06. **Cardiovascular and Renal**

06.001 **Effect vascular of ethanolic extract of leaves *Vitex polygama* Cham. (Lamiaceae)**.
Vidal MC, Carneiro A, Ferreira LLDM, Konno TUP[1], Guimarães DO[1], Leal ICR[1], Muzitano MF[1], Raimundo JM[1] [1]UFRJ

Introduction: The Restinga of Jurubatiba National Park (PARNA Jurubatiba), located in the northern Rio de Janeiro state, protects a region of great biodiversity. Among the plant species found at PARNA Jurubatiba is *Vitex polygama*, popularly known as tarumã, and used in folk medicine as a diuretic and emmenagogue1,2. The pharmacological activities of this specie have never been studied, although there are some biological activities described for other species of the genus *Vitex*. Thus, the objective of this study was to investigate the effects of the crude ethanolic extract of leaves of *Vitex polygama* on vascular smooth muscle and its mechanism of action. **Methods:** The vasodilatory effect of the extract was evaluated by using isolated aorta from male Wistar rats (220-280 g) prepared for isometric tension recording. Aortic rings were placed in vertical chambers filled with Krebs-Henseilet solution continuously oxygenated with carbogen gas (95% O_2/ 5% CO_2), at 37°C. After the equilibration period, vascular smooth muscle contraction was induced with 10 µM of phenylephrine and then cumulative concentrations of the extract were tested (1–1000 µg/ml). It was used aortas with and without endothelium, which was considered intact if the relaxation induced by acetylcholine (10 µM) was greater than 80%. Mechanical removal of endothelium was confirmed by the lack of relaxation in response to acetylcholine. All protocols were approved by the Animal Care and Use Committee Under License Macaé01. **Results:** The ethanolic extract of *Vitex polygama* leaves caused a concentration-dependent relaxation in aortas with endothelium. At 500 µg/ml, the extract produced a relaxation of 87.0 ± 3.5 % (n=5, $P<0.05$). The concentration of extract necessary to reduce phenylephrine-induced contraction of endothelium-intact aorta by 50% (IC_{50}) was 315.9 ± 24.8 µg/ml. To test whether the vasorelaxation was endothelium-dependent, the vascular effects of the extract were tested in endothelium-denuded aortic rings. Removal of endothelium partially inhibited the relaxation induced by *Vitex polygama* extract, increasing the IC_{50} to 559.7 ± 35.5 µg/ml ($P<0.05$). **Discussion:** The mechanism of action of the extract appears to involve the release of endothelial factors and a direct effect on vascular smooth muscle at high concentrations. The signaling pathways involved in the vasodilatory activity of the ethanolic extract of leaves of *Vitex polygama* are still being investigated. **References:**[1]GALLO, M.B.C., Beltrame, F.L., Vieira, P.C., Cass, Q.B., Fernandes, J.B., Silva, M.F.G.F.. Quantitative determination of 20-hydroxyecdysone in methanolic extract of twigs from Vitex polygama Cham. Journal of Chromatography B.832: 36-40, 2006. [2]GALLO,M.B.C.,Vieira,P.C.,Fernandes,J.B.,Silva,M.F.G.F.,Pires,F.R.S.. Compounds from Vitex polygama active against kidney diseases. Journal Chromatography.115: 320-32, 2008. **Financial support:** FAPERJ, FUNEMAC, UFRJ.

06.002 New agonist of adenosine receptor reduces cardiac and vascular dysfunction in rats with monocrotaline-induced pulmonary hypertension. Pereira SL[1], Alencar AKN[1], Ferraz EB[2], Tesch R[1], Nascimento JHM[2], Maia R[1], Fraga CAM[1], Barreiro EJ[1], Sudo RT[1], Zapata-Sudo G[1] [1]ICB-UFRJ, [2]IBCCF-UFRJ

