Prenatal alcohol exposure can change the expression of genes related to Osteogenesis in pre-osteoblasts of newborns rats. Carvalho ICS¹, Milhan NVM², Barros PP², Back-Brito GN², Jorge AOC², Godoi BH¹, Moraes CDGO¹, Rocha RF², Pacheco-Soares C¹
¹UNIVAP – Biologia Celular e Molecular, ²Unesp – Ciências Biológicas e Odontologia

Introduction: Alcohol exerts teratogenic effects and its consumption during pregnancy may cause deficit of bone development. The purpose of this study was to evaluate the genes expression related to osteogenesis, in pre-osteoblasts of newborns, after teratogenic doses of ethanol during pregnancy. **Methods:** Wistar rats were initially divided into three groups: Ethanol group which received Ethanol 20% V/V in liquid diet and solid diet *ad libitum*; Pair-fed group that received the equivalent amount of carbohydrate solution and solid diet, and Control group, which received solid diet and water *ad libitum*. Each group received this specific diet for eight weeks prior to breeding and during the three weeks of gestation. The newborns were euthanized on the fifth day of life. After, the calvaria was removed, and the cells were isolated by sequential enzymatic digestion. The pool of cells from each experimental group were frozen at - 80°C in aliquots of 1×10^6 cells for later use. Total RNA was extracted, quantified and transcribed in the cDNA. Diluted cDNAs were subjected to PCR amplification in real time using Osteogenesis RT² Profiler Rat PCR array (Qiagen). The qPCR data were evaluated by a software available on the company website. A total of 84 genes related to different processes of osteogenesis were evaluated. **Results:** In this study the expression profiles by microarray to the process of osteogenesis were variables in the analyzed groups. Most of the genes were up regulated in the Pair-fed and Control groups, and down regulated in the Alcohol group. By the other hand, many genes were up regulated in the Alcohol group and down regulated in the Pair-fed and Control groups, such as Runx2, Sp7, Col1a1, Spp1, Bglap and Alpl, which are mainly related to osteoblasts differentiation and ossification process. **Conclusion:** Based on the results we may suggest that prenatal alcohol exposure modified the profile of gene expression in the osteogenesis process in rats, and has a direct effect on pre-osteoblasts of newborns. **Financial support:** FAPESP (Grant 2012/10643-3) and CAPES. The Ethical Committee in Research of São José dos Campos Institute of Science and Technology, UNESP – Universidade Estadual Paulista approved the study protocol (01/2012-PA/CEP).

01.017 Treatment of diet-induced obese mice with pioglitazone causes decrease of MIR-23b by adiponectin-independent pathway in skeletal muscle. Mendonça M, Sousa E, Rodrigues AC ICB-USP – Farmacologia

Introduction: Pioglitazone, an agonist of peroxisome proliferator-activated receptor (PPAR) alpha and gamma, induces the production of adiponectin and ameliorates insulin resistance in diet-obese mice. Recently, there are evidences that adiponectin gene and its receptors are regulated by microRNAs (miRs)¹, therefore a treatment that regulates the expression of these miRs can be effective in the improvement of insulin resistance. Recent studies of our group performed in C57BL/6 mice fed a high fat diet for 8 weeks to induce insulin resistance, showed increased mRNA expression of adiponectin in skeletal muscle after treatment with pioglitazone associated to a decreased expression of miR-23b. Therefore, this study aims to evaluate if pioglitazone-induced decrease of miR-23b and increased insulin sensitivity is dependent on adiponectin plasma levels. **Methods:** Eighteen male C57BL/6 mice adiponectin knockout (adipoko), 8 weeks old and 20g on average were fed a balanced diet (BD) or high fat diet (HFD) for 6 weeks to induce obesity and, then part of the mice of the HFD group was treated for 2 weeks with pioglitazone 35mg/kg/day (group HFD+P). After treatment, insulin tolerance test was performed for insulin resistance evaluation by glucose decay rate (kITT). At the end of treatment, animals were euthanized and their soleus muscles were collected for analysis of miR-23b expression by Real-Time PCR and protein expression by Western Blot analysis. The data were analyzed by ANOVA One-way/Two-way followed by Tukey's/ Bonferroni post-test. **Results:** The kITT demonstrated that HFD animals had lower insulin sensitivity compared to BD and treatment with pioglitazone improved insulin sensitivity (kITT- BD: $6.6 \pm 0.8\%/min$ vs HFD: $4.4 \pm 1.3\%/min$ vs HFD+P: $6.7 \pm 1.0\%/min$; $p < 0.05$). miR-23b was up regulated in obese mice, and treatment with pioglitazone reversed its expression to similar levels found in BD group (BD: 1.00 ± 0.07 vs HFD: 1.64 ± 0.11 vs HFD+P: 0.67 ± 0.05 ; $p < 0.05$). Pioglitazone is known to activates AMPK pathway², so Western-Blot analysis were made to phosphorylated AMP-activated protein kinase (pAMPK) and Sirtuin1 (Sirt1), a target of miR-23b. We observed an increased expression of pAMPK in HFD+P group, comparing to HFD group, (BD: 1.00 ± 0.05 vs HFD: 0.85 ± 0.04 vs HFD+P: 1.60 ± 0.14 ; $p < 0.05$) and an increased expression of Sirt1 in HFD+P group comparing to BD and HFD groups (BD: 1.00 ± 0.07 vs HFD: 0.75 ± 0.09 vs HFD+P: 4.15 ± 0.62 ; $p < 0.05$). **Conclusion:** Pioglitazone-induced amelioration of insulin resistance occurs adiponectin independently, but by a mechanism dependent of miR-23b in skeletal muscle. **References:** 1-Lustig Y. Diabetes .(63):433; (2014). 2-Saha AK. BBRC. (314):580; (2004). **Financial support** : FAPESP (2015/24650-0) Animal Research Ethical Committee Process Number: 137/2015

01.018 Cilomilast inhibits elastase-induced lung emphysema in mice Cunha LCL¹, Souza ET², Martins MA², Silva PMR² ¹UERJ – Ciências Biológicas, ²Fiocruz – Farmacologia Bioquímica e Molecular

Introduction: Chronic obstructive pulmonary disease (COPD), manifested as chronic airway obstruction, bronchitis, and emphysema, is considered as a major public health problem worldwide. There is no effective treatment available for this disease. To identify potential therapeutic targets, rodent models have been established including the administration of porcine pancreatic elastase (PPE). **Aim:** This study was undertaken to investigate the potential effect of phosphodiesterase (PDE) type 4 inhibitor cilomilast on PPE-induced emphysema in mice. **Methods:** Female C57BL/6 mice were used and PPE (0.2 and 0.6 IU) or saline were instilled intranasally in anaesthetized animals. Cilomilast was administered daily, at the doses of 10 and 30 g/kg (p.o.), from day 7 to 13 and the analyses were performed 24 h after the last dose. The parameters included lung function and airway hyper-reactivity to aerosolized methacholine by invasive plethysmography (Finepointe, Buxco System) and morphology/morphometry by automatic scanner (3DHISTECH) and Panoramic Viewer Software. All procedures were approved by the Ethics Committee for Animal use (CEUA - number of license LW57/14). **Results:** First a dose response of PPE was performed. Microscopic analysis revealed that 24 h after PPE (0.2 IU), there was an inflammatory response with a marked leukocyte infiltration. At 14 days, destruction of the alveolar wall, with clear signs of hyper-insufflation area, and peribronchiolar fibrosis were noted in C57BL/6 mice, in comparison with normal alveolar structure of the controls. This response correlated with increased airways resistance and reduced lung elastance. Because the dose of 0.6 IU caused animal death, later studies were performed with 0.2 IU. The administration of the PDE inhibitor cilomilast to PPE-challenged mice attenuated the emphysematous response including both hyperinsufflation area and fibrosis. **Conclusion:** Our findings show that elastase-induced lung emphysema exhibits important features of the clinical disease, a response sensitive to treatment with cilomilast. This is indicative that the blockade of PDE4 enzyme seems to be a promising therapeutic tool for application in the treatment of COPD. **Financial support:** FIOCRUZ, CNPq, FAPERJ and CAPES (Brazil).