Introduction: Pulmonary hypertension (PH) is a serious disease which is characterized by enhanced pulmonary vascular resistance and subsequent vascular and cardiac remodeling. This work investigated the vascular reactivity and ventricular function in rats with PH treated with (E)-*N*'-(3,4-dimethoxybenzylidene)-4-methoxybenzohydrazide (LASSBio-1386), a novel agonist of the adenosine receptor A_{2A}. **Methods:** The protocols used in the present study were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro under #DFBCICB059. Male Wistar rats (200 - 250 g) received a single intraperitoneal injection of monocrotaline (MCT) (60 mg/kg) for PH induction. Animals were randomly divided in the following groups: 1) saline (control), 2) MCT + vehicle (DMSO), 3) MCT + LASSBio-1386 (50 mg/kg, p.o.). At the end of treatment period (14 days), some parameters were evaluated: right ventricular systolic pressure (RVSP); relation between right ventricle to body weight (RV/BW); RV wall thickness, pulmonary artery diameter and acceleration time (PAT) measured using transthoracic echocardiography; pulmonary artery wall thickness; vascular reactivity to acetylcholine of pulmonary artery. All data were expressed as mean ± standard error of the mean. For the comparison of groups, analysis of variance (ANOVA) followed by Newman-Keuls' test was used and differences were considered significant when P was <0.05. **Results and discussion:** RVSP (mmHg) increased from 26.0 ± 2.0 (control) to 49.5 ± 5.0 (MCT + vehicle; $P< 0.05$) and was recovered to 23.0 ± 1.0 (MCT + LASSBio-1386; $P< 0.05$) when animals with PH were orally treated with vehicle and LASSBio-1386, respectively. RV/BW (mg/g) increased from 0.66 ± 0.02 (control) to 1.63 ± 0.16 (MCT + vehicle; $P< 0.05$) and decreased to 0.65 ± 0.41 in MCT + LASSBio-1386 group ($P< 0.05$). RV wall thickness (cm) increased from 0.10 ± 0.02 (control) to 0.15 ± 0.09 (MCT + vehicle group; $P< 0.05$) and reduced to 0.10 ± 0.01 in MCT-injected rats treated with LASSBio-1386 ($P< 0.05$). Pulmonary artery diameter (cm) was enhanced from 0.29 ± 0.01 (control) to 0.41 ± 0.01 (MCT + vehicle group; $P< 0.05$) and reduced to 0.29 ± 0.03 in MCT + LASSBio-1386 group ($P<0.05$). PAT (ms) was reduced from 44.2 ± 0.7 (control) to 25.5 ± 1.3 in MCT + vehicle group ($P< 0.05$) and restored to 41.9 ± 1.2 in MCT + LASSBio-1386 group. The wall thickness of pulmonary arterioles (%) was increased from 74.1 ± 1.3 (control) to 90.2 ± 2.7 (MCT + vehicle; $P< 0.05$) and recovered to 72.18 ± 2.20 in MCT + LASSBio-1386 group. Acetylcholine-induced maximum relaxation of pulmonary arteries (%) was reduced from 62.7 ± 1.5 (control) to 43.6 ± 1.2 in the group MCT treated with vehicle and restored to 68.4 ± 3.5 when treated with LASSBio-1386, indicating that PH induced an endothelial dysfunction in pulmonary artery rings which was recovered by treatment with the compound. LASSBio-1386 reversed right ventricular hypertrophy and pulmonary vascular remodeling in rats with MCT-induced PH. **Financial agencies:** CNPq, FAPERJ, CAPES, INCT, PRONEX.

06.003 Estrone acutely relaxes rat aorta through an endothelium-dependent mechanism: **role of nitric oxide**. Oliveira TS, Oliveira LM, Filgueira FP, Ghedini PC UFG – Fisiologia e Farmacologia

Introduction: Hormonal replacement therapy (HRT) with conjugated estrogens has been suggested to be cardioprotective; however, recent clinical trials do not support these benefits. Several explanations have been proposed for this controversy, including the fact that, in these trials, patients were treated with the conjugated equine estrogens (CEEs), whereas the majority of animal and clinical studies evaluated only the effects of 17β-estradiol, the most abundant circulating estrogen found in humans. Considering that estrone (E1) is the major component of CEE, corresponding to approximately 40% of its content, the present study was designed to investigate the direct relaxing effects of this estrogen compound. **Methods:** The vasorelaxant effect of E1 was evaluated in thoracic aorta from Wistar rats (200-300g) using organ chambers. Concentration-response curves to E1 (0.1μM – 100μM) were constructed in phenylephrine pre-contracted aortic rings with either intact or denuded endothelium. Relaxation responses to E1 in intact endothelium rings were performed in the absence or presence of the nitric oxide synthase inhibitor (L-NAME – 100μM), the soluble guanylate cyclase inhibitor (ODQ - 10μM), the cyclooxygenase inhibitor (Indomethacin - 10μM) or the non-selective K^+ channel blocker (TEA – 1mM). All experiments were conducted in accordance with the Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL) and were approved by the local Ethics in Research Committee (Protocol CEUA/UFG 20/2013). Data are presented as mean±SEM of 5-7 experiments and compared by Student's t-test or one-way ANOVA when appropriate. P values less than 0.05 were considered significant. **Results and Discussion:** E1 induced concentration-dependent relaxation in endothelium-intact aortic rings (E_{max} = 41.04 ± 1.89%), whereas in endothelium-denuded vessels it did not have any effect. Aorta incubation with L-NAME or ODQ significantly reduced E1-induced relaxation (E_{max} = 7.97 ± 2.63% and 7.12 ± 1.36%, respectively). The incubation with indomethacin or TEA did not inhibit the effect of E1 (E_{max} = 45.53 ± 4.34% and 46.76 ± 9.61%, respectively). These results suggest that vasorelaxant effect of E1 is endothelium-dependent and involves stimulation of the nitric oxide/cyclic GMP pathway. These findings provide support for a possible protective effect of E1 against cardiovascular diseases. **Financial Support:** CAPES, FAPEG, CNPq

06.004 Evaluation of cardiac and renal markers function in Wistar rats treated with different clozapine-loaded nanosystems. Güllich AAC[1], Coelho RP[1], Pereira MP[1], Mezzomo J[1], Pilar BC[1], Ströher DJ[1], Galarça LASL[1], Piccoli JCE[1], Haas SE[1], Puntel RL[2], Manfredini V[1]
[1]Unipampa – Estresse Oxidativo, [2]Unipampa – Nutrição, Saúde e Qualidade de Vida

Introduction: Clozapine is an atypical antipsychotic used as first-line treatment in refractory schizophrenia. However, most of the adverse effects occurring in the first weeks of treatment, some devastating, such as cardiomyopathy and sudden death of patients, thus limiting their use. Therefore, the nanosystems are a pharmaceutical form able to reduce undesirable effects through vectorization of the drug for central nervous systems. This study aimed to evaluate of cardiac and renal markers function in Wistar rats treated with different clozapine-loaded nanosystems. **Methods**: In this study, we used 48 adult male Wistar rats were divided into eight groups with six animals in each group, animals received the following treatments: saline (NaCl 0,9% 1,0 mL/kg ip), free clozapine (25 mg/kg ip), blank uncoated nanosystems, clozapina-loaded uncoated nanosystems (25 mg/kg ip), blank chitosan-coated or polyethyleneglycol-coated nanosystems, clozapina-loaded chitosan-coated or polyethyleneglycol-coated nanosystems (25 mg/kg ip). The animals received the formulation once daily for seven consecutive days, the volume of administration shall not exceed 1 mL/kg and euthanized on the eighth day. The formulations nanocapsules have an organic phase comprising the polymer Poly (e-caprolactone), medium chain triglyceride, Lipoid S45 ® surfactant, when loaded clozapine, acetone, and an aqueous phase surfactant consists of Tween 80 ® and Milli-Q water . For the formulation coated with polyethylene glycol, it was added to the aqueous phase of the suspension. For the coating quitosona, an acidic solution of the polysaccharide was added to the nanocapsule suspension prepared under constant agitation for 1 hour. We evaluated the cardiac markers function homocysteine, CK and CKmb and renal markers function creatinine and urea. This study was approved by the Ethics Committee on Animal Use - CEUA (Federal University of Pampa - Protocol n ° 034/2012), which is affiliated to the Brazilian College of Animal Experimentation (COBEA), in accordance with international standards of ethics research. The results were expressed as mean ± standard deviation (SD). Test was used for analysis of variance (ANOVA), two-way, followed by post hoc Duncan, with significance level $p < 0.05$. **Results and Discussion**: The evaluation of cardiac markers function revealed that there was a significant increase ($p < 0.05$) of the evaluated parameters induced by clozapine free (38.7 ± 0.71). These results corroborate with the literature where most effects of the drug occurs in the first weeks of treatment. The groups treated with different nanosystems showed a significant improvement in cardiac markers function, and the group treated with clozapina-loaded chitosan-coated nanosystems (17.7 ± 0.76) showed better results. These results demonstrate that the drug when bound to different nano is able to mitigate the harmful effects of the drug making it safer. For markers creatinine and urea results are similar to saline group (52 ± 2.6) suggesting that the nanoparticle systems were not nephrotoxic. And, clozapina-loaded polyethyleneglycol-coated nanosystems (44.2 ± 3.4) demonstrate a decrease in urea levels indicating renal function improvement. The findings show that different coatings can act so diverse and specific to each organ or tissue in which they have greater affinity. The nano-medicine may be an alternative to the administration of clozapine and its nanoencapsulation in polymeric systems is a promising therapeutic tool. References: BERNARDI, A. Br J Pharmacol, v. 158, p. 1104, 2009. DASKALAKIS, Z. J. Clin Pharmacol Ther, v. 86, p. 442, 2009. GASZNER, P. Prog Neuropsychopharmacol Biol *Psychiatry*, v. 28, p. 465, 2004.

06.005 Cardioprotective activity of subcutaneously administered pyridostigmine. Souza ACM[1], Barcellos NMS[1], Frezard FJG[2], Guimarães HN[3], Castro QJT[1], Pereira SC[1], Grabe-Guimarães A[1] [1]UFOP – Farmácia, [2]UFMG – Fisiologia e Biofísica, [3]UFMG – Engenharia