01.019 Evaluation of an *in vitro* cell culture bioassay for the potency assessment of recombinant human erythropoietin. Perobelli RF, Xavier B, Maldaner FPS, Walter ME, Motta LGJ, Dalmora SL UFSM – Farmácia Industrial

Introduction: Human erythropoietin (EPO) produced by recombinant DNA technology (rhEPO) is marketed worldwide to treat anemia. The hormone consists of a 165 amino acids polypeptide chain, heavily glycosylated at three N-linked and one O-linked glycosylation sites, with a molecular mass of 30.4 kDa. The aim of this study was to validate an *in vitro* TF-1 cell proliferation assay for the potency assessment of rhEPO in biopharmaceutical formulations.

Methods: The assay was based on the growth-promoting activity of the factor-dependent human cell line, TF-1 (ATCC CRL-2003). The cells were maintained in RPMI 1640 culture medium, supplemented with 10% (v/v) fetal bovine serum. The cells were seeded in 96-well cell culture plates at a density of 1.0×10^5 cells/mL, and dosed upon seeding with five concentrations of rhEPO between 0.10 – 2.0 IU/mL, in triplicate, as parallel assays. The Ph Eur BRP rhEPO was used as the standard, and the control was RPMI 1640 medium. The plates were incubated at 37°C, 5% v/v CO₂, for 48 h. Then, 25 µL of a 1 mg/mL tetrazolium sodium salt (XTT) solution per well was added, and the plates were incubated (37 °C, 5% v/v CO₂) overnight. The absorbance at 450 nm was measured with microplate reader.

Results: The TF-1 cell culture assay was validated by using samples of rhEPO (4 000 IU/mL). The specificity was established with rhEPO formulations spiked independently with known amounts of rhGH, rhGCSF, interleukin-11 and neuraminic acid with showed non-significant differences ($p>0.05$) related to the un-spiked sample. The analytical curves were found to be linear over the range 0.0125 – 6.4 IU/mL, the determination coefficient calculated from $y = 0.171x + 0.806$, was $r^2 = 0.9944$, which confirmed the linearity of the curve. The limit of quantitation was 0.0125 IU/mL. The accuracy was 98.93% with bias lower than 0.60%. Moreover, method validation demonstrated acceptable results for precision and robustness. The method was applied for the analysis of recombinant human erythropoietin in biopharmaceutical formulations giving potencies between 87.30% and 116.20%, related to the potencies claimed by the manufacturers.

Conclusion: The TF-1 cell culture assay represents an advance toward the establishment of *in vitro* approaches, in the context of the Three Rs. The assay was validated and applied for the potency assessment of rhEPO in biopharmaceutical formulations, contributing to establish alternative which improve the quality control assuring the therapeutic efficacy.

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Acknowledgments: CNPq and FATEC for the financial support.

01.020 Investigation of the role of endothelial P2Y₂ and P2Y₆ receptors in leukocyte adhesion during mesenteric inflammation caused by schistosomiasis. Pereira LM, Silva CLM UFRJ – Farmacologia e Inflamação

Introduction: Schistosomiasis is a chronic intravascular disease caused by *Schistosoma mansoni* and it is related to mesenteric vascular inflammation (Oliveira et al., 2011 Plos One 6(8):e23547). Endothelial cells play an important role during inflammation since, once activated, they release proinflammatory nucleotides and express adhesion molecules important for leukocyte adhesion. Constitutive nitric oxide (NO) production counteracts the later. Extracellular ATP activates P2Y and P2X purinoceptors (P2R) while ADP, UDP and UTP act through P2Y₁, P2Y₂ and P2Y₆R, respectively. Recently we showed that P2Y₁R-mediated leukocyte adhesion to endothelial cells is enhanced during schistosomiasis (Oliveira et al., 2016 Vascul Pharmacol 82:66), but the other P2YRs have not been investigated yet. The objective of this work was to investigate the putative role of endothelial P2Y₂R and P2Y₆R to leukocyte adhesion during schistosomal inflammation. **Methodology:** Newborn Swiss mice (male) were infected with *S. mansoni* as previously described. Animals (75 days old, control and infected) were anesthetized; the mesenteric vessels were removed, cut into small segments, and kept in DMEM enriched with 20% fetal bovine serum (37°C, 5% CO₂) until confluence. Cultured endothelial cells were plated to leukocyte adhesion assay and nitric oxide (NO) measurement. Mononuclear cells from both groups were isolated from peripheral blood using Percoll gradient (Oliveira et al., 2011 Plos One 6(8):e23547). Confluent endothelial cells were stimulated with 100 µM UTP (P2Y₂R) or 30 µM UDP (P2Y₆R) for 5h, in the absence (basal) or presence of the P2YR antagonists suramin (P2Y₂R) and MRS2578 (P2Y₆R), respectively; and then co-incubated with mononuclear cells for 30 min. Next cells were washed. Four fields/well were chosen and the number of adherent cells was defined using an optical microscopy. Alternatively, endothelial cells were treated for 1h or 12h to measure NO and IL-1β, respectively. Experiments were performed in triplicate using different cultures. **Results:** In the control group the treatment of endothelial cells with 100 µM UTP (P2Y₂R) increased leukocyte adhesion from 4.3 ± 0.1 to 7.7 ± 0.3 cells/field (n = 4, P < 0.001). Suramin (50 µM) pretreatment blocked the agonist effect (4.4 ± 0.2 cells/field). However, in the infected group UTP (100 µM) increased leukocyte adhesion from 10 ± 0.8 to 17 ± 1.1 cells/field (n = 3, P < 0.001), and suramin also blocked the effect. In the control group UTP (100 µM) increased NO production only by 10% and its effect was smaller than 1 µM bradykinin used as positive control (33.8 ± 1.9%, n = 2), suggesting that P2Y₂R are not involved in constitutive endothelial NO production. Furthermore, UTP had no effect in the infected group (P = 0.13). In the control group UDP (30 µM) also increased leukocyte adhesion (P < 0.05). Preliminary data suggest that basal production of IL-1β is increased in endothelial cells from infected mice (17.1 ± 2.2 pmol/ml) as compared to control (8.5 ± 1.5 pmol/ml, n=2), and in the latter group UDP (30 µM) increased IL-1β production to from 8.5 to 23.55 pmol/ml. **Conclusions:** The stimulation of endothelial P2Y₂ and P2Y₆ receptors increases leukocyte adhesion in control and infected groups, but the final number of adhered leucocytes to endothelial cells is higher in the infected group, suggesting an upregulation of inflammatory purinergic signaling during schistosomiasis. **Financial Support:** CAPES, FAPERJ and CNPQ. **Ethics Committee:** CEUA UFRJ 048/16

01.021 Melanoma-derived microvesicles induce human neutrophils polarization.
Guimarães-Bastos DA¹, Frony AC¹, Saldanha-Gama R¹, Moraes JA^{1,2}, Barja-Fidalgo TC¹
¹UERJ – Biologia Celular e Molecular, ²UFRJ – Farmacologia Bioquímica e Celular

Introduction: The understanding of the mechanisms involved in tumor growth runs through the knowledge on its microenvironment, formed by extracellular matrix components, growth factors, cytokines and different cell types. Immune cells present in tumor microenvironment have their pro-inflammatory functions modified to support tumor growth. Recent studies have provided evidence on two phenotypes of tumor-associated neutrophils (TAN): a protumoral, TAN-N2 or an antitumoral, TAN-N1. Evidence also shows that tumor microenvironment is rich in microvesicles (MV), produced abundantly by the tumor. Most MV can not only carry molecular information from their cellular origin, but could also modulate activity of target cells. Although tumor-derived MV were shown to interact with different cells in the microenvironment, there are no reports on its contribution to modulate TAN. Now, we investigate whether MV produced by a human melanoma cell line modulate human neutrophil activity, shifting it to a N2-like phenotype.

Methods: MV were obtained from conditioned media of human melanoma cell line MV3 cultures and quantified by annexin-V staining. Human neutrophils (PMN), isolated by Percoll gradient, were incubated in the presence or absence of MV (10%v/v) or LPS (10µg/mL) at different times. Chemotaxis was assayed in a 48-wells modified Boyden Chamber. Apoptosis was assessed through cytometry analyses. Neutrophil extracellular traps (NETs) formation was evaluated by DNA quantification and immunofluorescence. Intracellular ROS, NO and peroxynitrite production were measured through the oxidation of CM-H2DCFDA, DAF-FM and HPF probes, respectively. MV3 cells co-cultured with PMN viability was assessed by MTT assays. Molecular markers gene expression were obtained by qRT-PCR. CXCR4 and arginase contents were obtained by cytometry and immunoblotting, respectively. **Results:** MV induced PMN chemotaxis, and this effect was inhibited by pertussis toxin and is dependent on PI3K-AKT pathway activation. We also observed that MV effect on PMN chemotaxis relies on CXCR2, once its inhibitor, SB225002, abrogated PMN migration. MV delayed PMN spontaneous apoptosis, triggered NETs formation and induced intracellular ROS production. Treatment with MV decreased NO basal production, and increased CXCR4 and arginase protein expression and also augmented the mRNA content of several TAN-N2 molecular markers, such as arginase, CXCR4 and VEGF in PMN. Finally, PMN treated with MV did not affect melanoma cells viability in co-culture. **Conclusion:** Our data indicate that melanoma-derived MV may have an important role on shaping the phenotype of PMN in tumor microenvironments towards a TAN-N2 phenotype, which shows an increased expression of pro-tumor molecular markers and presents low cytotoxic properties. **Funding support:** CAPES, CNPq, FAPERJ Study approval from Ethics Committee of Pedro Ernesto Hospital: 38257914.7.0000.5259

01.022 Neutrophil microparticles: generation and role on inflammation. Frony AC¹, Moraes JA², Barcellos-de-Souza P³, Cunha M, Boisson-Vidal C⁴, Barja-Fidalgo TC¹ ¹UERJ, ²UFRJ, ³INCa, ⁴INSERM

Introduction: During an inflammatory process, microparticles (MP) released by stimulated cells can be found in bloodstream, acting as biomarkers and bioeffectors. The migration of polymorphonuclear neutrophils (PMN) is triggered by a gamma of soluble mediators that induce cell activation and is primarily modulated by adhesion molecules. Among them, L-selectin mediates PMN rolling on endothelial surface. Fucoidans are sulfated polysaccharides able to inhibit selectin-mediated events. A low-molecular-weight fucoidan fraction extracted from the brown algae *Ascophyllum nodosum* (LMW-Fuc) is a potent antithrombotic, with low anticoagulant activities, and potent proangiogenic properties. We have early shown that LMW-Fuc decreased LPS/fMLP-induced activation on human PMN, such as chemotaxis, apoptosis, NET and ROS production. In this work, we evaluated the effect of LMW-Fuc on MP production by activated PMN, and also investigated the effects of these MP on PMN and macrophages.

Methods: Human PMN and monocytes were isolated in Percoll gradient. MP were isolated from PMN supernatant by ultracentrifugation at 100000xg for 4h, and quantified by flow cytometry. Proteins expression was evaluated by immunoblotting. ROS production was quantified by Lucigenin (5 uM) and CM-H₂DCFDA (10 uM). **Results: (1)** PMN primed with LPS (1 µg/mL) and further treated with fMLP (100 nM) for 1 h released high amounts of MP. **(2)** This process was dependent on L-selectin, once it was inhibited by LMW-Fuc (10 µg/mL). **(3)** LPS/fMLP-induced MP generation was also dependent on MLC pathway, whose activation relies on L-selectin. **(4)** PMN activated by LPS/fMLP produced MP enriched in p47^{phox}, which were able to induce extracellular ROS production on fresh PMN and macrophages. **(5)** To investigate the role of the p47^{phox} derived from MP on this effect, macrophages were silenced for NOX2 and treated with pro-inflammatory MP. Silenced cells did not produce ROS, indicating that the MP effect requires the whole NOX2 complex to induce ROS release. Furthermore, macrophages without p47^{phox} activity that were treated with pro-inflammatory MP were able to produce extracellular ROS production, indicating that p47^{phox} is transferred from MP to macrophages. **Conclusion:** Our data point to an important pro-inflammatory role of activated PMN-derived MP during inflammatory process, modulating ROS production by phagocytes. The results also show that the treatment with LMW-Fuc could attenuate PMN activation during infectious process, decreasing the production of pro-inflammatory MP, suggesting a new anti-inflammatory approach. **Funding support:** CAPES, CNPq, FAPERJ **Study approval from Ethics Committee of Pedro Ernesto Hospital:**38257914.7.0000.5259

01.023 Expression of the estrogen receptors in DU-145 human prostate cancer cells.
Souza DS, Lombardi APG, Lucas TFG, Porto CS¹Unifesp-EPM – Farmacologia

Introduction: Androgens, estrogens and stromal-epithelial interactions are involved in the development of prostate cancer (reviewed in Prins & Korach, *Steroids* 73:233, 2008). Prostate cancer initially responds well to androgen-deprivation therapies, but the majority of tumors evolve from an androgen-sensitive to an androgen-independent form of the disease, also known as castration-resistant prostate cancer (CRPC), which presents a poor prognosis and no effective therapy (reviewed in Parray et al., *Biologics: Targets and Therapy* 6:267, 2012). Estrogens, acting through classic estrogen receptors ESR1 (ERalpha) and ESR2 (ERbeta), may play an important role in this mechanism. Thus, the aim of this study was to identify the expression and cellular localization of the ESR1 and ESR2 in the androgen-independent prostate cancer cell line DU-145, used as CRPC models. In addition, we evaluated the relative contribution of these receptors to the activation of the ERK1/2 (extracellular signal-regulated protein kinases) signaling pathway. **Methods:** The experimental procedures were approved by the Research Ethical Committee at EPM-UNIFESP (#2014/05292-2). DU-145, androgen-independent prostate cancer cell, and PNT1A, human post pubertal prostate normal cells, used as a control of some experiments, were grown in RPMI 1640 medium without phenol red, supplemented with 10% of fetal bovine serum, HEPES (5.95 mg/ml) and gentamicin (0.02 mg/ml). After 24 hours of serum removal, DU-145 cells were incubated in the absence (control) and presence of 17beta-estradiol (E2, 0.1 nM), ESR1-selective agonist PPT (10 nM) or ESR2-selective agonist DPN (10 nM) for different times of incubation and Western blot assays were performed for detection of ESR1, ESR2, ERK1/2 and phospho-ERK1/2. Cells were also untreated (control) or pretreated with CRM1 (exportin 1) inhibitor leptomycin B (4.5 nM) for 24 h and immunofluorescence assays for detection of ESR1 and ESR2 were performed as previously described (Pisolato et al., *Steroids* 107:74, 2016). **Results:** ESR1 immunostaining was predominantly nuclear in PNT1A. ESR1 and ESR2 immunostaining were localized preferentially in extranuclear regions of DU-145 cells. ESR1 and ESR2 were detected as a single protein band of about 66 kDa and 56 kDa, respectively, by Western blot. The unusual cytoplasmic localization of both receptors in DU-145 cells differs from nuclear localization in the majority of estrogen target cells and suggests that rapid signaling pathways may be preferentially activated. In fact, E2 induced a rapid and transient increase in the phosphorylation state of ERK1/2 in the DU-145 cells. Peak ERK1/2 phosphorylation levels occurred at 5 min (1.7-2.2-fold increase). PPT and DPN also induced an increase in the phosphorylation state of ERK1/2. In the presence of CRM1 inhibitor leptomycin B, immunostaining for ESR1 and ESR2 were found in the nuclei of DU-145 cells, suggesting the involvement of CRM1 protein in the preferential cytoplasmic localization of ERs. No immunostaining was observed in the negative control, performed with the primary antibody preadsorbed with its respective blocking peptide. The identification of estrogen receptors and new signaling pathways is important to understand the estrogen-mediated functions in the prostate cancer and the possible role of this hormone in the progress of the disease to an androgen-independent phenotype. **Financial Support:** FAPESP, CNPq, CAPES.