Pyridostigmine (PIR), a reversible anti-cholinesterase agent, had its cardioprotective effects demonstrated by reduction of the QT interval of ECG dispersion at rest in patients with heart disease and QT prolongation induced by stress or experimentally by adrenergic stimulation, administered orally or I.V. However, its short half-life limits its long-term use. This study was aimed to investigate the cardioprotective effect of PIR administered subcutaneously (S.C.), as one more possible route with potential to be used to prevent adrenergic hyperactivity on cardiac function. The ability to prevent arterial pressure (AP) and ECG parameters alterations induced by I.V. administration of noradrenaline (NA; 1, 3 and 10 mg/kg) was evaluated in vivo in anaesthetized rats. The use of animals was approved by the Ethics Committee on Animal Use UFOP (CEUAUFOP) under at 2011/52. For obtaining the signals BP and IV administration of the formulations, polyethylene catheters were inserted into the femoral artery and vein, respectively. The catheter inserted in the femoral artery was then connected to a pressure transducer TruWave ® (Edwards Life science, Canada) for the acquisition of the signals BP. To enable measurement of the potential difference on the DII ECG stainless steel hypodermic needles were inserted in subcutaneous tissue of animals. The ECG sensor and transducer were connected to a PA conditioning system that provides real-time signal at a frequency of 1200 Hz, processed by an analog to digital converter board (DaqBoard/2001, USA).PIR was given S.C. at a dose of 1 mg/kg and after 1, 2, 4 and 6 hours of administration, the signals were continuously obtained. The PIR did not cause changes in the baseline AP and ECG signals. After NA 10 ug/kg, PIR was able to inhibit the QT interval prolongation (4, 7 ± 2, 0) when compared to the control group (23, 7 ± 0, 9), as for the QTc interval (7, 7 ± 3, 6 for treated X 28, 0 ± 1, 8 for control), after 2 hours of its administration. When given a dose of NA 3ug/kg, PIR was able to inhibit the prolongation of the QT interval for 5, 2 ± 2, 7 when compared to the control group 16, 3 ± 2, 1, as for the QTc interval (6, 2 ± 2, 5 for treated X 20, 2 ± 2, 2 for control), also after 2 hours of its administration. The other ECG parameters (PR and QRS intervals) were not different between the PIR treated and control groups. PIR was able to inhibit the systolic AP increases induced by 10 ug/kg NA (36, 5 ± 8, 6 for treated X 79, 9 ± 4, 7 for control group). In conclusion, the PIR administered S.C. also presents the cardioprotective effect demonstrated by the ability to inhibit the QT interval prolongation.

06.006 Effect of ethanolic extract the fruit peel from *Platonia insignis* on the cardiovascular system in rats. Mendes MB, Santos MEP, Azevedo PSS, Sabino CKB, Arcanjo DDR, Chaves MH, Oliveira AP UFPI – Plantas Medicinais

Introduction: The *Platonia insignis* Mart is a fruit tree Amazon that dispersed in marginal clenched in northeastern Brazil. Its fruit which is called "bacuri" is consumed raw, as juice (PORTE et al, 2010). The present study evaluated the effects of the ethanol extract of the fruit rind from *Platonia insignis* Mart.(Pi-EtOH) on the cardiovascular system in rats. **Material and methods:** Male Wistar rats (270 ± 30 g) were used for all experiments (Animal Research Ethics Committee/UFPI - 015/2012). In vivo approach: Measurements of mean arterial pressure assessment were performed as described by Oliveira et al. [2006]. Under sodium thiopental anesthesia (45mg/kg, i.v.), the lower abdominal aorta and inferior vena cava were canulated via left femoral artery and vein using a polyethylene catheter. Thereafter, the catheter was led under the skin to emerge between the scapulae. Arterial pressure was measured after 24 h by connecting the arterial catheter to a pressure transducer (Statham P23 ID; Gould, Cleveland, OH, USA) coupled to an amplifier (Model AECAD 1604, AVS Projetos, São Paulo, Brazil) and connected to a computer equipped with AQCAD 2.02 software (AVS Projetos, São Paulo, Brazil). In vitro approach: The superior mesenteric artery was removed and cleaned from connective tissue and fat. Rings (2–3 mm) were obtained and suspended by cotton threads in organ baths at 37˚C containing 10 ml of Tyrode's solution and gassed (95% O2 and 5% CO2).Then, they were stabilized with a resting tension of 1.0 g (1h).The isometric tension was recorded by a force transducer coupled to a data acquisition system (AECAD 1604, AQCAD 2.8.0., AVS Projects, SP, Brazil) described by Arcanjo et al. [2011]. All values were expressed as mean ± S.E.M. Student's t-test and ANOVA-one way Bonferroni post-test were used in the data analysis and results were considered significant when $p<0.05$. (GraphPad™Prism 5.0). **Results and discussion:** Pi-EtOH (12.5, 25 and 50 mg/kg, i.v.) induced hypotensive effect (-11.6 ± 0.89; -7.4 ± 0.85; -17.6 ± 1.97 mmHg, respectively, n = 5).The hypotensive response of EtOH-*PI* was not attenuated when used in pretreatment with L-NAME (20 mg/kg).In mesenteric rings Pi-EtOH (0.1 - 750 µg/mL) promoted a concentration-dependent vasorelaxation on tonic contractions evoked by phenylephrine (Phe) (10 µmol/mL) in endothelium-intact (pD_2 = 2.3 ± 0.03, n = 5) or endothelium-denuded (pD_2 = 2.1 ± 0.04, n=5) mesenteric artery rings. A similar effect was obtained in preparations pre-contracted with KCl 80 mM in absence of endothelium (pD_2 = 1.8 ± 0.1, n = 6).Likewise, in a Ca^{2+}-free depolarizing medium Pi-EtOH (243, 500 e 750 µg/mL) inhibited $CaCl_2$ (10^{-6} – $3x10^{-2}$ M)-induced contractions and promoted a concentration-dependent rightward shifting of the concentration-response curves, indicating that Pi-EtOH inhibited the contractile mechanisms involving extracellular Ca^{2+} influx. Our results suggest that Pi-EtOH causes an hypotensive effect and induces relaxation in rat mesenteric rings through an endothelium-independent pathway, involving blockade of Ca^{2+} channels. **Keywords:** *Platonia insignis*, hypotension, vasorelaxation. **Financial Support:** This work was supported by UFPI/FAPEPI/CNPq.