01.024 Obese adipose tissue contributes to increase proliferation, migration and invasion in breast cancer cells. Andrade IR¹, Renovato-Martins M¹, João JA², Matheus ME², Silva SV¹, Bouskela E¹, Souza AP², Cláudio-da-Silva C², Barja-Fidalgo TC¹ ¹UERJ, ²UFRJ

Introduction: Obesity is characterized by a chronic low-grade inflammation and is a major problem of public health, especially because of its association with several diseases, including cancer. Many studies have shown a higher incidence and a worse prognosis of breast cancer in obese women. Aiming to evaluate the influence of adipose tissue on tumor cells during obesity, we used an experimental model, in vitro, in which the conditioned media of cultures of adipose tissue (AT) explants of obese and non-obese individuals were used to stimulate two cell lines of human breast adenocarcinoma, MCF-7 and MDA-MB-231 (more metastatic than MCF-7). We have evaluated the influence of factors secreted by AT on the behavior of these cells, and the molecular mechanisms involved in those effects that could contribute to a worse prognosis in cancer. **Materials and Methods:** Tumor cells were stimulated with 20% of conditioned medium (CM) or with microparticles (MP) derived from AT explants obtained from obese patients or lean individuals. In some experiments anti-MIP-1 α or anti-VEGF were added to AT explant cultures. Cell viability (24 and 48 h stimulation) was assayed by MTT method; Cell cycle assay (24 h) was evaluated in flow cytometry; Cell migration assayed by wound healing method after 24 h stimulation; For tubulogenesis assays on MatriGel, endothelial cells (HMEC) were incubated with the supernatant of cultures of tumor cells treated (24 h) with the conditioned medium obtained from obese or lean AT. Cell invasion was assessed through transwell migration after 24 h of stimulation. MMP9 RNA expression was evaluated by real-time PCR. **Results:** The MCF-7 cells, but not MDA-MB-231 cells, when stimulated with CM or with MP derived from obese AT showed higher proliferation ($p < 0.05$), compared to cells treated with CM- or MP-derived from lean AT. When anti-MIP-1 α was added to obese AT explant cultures, the increasing proliferative effect of CM, but not of MP, was impaired in MCF-7 cells. On the other hand, CM and MP from obese AT enhanced the migration capacity of MDA-MB-231 cells ($p < 0.005$), but not of MCF-7 cells. The treatment of MDA-MB-231 cells with CM or with MP from obese AT explants enhanced their ability to induce tubulogenesis ($p < 0.01$) and invasion ($p < 0.05$). Both effects were blocked when anti-VEGF was added to obese AT explant cultures ($p < 0.005$). Furthermore, MP derived from obese AT increased ERK phosphorylation in MCF-7 cells ($p < 0.05$), whereas increased AKT phosphorylation in MDA-MB-231 cells ($p < 0.05$). **Conclusion:** Our results demonstrated that MP and molecules secreted by obese adipose tissue, like MIP-1 α , VEGF, can improve the functionality of breast tumor cells, increasing proliferation, migration, invasion and their angiogenic properties, what might contribute to enhance malignancy. **Financial support:** CAPES, CNPq, FAPERJ This study was approved by Ethic Committee from Pedro Ernesto hospital (CAAE 36880914.0.0000.5259).

01.025 Annexin A1 Modulates Peroxisome Proliferator-Activated Receptor γ Expression in BV2 murine microglial cell line. Rocha GHO, Pantaleão LN, Farsky SHP USP – Análises Clínicas e Toxicológicas

Introduction: Peroxisome proliferator-activated receptor γ (PPAR γ) is an important nuclear protein involved with energetic metabolism in adipocytes, but is also expressed in both mononuclear and polymorphonuclear cells, including those located in the central nervous system (CNS). Recent research suggests that PPAR γ also displays anti-inflammatory actions and might contribute to alleviation of inflammatory damage in the CNS. Annexin A1 (ANXA1) is an anti-inflammatory protein that plays a key role in glucocorticoid-mediated effects and is also expressed by immune cells in the CNS. While it is well-known that ANXA1 is vital for the resolution of inflammation, all the underlying mechanisms and interactions with other anti-inflammatory factors that lead to such action are still not fully understood, especially in the CNS. Using BV2 murine microglial cell line as the experimental model, we aimed to investigate whether PPAR γ expression and also its anti-inflammatory effects could be modulated by ANXA1 in the CNS. **Methods:** Cultured BV2 cells were treated with ANXA1 at increasing concentrations (ranging from 10 to 100 nM) for 60 minutes and the proteic content was then extracted; proteic expression of PPAR γ was evaluated by Western Blotting. Other set of BV2 cells were treated with ANXA1 (100 nM) for 30 and 60 minutes and gene expression was assessed by Real Time Polymerase Chain Reaction (RT-PCR). In order to investigate the effects of the ANXA1-PPAR γ interaction, BV2 cells treated with either 100 nM of ANXA1, 10 μ M of pioglitazone (PPAR γ agonist) or both, for a period of 60 minutes, were co-cultured with apoptotic PC12 cells (murine neuron-like cell line); phagocytosis and the expression of CD-36, a surface protein involved with phagocytosis and directly modulated by PPAR γ , were assessed by flow cytometry. **Results:** Data obtained demonstrated that ANXA1 increases both protein and gene expression of PPAR γ in BV2 cells by almost 2-fold. Treatment with ANXA1 did not seem to be involved with PPAR γ -associated phagocytosis effects however, as simultaneous treatment with both ANXA1 and pioglitazone was ineffective in increasing expression of CD36 or increasing the amount of phagocytized apoptotic PC12 cells. **Conclusion:** Our data show that ANXA1 evidently modulates PPAR γ expression in BV2 microglial cells, but such modulation does not seem to induce any improving effects on the PPAR γ -mediated phagocytic capabilities of BV2 microglial cells. Further studies are still required to better address the anti-inflammatory effects that might originate from a PPAR γ -ANXA1 interaction. The first author received scholarship from *Conselho Nacional de Pesquisa e Desenvolvimento* (CNPq). The present work received funding from *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, nº 2014/07328-4).

01.026 Ouabain-induced hypertension promotes unique alterations of Na/K-ATPase from different rat organs. Feijó PRO¹, Neto AF², Rossoni LV², Noël F¹, Quintas LEM¹ ¹ICB-UFRJ, ²USP – Farmacologia

Introduction: Organ-specific changes of the activity and expression of Na⁺/K⁺-ATPase (NKA), the receptor of cardiotonic steroids, are observed in experimental hypertension. Ouabain is now considered as an endogenous cardiotonic steroid related to hypertensive states, although its function is still elusive. Ouabain induces hypertension in rats when administered chronically, but the effect on NKA of organs involved in controlling blood pressure is poorly understood. Moreover, the putative separate roles of ouabain and hypertension are unknown. To evaluate the effect of chronic administration with ouabain on the activity and expression of NKA isoforms from rat heart, kidney and brain, and the concomitant treatment with antihypertensives.