References: ARCANJO, DDR, OLIVEIRA, NNPM, FERREIRA-FILHO, ES, COSTA, DA, CHAVES, MH, BORGES, ACR, OLIVEIRA, AP, OLIVEIRA, RCM. Vasorelaxant response induced by *Sida santaremnensis* H. Monteiro ethanol extract on rat superior mesenteric artery. *African Journal of Biotechnology* Vol. 10(65), pp. 14587-14597, 2011. OLIVEIRA, AP, FURTADO, FF, SILVA MS, TAVARES, JF, MAFRA, RA, ARAUJO, DAM, CRUZ, JS, MEDEIROS, IA. Calcium channel blockade as a target for the hypotensive and spasmolytic effects induced by the 8 (17), 12E, 14-labdatrien-18-oic acid (Labdane-302). *Vascul Pharmacol* 44:338–44, 2006. PORTE, A; REZENDE CM.; ANTUNES, OAC.; MAIA, LH. Redução de aminoácidos em polpas de bacuri (*Platonia insignis* Mart), cupuaçu (*Theobroma grandiflorum* Willd ex-Spreng Schum) e murici (*Byrsonima crassifolia* L.) processado (aquecido e alcalinizado) .**ACTA AMAZONICA** VOL. 40:3 573 – 578, 2010.

06.007 Hypotensive effect of ethyl acetate fraction the fruit peel from *Platonia insignis* Mart. in rats. Mendes MB, Silva-Filho JC, Arcanjo DDR, Chaves MH, Oliveira RCM, Oliveira AP, Moura LHP, Campelo RT, Resende-Junior LM UFPI – Plantas Medicinais

The bacuri fruit *(Platonia insignis* Mart) is native of the Amazon region, primarily found in the state of Piauí, Maranhão and Pará (Brazil), and is composed by pulp, shell and seed. The fruit pulp is used as a flavoring agent in ice creams, jams and yogurts (Souza et al., 2000). The present study evaluated the effects of the ethyl acetate fraction of the fruit peel of *Platonia insignis* Mart. (*Pi*-EtOAc) on the blood pressure in rats. **Material and methods:** Male Wistar rats (270 ± 30 g) were used for all experiments (Animal Research Ethics Committee/UFPI - 015/2012). The measurements of the mean arterial pressure were performed as described by Lahlou et al. [2002]. All values were expressed as mean ± S.E.M. Student's t-test and ANOVA-one way Bonferroni post-test were used in the data analysis and results were considered significant when $p<0.05$ (GraphPad™ Prism 5.0). **Results and discussion:** *Pi*-EtOAc was diluted in saline and administered at doses of 12.5, 25 and 50 mg/kg, i.v. inducing hypotensive effect (-11.2 ± 1.03, -14.48 ± 1.35 and -29.89 ± 2.67 mmHg, respectively, n=5). The hypotensive response from *Pi*-AcOEt was not attenuated when used in the pretreatment with L-NAME, verapamil, propranolol, hexamethonium and prazosin; but when using yohimbine, the hypotensive effect was inhibited (-4.42 ± 1.28*, -3.29 ± 0.99* and 2.06 ± 1.18* mmHg, respectively, *$p<0.01$, versus control, one-way ANOVA). It is concluded that *Pi*-EtOAc seems to act on the CNS in a way that is similar to the α_2-adrenergic agonist.

06.008 Vasorelaxant effect induced by thymol in porcine coronary artery rings. Mendes-Neto JM[1], Nascimento RS[1], Diniz JM[1], Guedes DN[1], Medeiros IA[2], Costa KVMC[1], Gonçalves IGA[3], Albuquerque KLGD[1], Correia NA[1] [1]UFPB – Fisiologia e Patologia, [2]UFPB – Biotecnologia, [3]UFPB – Ciências Farmacêuticas

Introduction: Thymol is the major constituent of the essential oil from various aromatic plants such as *Thymus vulgaris* l. (Lamiaceae),*Origanium compactum* (Labiatae), *Acalypha phleoides* (Euphorbiaceae) and *Lippia sidoides* (Verbenaceae). Furthermore, thymol possess multiple biological properties such as anti-inflammatory (RUBERTO, 2000), antifungal (LACOSTE, 1996), antispasmodic activity in the isolated ileum and endothelium-independent relaxation in rat isolated aorta (VAN-DEN-BROUCKE, 1982; PEIXOTO-NEVES, 2010) . The aim of this study was to investigate the vascular effect of thymol on porcine left anterior descending coronary artery. **Method**: Porcine coronary artery rings (2-4 mm) were removed and cleaned from connective tissue and fat and suspended by rod metal in organ baths containing 10 mL Kreb´s solution, maintained at 37 ºC and gassed with carbogenic mixture (95 % O_2 and 5 % CO_2), pH 7.4, stabilization period of 3 hour under a resting tension of 2.0 g. During this time, the Kreb's solution was changed every 15 min. to prevent the accumulation of metabolites. **Results**: In isolated coronary artery rings in the absence of endothelium pre-contracted with U46619 (10^{-7}M), increases concentrations of thymol (10^{-6} to $7x10^{-4}$ M) induced concentration-dependent relaxations [pD_2=4.06±0.06; Emax.=91,82±1,81 % ; n=5]. Similar results were obtained in coronary artery rings pre-contracted with KCl (60 mM) [pD2=4.16±0.03; Emax.=100±4.1%; n=5]. In addition, increases concentrations of thymol (10^{-6} to $7x10^{-4}$ M) were not able to modify the resting tone in these rings. Furthermore, thymol (10^{-6} to $7x10^{-4}$ M) was able to antagonize $CaCl_2$-induced contractions in porcine coronary artery rings without endothelium in 10^{-3}M (30,1±7,81). **Conclusion**: In conclusion, these results suggest that thymol exerts an endothelium-independent relaxation in porcine isolated coronary artery rings, an effect that seems mediated through some mechanisms probably involving a common transduction pathway between the contraction induced by U46619 and KCl 60 mM which appears to be mediated by intracellular calcium decrease probably due to Ca^{2+} influx inhibition by voltage operated calcium channels. **Financial Support:** CAPES/CNPq. **Licence number:** 0406/2013 - CEUA/ UFPB. **Key words:** coronary artery, essential oils, vasorelaxation. **References:** LACOSTE E. Ann. pharmac. Fr.(54)228, 1996. PEIXOTO-NEVES. Fund. clín. Phamac.(24)341,2010. RUBERTO G. Food chem. (69)167,2000. VAN DEN BROUCKE. Plant.med(45)188,1982.

06.009 Reduction in CINC-2 and IL-18 expression is related to improvement of renal function, in intrauterine undernourished rats. Landgraf MA[1,2], Hirata AE[3], Landgraf RG[2], Correa-Costa M[4], de Marco DTK[3], Semedo P[5], Gil FZ[3], Câmara NOS[4] [1]Unifesp – Pharmacology, [2]Unifesp – Inflammation and Vascular Pharmacology, [3]Unifesp – Physiology, [4]USP – Immunology, [5]Unifesp – Nephrology

Introduction: Maternal undernutrition can induce a range of fetal adaptations, which can lead to permanent alterations in adulthood *(Godfrey, Am Soc Clin Nutr, 71: 1344, 2000).* Interleukin (IL)-18 play an integral role in tubular injury and the development of renal dysfunction during a variety of inflammatory processes *(Han, Curr Opin Crit Care, 10:476, 2004)*. In this work, we have investigated the impact of intrauterine undernutrition on the inflammatory markers, and the correlation of these markers with the renal dysfunction; the effect of L-arginine administration on those parameters also was investigated. Methods: Female Wistar rats were randomly divided into 2 groups: nourished (ad libitum diet) and undernourished (50% food restriction). After birth, each litter was left with the mother for 28 days. Some of the offspring received a 2% L-Arg solution in drinking water (groups NR+L-Arg and UR+L-Arg). Proteinuria and glomerular filtration rate (GFR) were determined. IL-18 and cytokine induced neutrophil chemoattractant-2 (CINC-2) were evaluated by Bioplex, in serum (CEP 1666/09). Results: In UR, the proteinuria (63%), IL-18 (121%) and CINC-2 (61%) were increased, when compared to NR, and the L-arg treatment abolished these effects; the GFR was decreased in UR (43%), and the L-arg treatment prevented this reduction. Conclusions: In UR, the inflammatory markers, such as IL-18, are present in an early stage after birth, probably contributing for the development of renal injury in this model. The lower expression of these markers seems to be directly related to improvement of renal function. Our results suggest that these inflammatory markers can be attenuated by L-arginine. Supported by FAPESP, CNPq, and INCT Complex Fluids

06.010 Compensatory cardiac leptin receptor upregulation and P-Type ATPases modulation in rats submitted to neonatal leptin treatment. Marques EB, Silva RM, Graça RO, Scaramello CBV UFF – Farmacologia Experimental