Methodology: Male Wistar rats weighing between 320-450g (6 weeks, n=6) were treated with ouabain (OUA, 8 µg/day subcutaneously), ouabain and antihypertensives (OUA+HH, hydrochlorothiazide 9.4 mg/kg + hydralazine 44 mg/kg), vehicle (VEH) or vehicle and antihypertensives (VEH+HH) for 5 weeks. Kidneys, brain and heart ventricles were dissected, weighed and stored at -80°C. Then, they were homogenized and the pellets from ultracentrifugation were resuspended and used for enzyme activity experiments (NKA), and protein expression determined by Western blot technique. All animal procedures were approved by the Ethics Committee on Animal Experiments of ICB/USP (034/2012). Values are expressed as mean ± SEM and statistical analysis was performed by Student *t* test. **Results:** Rats from OUA group were hypertensive compared to VEH, VEH+HH and OUA+HH (*135.5 ± 0.6, 118.3 ± 0.7, 116.5 ± 0.8 and 116.1 ± 0.9 mm Hg, respectively; *p<0.05 compared to all groups, n=6). There was no cardiac and renal hypertrophy in this model. Only renal NKA activity was altered by ouabain (VEH: 9.1 ± 0.5 and OUA: *12.7 ± 0.8 µmol Pi/mg/h, *p<0.05 compared to VEH, n=6) but this was impaired by antihypertensives. Curiously, NKA activity from kidneys (VEH+HH: **30.6 ± 2.9 and OUA+HH:**30.9 ± 2.6 µmol Pi/mg/h, **p<0.05 compared to VEH and OUA, n=6) and brain (VEH: 6.7 ± 0.8, OUA: 7.0 ± 1.2, VEH+HH: **42.7 ± 2.3 and OUA+HH: **40.5 ± 1.7 µmol Pi/mg/h, **p<0.05 compared to VEH+HH and OUA+HH, n=6) of animals treated with antihypertensives were higher than untreated groups. Ouabain-treated rats showed higher expression of renal NKA alpha1 isoform (VEH: 100 ± 21.6 and OUA: *187 ± 16%, *p<0.05 compared to VEH, n=5-6) and left cardiac ventricle alpha2 isoform (VEH: 100 ± 8.3 and OUA: *165 ± 27.6, *p<0.05 compared to VEH, n=5-6) as well as lower brain expression of alpha1 and 3 (VEH: 100 ± 4.9 and OUA: *41.8 ± 4.7, and VEH: 100 ± 5.5 and OUA: *70.0 ± 7.6, respectively; *p<0.05 compared to VEH, *p<0.05 n=5-6). **Conclusion:** Ouabain-induced hypertension increased the activity and expression of renal NKA and may be involved in enhanced Na⁺ reabsorption and blood pressure, while increase of cardiac alpha2 may be an adaptive mechanism to Ca²⁺ overload. Hypertension appears to be responsible for the altered activity. On the other hand, antihypertensives enhance kidney and brain NaK activities yet the reasons are still inconclusive. Experiments for the evaluation of cardiac and brain NaK expression and the activation of intracellular signaling pathways are in progress. **Support:** CAPES, FAPERJ, FAPESP and CNPQ.

01.027 Extracellular cyclic AMP-adenosine pathway: a promising therapeutic target for treating muscle wasting disorders Eloi FR, Chiavegatti T, Andrade-Lopes AL, Godinho RO
Unifesp-EPM – Farmacologia

Introduction: The development of new therapeutic approaches for the prevention or treatment of exacerbated skeletal muscle catabolism has a great clinical relevance because skeletal muscle wasting and atrophy increases the morbidity and mortality associated with pathological conditions like muscular dystrophies and systemic disorders such as cancer, diabetes and sepsis. The adenylyl cyclase/cAMP pathway constitute a particularly interesting therapeutic target since activation G protein coupled receptors (GPCR) linked to the stimulatory G protein attenuates skeletal muscle proteolysis. Considering that in the skeletal muscle, intracellular formation of cAMP is followed by its efflux and extracellular degradation into adenosine, the aim of the present study was to evaluate the possible modulatory effects of the “extracellular cAMP-adenosine pathway” on skeletal muscle proteolysis. **Material and Methods:** L6 rat cell line (3×10^5 cells/ mL) were grown in DMEM supplemented with 10% fetal calf serum on 35 mm dishes. Myogenic differentiation was induced with DMEM plus 5% horse serum, at day 3. The effect of cAMP, adenosine, CGS-15943 (non-selective adenosine receptor antagonist) and DMPX (selective A_2 receptor antagonist) on muscle proteolysis was evaluated on 7-11-day-old L6 myotubes pre-incubated with 0.5 mM cycloheximide at 37°C. After 1 to 5 h treatment, myotube proteolysis was evaluated by measuring the rate of tyrosine release into the incubation medium using a fluorometric method (at 485/590 nm) (Bergantin et al., J Appl Physiol 111: 1710, 2011) (n=4). Activation of GPCR was evaluated using the functional [35 S]GTP γ S binding assay. Briefly, L6 membrane fractions (50 μ g/well, n=4-5) were incubated with 0.1 nM [35 S]GTP γ S (non-hydrolysable GTP analogue) and 50 μ M GDP, in the absence or presence of cAMP or adenosine. Nonspecific binding was determined with 50 μ M unlabeled GTP γ S. **Results and Conclusion:** 30-300 μ M cAMP increased by up to 150% the basal binding of [35 S]GTP γ S to L6 myotube membranes (1.17 ± 0.1 fmol/ mg of protein). The stimulatory effect of cAMP on G protein activation was mimicked by either 10-100 μ M adenosine or its analog NECA (1-100 μ M). Treatment of cultures for 4h with 0.1-1 mM cAMP reduced by up to 18% the basal rate of tyrosine release (13.07 ± 0.19 nmol / dish). The anticatabolic effect of cAMP was sustained for up 5h and mimicked by incubation of cells with 0.1-10 μ M adenosine, which reduced by up to 28% the basal rate (12.58 ± 0.19 nmol / dish) of tyrosine released. Interestingly, pre-treatment of cells with the adenosine receptor antagonist CGS-15943 or DMPX completely abolished the antiproteolytic effects of either cAMP or adenosine treatment. These data show that extracellular cAMP can modulate the skeletal muscle proteolysis through activation of adenosine receptor coupled to stimulatory G protein. This extracellular arm of cAMP signaling may represent an important feedback mechanism that amplifies the physiological anticatabolic function of intracellular cAMP, becoming a target for developing pharmacological strategies for treating skeletal muscle wasting/ atrophy. **Ethical Committee:** CEUA N $^{\circ}$ 5523140415 **Financial Support:** CNPq, Capes and FAPESP 2015/ 07019-4

01.028 Glucocorticoid Receptor Signaling in Wolffian Duct Morphogenesis. Thimoteo DS¹, Ribeiro CM¹, Silva EJR², Hinton BT³, Avellar MCW⁴ ¹Unifesp-EPM – Farmacologia, ²Unesp – Farmacologia, ³University of Virginia, School of Medicine – Cell Biology, ⁴Unifesp-EPM – Farmacologia