Introduction: Many studies show cardiac diseases related to altered leptin activity (Schulze & Kratzsch. Clin Chim Acta, 362: 1, 2005; Triverdi *et al*., J Cell Cycle,7: 560, 2008) and hypothalamic leptin resistance, in addition to metabolic changes, in adult rats due to neonatal leptin treatment (Toste *et al*. Br J of Nutrition, 95: 830, 2006). Intracellular Ca^{2+} homeostasis is essential for cardiac function and alteration of Ca^{2+}-regulatory protein may be associated with myocardial dysfunction (Yang *et al*. Eur J Heart Fail, 11: 6, 2009). Previous data of our group shows that hyperleptinaemia neonatal increases cardiovascular risk, enhances heart sympathetic activity and induces a cardiac dysfunction in rat adulthood (Marques *et al*., FeSBE2011, panel 24.033; Marques *et al*., SBFTE2012, panel 06.012). The aim of this work was to investigate if neonatal leptin treatment could modulate this adipocin receptor expression in the rat heart, such as it does in the hypothalamus, and also modulate cardiac P-type ATPases expression/activity. **Methods:** Pups were divided into two groups: Leptin group (L), injected daily with leptin (8µg/100g sc) for the first 10 days of lactation and Control group (C), injected saline at the same period. At 5 months-old rats were euthanized, the hearts were removed and cardiac homogenates were obtained (Bambrick *et al*., J Pharmacol Meth, 20: 313, 1988). Homogenates' protein concentration ($mg.mL^{-1}$), P-type ATPases activity ($nmolPi.mg^{-1}$ in 1h) and SERCA, Na^+/K^+ATPase and cardiac leptin receptor (OB-R) protein level (arbitrary units) were determined (Lowry *et al*. J Biol Chem, 193:265,1951; Fiske &Subbarow. J Biol Chem, 66: 375, 1925; Laemmli. Nature, 227: 680, 1970). Data are presented as mean and standard error of the mean (at least 3 observations), analyzed by Student *t* test and considered statistically different if $P<0.05$(*). Tendency studies (Cohen. Statistical power analysis for behavioral sciences, 1977) were also applied and the intervention effect size seems to be great if $d>0.8(^d)$. The use of animals was approved by Ethics Committee (CEPA/UFF00123-09). **Results**: It was observed a significant increase of SERCA protein level (C=6.2±0.4 vs L=10.8±1.0*) and activity (C=1148±152 vs L=3822±675*) in adulthood due to neonatal leptin treatment. However Na^+/K^+ATPase activity was decreased (C=2587±567 vs L=1133±149*) without significant changes in this pump expression yet (C=9.1±2.1 vs L=6.5±2.9^d). Additionally, it was also found a significant increase of OB-R protein level (C=5.6±0.5 vs L=8.9±0.5*). **Discussion**: OB-R upregulation seems to be a compensatory mechanism due cardiac injury (Schulze *et a*l. Eur J Heart Fail. 5: 33, 2003; McGaffin *et al*. Cardiovasc Res. 77: 54, 2008). Increased SERCA activity and decreased Na^+/K^+ATPase activity are related to cardiac performance improvement (Talukder *et al*. Cardiovasc Res. 84: 345, 2009). So, our data suggest a compensatory change in P-type ATPases activity secondary to OB-R upregulation in response to cardiac dysfunction programmed by neonatal hyperleptinaemia. SERCA activity was increased due to an upregulation of its expression. More assays need to be perform to ensure the mechanism involved in Na^+/K^+ activity decrease. **Financial Support**: FAPERJ, CNPq, CAPES, PROPPI/UFF.

06.011 Effects of the selective TRPV4 Modulators GSK1016790A and HC-067047 in isolated arteries from several species. Silva JDP, Alves Filho FC, Ballejo G FMRP-USP – Pharmacology

Introduction: Production and release of relaxing factors by endothelial cells (EC) require calcium influx; the Ca^{2+}-permeable channels involved, however, remain unidentified. Previously, we obtained evidence that TRPV4 channels (calcium-permeable non selective cation channels) are expressed in EC of rat aorta, but the lack of selective modulators hindered the elucidation of their role in EC functions. The aim of this study was to characterize the effects of the recently described TRPV4–activator GSK101 and –blocker HC-067 in isolated arteries. For comparison purposes we also examined the effects of 4αPDD and ruthenium red (RR). **Methods:** Arterial rings from several species were mounted in an isolated organ bath for recording isometric contractions evoked by phenylephrine (PE, 100nM) or U46619 (30-100nM). Experimental procedures were approved by CETEA (024/2011). **Results:** GSK, PDD, HC or RR did not have any effect on arterial rings under basal tone. GSK caused concentration-dependent relaxations (maximal effect corresponded to 100% relaxation) in pre-constricted thoracic aortic rings (*TAR*) from rat (**EC_{50}=**0.5nM, **95%CI=**0.35-0.72nM, n=7), rabbit (4.3nM, 3.58-5.14nM, n=5), mouse (1.4nM, 0.85-2.24nM, n=3) and guinea-pig (0.2nM, 0.12-0.22nM, n=4) as well as in rings from rabbit abdominal aorta (6.5nM, 3.7-11.3nM, n=3) and femoral artery (17nM, 16.8-18.7nM, n=4); PDD (single concentrations) caused relaxations in rat (72% at 3μM, n=30), rabbit (70% at 10μM, n=6) and guinea-pig (24% at 1μM, n=3) *TAR*. GSK- and PDD-induced relaxations started 1-3min after their addition, reached a steady-state in 6-8min and were reversible after washout of the drugs; no *tachyphylaxis* to GSK and PDD was observed when responses were obtained at 20-30min intervals. Furthermore, GSK- and PDD-elicited relaxations were strictly endothelium- and extracellular Ca^{2+}-dependent. HC(1-3μM) and RR**(**1μM) fully reverted all relaxations caused by GSK or PDD (but in rat aorta HC did not revert PDD-induced relaxation in 3/20 responding rings). In rat *TAR*, HC pre-incubation (0.1-1μM, 5min) caused a concentration-dependent parallel rightward shift of the GSK concentration-response curve with no alteration of the maximum response. In contrast, RR (1μM) caused an unsurmountable antagonism of GSK (1-30nM). Noteworthy, the effect of GSK remained blocked after 1h of washing out HC/RR, whereas the effect of PDD regained the magnitude observed under control conditions. HC/RR (1-10μM) did not affect the magnitude of PE contraction in endothelium-denuded rings. Intriguingly, in rings of anterior interventricular porcine coronary artery (relaxation to bradykinin>80%, n=11) GSK (10nM) caused only transient relaxations. Finally, the endothelium- and Ca^{2+}-dependent relaxations elicited by acetylcholine, histamine and thapsigargin were not affected by HC(3μM) or RR(1μM). **Discussion:** Pharmacological activators of TRPV4 channels cause endothelium- and Ca^{2+}-dependent relaxations (EDR) in arteries from all species studied. HC selectively blocks TRPV4, in an apparent competitive manner, whereas RR blockade was non-competitive. PDD appears to produce EDR through both TRPV4 and non-TRPV4 mechanisms indicating that it might not be a pure selective TRPV4 agonist. Considering these findings we infer that TRPV4 channels could be involved in the regulation of EC functions under physiological or pathophysiological conditions. **Support:** CAPES