Introduction: Practice of prenatal glucocorticoid therapy has improved the outcomes of preterm births by accelerating fetal lung maturation. In rats, in vivo treatment of the pregnant females with glucocorticoids negatively impact several reproductive outcomes along the postnatal life of male offspring. The effect of glucocorticoids on the development of Wolffian duct (WD) through a signaling via glucocorticoid receptor (GR) is scarcely known. Our group has previously demonstrated a differential expression and immunolocalization of GR during rat WD morphogenesis. **Aim:** In this present study we evaluated the functionality of GR in rat WD morphogenesis using an organotypic culture as experimental model. **Methods:** Embryos were collected from male Wistar rats at embryonic age of 17.5; the urogenital tract was dissected and the WD isolated and cultured for up to 96h on cell inserts floating in basal medium containing or not the following drugs: testosterone (10 nM); hydroxyflutamide (synthetic androgen receptor antagonist, 10 μ M); dexamethasone (selective synthetic agonist for GR, 10 nM, 100 nM and 1 μ M) and RU486 (synthetic GR antagonist, 1 μ M). Medium was changed every 24 h. Gross morphology of WD morphogenesis was evaluated by the acquisition of whole WD images in an inverted microscope every 24 h. **Results:** Gross morphology analysis indicated that dexamethasone induced a time- and concentration-dependent effect on the development of cultured WDs. These effects were similar to tissues incubated with testosterone (10 nM). These effects were abrogated by RU486, but not hydroxyflutamide. WDs co-incubated with testosterone (10 nM) and dexamethasone (100 nM) exhibited increased duct length and number of curvatures when compared to tissues incubated with testosterone alone. Co-incubation of testosterone and RU486, but not dexamethasone with hydroxyflutamide, had slight effect on the morphological differentiation of the cultured WD. **Conclusions:** Collectively, our data indicate that GR is expressed and functional in the rat WD. The outcome of GR/glucocorticoids signaling may be relevant to the understanding of the role of glucocorticoids in the masculinization of the male reproductive tract and male fertility at adulthood. **Financial support:** CAPES, CNPq/Science Without Borders (PVE 401932/2013-3; PDJ 150040/2015-6), FAPESP (#2014/19378-6), NIH-NICHD #069654. Approved by the Research Ethics Committee from UUNIFESP/EPM (#4637290115).

01.029 Calcium mobilization in smooth muscle and endothelial cells cultures from rats with different plasmatic Angiotensin I Converting Enzyme (ACE) activity phenotypes

Pisano Dias ASES¹, Nering MB¹, Fernandes L², Souccar C¹, Lapa AJ³, Lima-Landman MTR¹
¹Unifesp-EPM – Farmacologia, ²Unifesp-Diadema – Farmacologia e Inflamação, ³UEA

Introduction: In previous studies it was demonstrated that plasmatic ACE activity phenotype influenced the pressoric response to Renin Angiotensin Aldosterone System (RAAS) agonists and antagonists. The greater hypertensive response to Ang I and Ang II detected in rats with low plasmatic ACE activity (ACEI) compared to rats with high plasmatic ACE activity (ACEh) as well as captopril (CAP) and losartan (LOSAR) responses are distinct between the two phenotypes indicates that the difference between ACEh and ACEI rats could be exerted at the receptor level or posterior to receptor activation. **Aims:** To evaluate calcium mobilization in smooth muscle and endothelial cells from ACEh and ACEI rats. **Methods:** Adult male rats (Wistar INFAR/ACEh and INFAR/ACEI) were used to determine the plasmatic ACE activity (nmol/min/mL) by FRET method. The smooth muscle and endothelial cells culture were performed as described by Macia et al (*Arch Gerontol Geriatr.*, 35(1):51-7, 2002) and Loiola et al (*Experimental cell research.*, 319:1102–1110, 2013), respectively. The calcium mobilization kit FLIPR® Calcium-4 (Molecular Devices) was used as described by manufactures. The cytosolic calcium mobilization (in units of arbitrary fluorescence - UAF) in smooth muscle cells was determined by KCl (40 mM) injection, before and after verapamil incubation (1 - 30 μ M); or by Ang II (10^{-5} - 10^{-7} M), before and after losartan incubation (10^{-8} - 10^{-6} M). In endothelial cell cultures BK (10^{-9} - 10^{-7} M) effect was determined before and after HOE-140 incubation (3 - 100 nM). The results were expressed as means and s.e.m. and analysed by ANOVA followed by the Tukey test ($p < 0.05$). **Results:** Confirming the phenotypic pattern of the rats, the plasmatic ACE activity was 82.3 ± 6.2 and 40.5 ± 3.4 nmol/min/mL ($n=9$), in ACEh and ACEI rats, respectively. The calcium mobilization induced by KCl stimulation in the absence (24.5 ± 2.5 UAF, $n=3$, for ACEh and 26.9 ± 0.9 UAF, $n=3$, for ACEI), or in the presence of all tested concentration of verapamil was similar in both phenotypes. After Ang II (10^{-7} M) administration, Ca^{2+} mobilization was 62 % higher in ACEh (14.4 ± 1.4 UAF, $n=5$) rats than in ACEI rats, but this difference was not maintained at 10^{-5} M, when ACEI mobilized 42 % more than ACEh rats. LOSAR 10, 30 and 100 nM blocked the Ang II response concentration-dependently only in ACEI rats, 35 %, 50 % and 65%, respectively (Ang II: 79.2 ± 4.1 UAF, $n=7$). Endothelial cells stimulated by BK promoted an increase in Ca^{2+} mobilization, in both rat phenotypes, but bigger in ACEI than in ACEh rats. **Conclusions:** The cytosolic calcium increase promoted by KCl indicated that the L-type calcium channels are not under influence of ACE phenotype. The different response to Ang II or to AT1 receptor blockade by LOSAR, in ACEI rats, reinforces the hypotheses that the activation or blockade of the RAAS is under phenotypic influence and is putatively exerted at the receptor binding site or in calcium mobilization. The BK signaling pathway indicates that also the behavior of the endothelial cells is influenced by the ACE phenotype. **Financial support:** CNPq, Fapesp, Capes Animal Investigation Ethics Committee Protocol (CEUA) N^o 8029280215

01.030 From discovering “calcium paradox” to Ca²⁺/cAMP intracellular signaling interaction, and its impact in human health and disease. Bergantin LB, Caricati-Neto A
Unifesp-EPM – Farmacologia

Introduction: The hypothesis of the so-called “calcium paradox” phenomenon in the sympathetic neurotransmission has its origin in experiments done in models of neurotransmission (rodent vas deferens) since 1970’s. Historically, “calcium paradox” originated several clinical studies reporting that acute and chronic administration of L-type voltage-activated Ca²⁺ channels (VACCs) blockers (drugs largely used for antihypertensive therapy, which reduce Ca²⁺ entry into the sympathetic neurons and cardiac/smooth muscle cells) such as verapamil and nifedipine, produces reduction in peripheral vascular resistance and arterial pressure, associated with a paradoxical increase in plasma noradrenaline levels and heart rate, typical of sympathetic hyperactivity. Despite this sympathetic hyperactivity has been initially attributed to adjust reflex of arterial pressure, the cellular and molecular mechanisms involved in this paradoxical sympathomimetic effect of the L-type VACCs blockers remained unclear for four decades. **Methods:** Also, experimental studies using isolated tissues richly innervated by sympathetic nerves (to exclude the influence of adjusting reflex) showed that neurogenic responses were completely inhibited by L-type VACCs blockers in high concentrations, but paradoxically potentiated in low concentrations, characterized as a “calcium paradox” phenomenon, which remained unclear for almost four decades. **Results:** We discovered in 2013 that this paradoxical increase in sympathetic activity produced by L-type VACCs blockers is due to interaction of the Ca²⁺/cAMP intracellular signaling pathways (Bergantin et al., Cell Calcium, 54:202-212, 2013; TOP 25 Hottest Articles - Biochemistry, Genetics and Molecular Biology - Cell Calcium - TOP 1 July to September 2013/ TOP 5 October to December 2013/ TOP 1 January to December 2013 full year/TOP 6 January to March 2014). **Conclusion:** Then, the pharmacological manipulation of this interaction produced by combination of the L-type VACCs blockers used in the antihypertensive therapy, and cAMP accumulating compounds used in the antidepressive therapy, could represent a potential cardiovascular risk for hypertensive patients due to increase in sympathetic hyperactivity. In contrast, this pharmacological manipulation could be a new therapeutic strategy for increasing neurotransmission in psychiatric disorders such as depression, and producing neuroprotection in the neurodegenerative diseases such as Alzheimer’s and Parkinson’s diseases. **Financial support** and acknowledgments: CNPq, CAPES and FAPESP (Bergantin’s postdoctoral fellowship FAPESP #2014/10274-3). Research approval by the Human or Animal Research Ethical Committee: CEP #1763/11; CEUA #9804030715

01.031 Hyperplastic prostate cell growth mediated through the transactivation of epidermal growth factor receptor by Alpha1-Adrenoceptors is inhibited by LDT5 *in vitro*. Nascimento-Viana JB¹, Alcántara-Hernández R², García-Sáinz JA², Romeiro LAS³, Noël F¹, Silva CLM¹ ¹UFRJ – Farmacologia Bioquímica e Molecular, ²UNAM – Instituto de Fisiología Celular, ³UnB – Desenvolvimento de Estratégias Terapêuticas

Introduction: G protein-coupled receptors (GPCRs) and receptor-tyrosine kinases such as the epidermal growth factor receptor (EGFR) regulate several cell signaling networks in physiologic and physiopathologic conditions. The transactivation of EGFR by GPCRs regulating cell proliferation is a topic of increasing interest (Wang, *Int J Mol Sci* 17:95, 2015; Prenzel et al., *Nature* 402:884, 1999). Benign prostatic hyperplasia (BPH) is a progressive disease related to an imbalance between cell proliferation and apoptosis (Roehrborn, *BJU Int* 2:13, 2008). The usual pharmacological treatment of BPH is based on the use of the α_{1A} -adrenoceptors (AR) antagonists which relax prostatic muscle favoring bladder emptying. Previous data of our group showed that LDT5 has high affinity for α_{1A} -AR, α_{1D} -AR and 5-HT_{1A} receptors acting as a multi-target antagonist that relaxes prostatic muscle *in vivo*. Moreover, LDT5 inhibits human hyperplastic prostate cell growth *in vitro* (Nascimento-Viana et al., *J Pharmacol Exp Ther* 356:212, 2016). The aim of this study was to evaluate the putative role of EGFR transactivation mediated by α_{1D} -AR to BPH cell growth, thereby elucidating the mechanism of action of LDT5.

Methods: Primary stromal cell culture from BPH patients was kindly provided by Dr L.E. Nasciutti (UFRJ/ICB) (Ethics Committee - CAAE-0029.0.197.000-05). Cells were cultured until confluence, starved from fetal bovine serum for 24h, and stimulated with 3 μ M phenylephrine for 48h in the absence or presence of LDT5, BMY7378 (α_{1D} -AR antagonist) or inhibitors of EGFR downstream cell signaling. Cell growth was evaluated by MTT and Trypan Blue exclusion. Western blot assays: BPH cells were lysed with RIPA buffer, centrifuged at 13,000g for 15 min and the supernatants were used for evaluation of activation (phosphorylation) of Mitogen Activated Protein kinases ERK1/2. **Results and conclusions:** Cell treatment with 3 μ M phenylephrine (48h) induced cell growth (145 \pm 8.3%, $P < 0.01$ vs. basal) comparable to EGF (0.1 μ M) effect (147 \pm 5%, $n=3$) used as control. Phenylephrine-induced cell growth was blocked by BMY7378 and LDT5 (50 nM), and also by GM6001 (10 μ M; metalloproteinase inhibitor), AG1478 (5 μ M; selective EGFR inhibitor) and MEK inhibitor PD98059 (1 μ M) suggesting that EGFR transactivation by α_{1D} -AR plays a role in BPH cell growth. However these inhibitors did not alter basal cell proliferation ($P > 0.05$). Then we evaluated p-ERK expression as a downstream signaling of EGFR transactivation. Phenylephrine (100 μ M, 15 min) increased p-ERK expression by 145 \pm 4.7%, $P < 0.01$ vs. basal) and cell treatment with LDT5 (1 μ M) inhibited this effect ($n=4$). Our data suggest that the blockage of α_{1D} -AR by LDT5 inhibits EGFR transactivation, p-ERK expression and BPH cell growth. If such results could be translated to the clinics, the multi-target antagonist LDT5 could both relax prostate and prevent BPH progression. Therefore we propose that LDT5 may have a better pharmacological profile than current α_{1A} -AR antagonists used for BPH treatment. **Financial Support:** CNPq, CAPES and FAPERJ. All protocols were approved by the Ethics Committee of UFRJ (DFBC/ICB011).

01.032 Immune response during differentiation of embryonic neural progenitor cells obtained from APP^{swe}/PS1^{dE9} Alzheimer's disease mouse model: role of the kinin-B2 receptor. Pillat MM, Ulrich H USP – Bioquímica

Introduction: Abnormal neurogenesis and neuroinflammation are processes inherent to pathogenesis of Alzheimer's disease (AD), and therefore signs that promote these processes can act as initial deleterious triggers. In this context, recent data show that the kinin system is stimulated by the pathologic β -amyloid (β A) in AD patients. The effects of this system are primarily mediated by bradykinin (BK) and its kinin-B2 receptor (B2R) and comprehend the direct action on neural stem cells inducing neurogenesis, permeabilization of the blood-brain barrier, and the strong mediation of inflammation. However, the role of BK/B2R in the processes inherent to AD pathogenesis (neurogenesis and inflammation) is unknown. Moreover, studies indicated that proteins involved in neurodegenerative disease, such as APP and PS, often play critical roles in early development of the central nervous system (1). However, it is unknown whether changes in inflammation start in embryonic stages of mutation carriers associated with familial AD. **Methods:** This project proposes to investigate these questions by using transgenic double mice as model of the familial AD (APP^{swe}/PS1^{dE9}). Neural stem and progenitor cells (NPCs) were obtained from wild type (WT) and APP^{swe}/PS1^{dE9} embryo telencephalon (E13). These cells proliferate as aggregates termed neurospheres in the presence of EGF and FGF-2, while retaining the ability to differentiate into neurons and glial cells. NPCs were differentiated in the absence or presence of the B2R antagonist HOE-140 or BK. Using microarray technology we studied the genome-wide gene expression profiles in the *in vitro* differentiated cells, obtained of WT and APP^{swe}/PS1^{dE9} embryo telencephalon, treated with BK or HOE-140. **Results:** It was observed the occurrence of a strong immune response with microglial activation in differentiated cells obtained of APP^{swe}/PS1^{dE9} embryos. In the presence of BK during APP^{swe}/PS1^{dE9} neurosphere differentiation, Iba-1 expression was increased, while treatment with HOE-140 decreased expression of this activated microglia marker, confirmed by real time PCR. Interestingly, cells obtained from APP^{swe}/PS1^{dE9} embryos expressed approximately 100-fold more CCL12, CCL5 (RANTES), CCL3, C3, CX3CR1, TLR2 and TNF than cells obtained from WT embryos. Moreover, HOE-140 affected, in the same way, expression level of these chemokines in APP^{swe}/PS1^{dE9} cells. In agreement, BK treatment during APP^{swe}/PS1^{dE9} neurosphere differentiation statistically increases expression of the CCL12, TLR2 and TNF. Furthermore, the secretome of APP^{swe}/PS1^{dE9} differentiated neurosphere induced significant chemoattraction of peripheral blood mononuclear cells (PBMCs) compared to secretome of WT cells, evidenced by transwell migration assay. While chronic treatment with HOE-140 during differentiation of APP^{swe}/PS1^{dE9} NPCs diminished chemoattraction of PBMCs, treatment with BK favored. **Conclusion:** Thus, our results suggested that mutated APP^{swe} and PS1^{dE9} proteins, involved in neurodegenerative familial AD, play critical roles in early development of the central nervous system, triggering inflammation and microglial activation. Moreover, the B2R blockade suggests that BK signaling mediates the immune response observed in the APP^{swe}/PS1^{dE9} neurospheres. **Reference:** Rogers, D (2010). *Ann Neurol* 67, 151-8. **Financial support:** FAPESP and CNPq. Research Ethical Committee IQ-USP:10/2013.

01.033 Estradiol improves endothelial function through estrogen receptor alpha.
Hermenegildo C, Mompeon A, Perez-Cremades D, Vidal-Gomez X, Oltra M, Novella S –
University of Valencia and INCLIVA Biomedical Research Institute – Physiology

Introduction: The incidence of cardiovascular diseases in women is lower than in age-matched men during their fertile stage, benefit that disappears after menopause. These sex-related differences point to sexual hormones, mainly estrogens, as possible cardiovascular protective factors. Most of the cardiovascular effects of estrogens are attributed to 17 β -estradiol (E2), which mediates genomic effects through estrogen receptors (ER), ER α and ER β , both presents in endothelial and smooth muscle cells. Endothelium regulates the production of vasoactive compounds, such as nitric oxide (NO), prostanoids (mainly prostacyclin PGI₂), angiotensin (Ang) II and Ang 1-7, which in turn control the vascular tone. Our goals are to analyze the regulation of endothelial production of the atheroprotective compounds NO, PGI₂ and Ang 1-7 by E2 and to study the involvement of ER α and ER β on these effects. **Methods:** Cultured human umbilical vein endothelial cells (HUVEC) were exposed to physiological concentrations of E2 (1-10 nM), to selective ER α agonist 4,4',4''-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT; 10 nM), and selective ER β agonist 2,3-bis(4-Hydroxyphenyl)-propionitrile (DPN; 10 nM). In some experiments, the unspecific ER antagonist ICI 182780 (1 μ M) or the specific ER α antagonist methyl-piperidino-pyrazole (MPP; 1 μ M) were added before E2 treatment. NO production was measured by fluorescence through the DAF-FM fluorescent probe, PGI₂ was measured by ELISA and Ang 1-7 was quantified by immunofluorescence. The regulatory pathways were analyzed by RT-PCR, Western blot and spectrofluorometric assays. **Results:** E2 promoted the release of vasoactive compounds, such as NO, PGI₂ and Ang 1-7. In the case of NO, E2 increased NO production up to 200% of control values through the increased expression and activity of endothelial NO synthase (eNOS). E2 induced both cyclooxygenase-1 and prostacyclin synthase expression, resulting in an enhanced PGI₂ production by 150% of control values. In the case of Ang 1-7, E2 increased angiotensin converting enzyme (ACE)1 and ACE2 protein expression and enzymatic activities (up to 200% of control values), causing an increased Ang 1-7 production by 160% of control values. All these E2-induced effects were completely abolished in the presence of both ICI 182780 and MPP, and were mirrored by PPT but not DPN. **Conclusion:** ER α , but not ER β , mediates E2-induced production of NO, PGI₂ and Ang 1-7 in HUVEC. Our results support a vascular beneficial role of ER α and reinforce the idea that a misbalance of ER β over ER α is generally associated with deleterious effects, such as higher oxidative stress and atherosclerotic plaque formation. Supported by the Spanish Min. Economía y Competitividad, Instituto de Salud Carlos III –FEDER-ERDF (grants FIS PI13/00617 and RD12/0042/0052). A.M. is a “Formación de Profesorado Universitario” fellow (FPU13/02235 Spanish Min. Educación, Cultura y Deporte). D.P-C. is an “Atracció de Talent” fellow (PREDOC13-110541, Univ. Valencia). This investigation conforms to the principles outlined in the Declaration of Helsinki, was approved by the Ethical Committee of Clinical Research of the INCLIVA, Hospital Clínico of Valencia, Spain, and written informed consent was obtained from all donors.

01.034 Regulation of Calponin-1 by Matrix Metalloproteinase (MMP)-2 contributes to hypertension-induced early vascular remodeling. Belo VA, Parente JM, Tanus-Santos JE, Castro MM FMRP-USP

Aims: Increased matrix metalloproteinase (MMP)-2 is implicated in the vascular remodeling of hypertension. Calponin-1 is a contractile protein, and its absence is associated with the VSMC phenotype switch, which leads to migration and remodeling. We evaluated whether MMP-2 may precede hypertension-induced chronic vascular remodeling by decreasing calponin-1 levels and inducing VSMC proliferation. **Methods:** Sham operated or two kidney-one clip (2K1C) rats were treated with either doxycycline at 30 mg/kg/day or vehicle (water). Treatment with doxycycline started 3 days post-surgery. Systolic blood pressure (SBP) was assessed in sham and 2K1C rats at 1 and 2 weeks following surgery by tail-cuff plethysmography. Gelatinolytic activity was determined in aortas by *in situ* zymography. Aortic MMP-2 levels and calponin-1 were determined by immunofluorescence and Western blot. Aortic myocardin were determined by Western blot. VSMC proliferation was determined by immunohistochemistry of Ki-67. Quantitative morphometry was performed in hematoxylin and eosin stained aortic sections to analyze structural changes. Double immunofluorescence and confocal microscopy analyzed the co-localization between aortic MMP-2 and calponin-1. Calponin-1 mRNA expression was analyzed by TaqMan real time PCR. **Results:** SBP was markedly increased in the 2K1C rats after 1 and 2 weeks post-surgery, and treatment with doxycycline was effective to reduce it only at 2 weeks of hypertension ($p < 0.05$). Increased activity and protein levels of MMP-2 was observed in aortas from 2K1C rats, followed by increased of VSMC proliferation at both 1 and 2 weeks of hypertension ($p < 0.05$ vs. Sham groups), without differences between the time of hypertension. Treating 2K1C rats with doxycycline abolished hypertension-induced increase in MMP-2 activity and VSMC proliferation in both weeks ($p < 0.05$). Increased aortic M/L is starting to emerge between Sham and 2K1C at 1 week ($p > 0.05$), and is established with 2 weeks of hypertension ($P < 0.05$). MMP-2 and calponin-1 are situated together and co-localized to each other in the cytosol of VSMC. Examination of aortas from 2K-1C rats in 1 week of hypertension showed a significant reduction in the 34 kDa calponin-1 ($p < 0.05$ vs. Sham and Sham + Doxy), and doxycycline prevented loss of calponin-1 in hypertensive aorta ($p < 0.05$). After 2 weeks of hypertension, calponin-1 was significantly upregulated in aortas of 2K1C ($p < 0.05$), and treatment with doxycycline decreased its levels ($p < 0.05$). Aortas from 2K1C rats did not show any alteration in the levels of myocardin in relation to the other groups in both weeks of hypertension. There was no difference in the mRNA of calponin-1 among all groups in 1 week of hypertension ($p > 0.05$). **Conclusion:** MMP-2 may contribute to the post-translational decreased of arterial calponin-1, thus may culminating in the chronic maladaptive arterial remodeling in hypertension. **Financial support:** Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); (# 4/2015, Human or the Animal Research Ethical Committee of Faculty of Medicine of Ribeirão Preto).