06.012 Acute ethanol intake induces endothelial dysfunction in rat aorta. Hipolito UV[1], Callera GE[2], Touyz RM[2], Batalhao ME[3], Carnio EC[3], Tirapelli CR[3] [1]FMRP-USP – Farmacologia, [2]Universidade de Ottawa, [3]EERP-USP

Introduction: Literature data show that the metabolism of ethanol leads to formation of reactive oxygen species (ROS), which may be the initial step in the cardiovascular dysfunction induced by ethanol. **Methods:** The experimental protocols were approved by the Ethical Committee from USP (10.1.235.53.0). Male Wistar rats initially weight 200-250 g were randomly divided into 4 groups: Control, Control + Vitamin C (250 mg/kg for 5 days), Ethanol (1g/kg; 10 mL/kg of 13% ethanol diluted in water) and Ethanol + Vitamin C. Vascular reactivity experiments using standard muscle bath procedures were performed on isolated thoracic aorta from Wistar rats. The mean arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and heart rate (HR) were recorded. Nitrate levels were measured in supernatants from total thoracic aorta homogenates and plasma. Activity of NAD(P)H was measured in aorta by lucigenin assay. Western blotting was used to asses: phospho-p38MAPK (Thr180/Tyr182), total p38MAPK, phospho-ERK1/2 (Thr202/Tyr204), total ERK1/2, phospho-SAPK/JNK (Thr183/Tyr185), total SAPK/JNK, phospho-Akt (Ser173), total Akt, phospho-eNOS (Ser1177), total eNOS and NOX 1. Cytosol/Membrane fraction to check, RHO A and RHO Kinase translocation was performed in aorta. **Results:** Compared with the corresponding control group, ethanol produced a significant decrease in MAP, SAP and DAP. On the other hand, ethanol administration did not affect HR. Vitamin C did not alter blood pressure or HR among groups ($p<0.05$/ANOVA). Acute ethanol intake increased phenylephrine-induced contraction in endothelium intact rings (1.6 g ± 0.06 n=9) when compared control group (1.2 g ± 0.07 n=8) Pre-treatment with vitamin C did not prevent this enhancement (1.5 g ± 0.08 n=8) ($p<0.05$/ANOVA). However, in endothelium denuded rings no differences were observed after ethanol administration among 4 groups experimental. Relaxation induced by acetylcholine reduced in aorta from ethanol-treated rats (76.7 g ± 4.4 n=9) when compared control group (100.7 g ± 3.7 n=5). Vitamin C prevented this impairment (111.1 g ± 3.4 n=4) ($p<0.05$/ANOVA). The lucigenin-derived luminescence was significantly higher in ethanol group (511.7 RLU ± 76.9, n=6) when compared to control group (223.9 RLU ± 58.9, n=6). The pre-treatment with vitamin C (5 days) did not prevent the increase in ROS generation induced by ethanol (549.1 RLU ± 54.6, n=7) ($p<0.05$/ANOVA). Ethanol significantly decreased ($p<0.05$) aortic nitrate levels. Pre-treatment with vitamin C did not prevent this decrease in nitrate levels in aorta Western blotting showed that protein phosphorylation JNK and ERK1/2 did not differ among the groups. The pre-treatment with vitamin C increased protein phosphorylation of p38, Akt and eNOS. NOX 1 expression in the 4 experimental groups was similar. Translocation (cytosol/membrane) assay showed that ethanol did not alter RHO A and RHO kinase translocation. **Discussion:**Acute ethanol intake increases superoxide anion generation in the aorta. This response may contribute to functional alterations that serve as a causative factor for cardiovascular diseases. **Supported by:** FAPESP.

06.013 Short pre-exposure to sodium nitrite does not induce tolerance in rat aorta. Banin TM, Bendhack LM FCFRP-USP – Física e Química

Introduction: The nitrite anion (NO_2^-) can be the major source of intravascular and tissue storage of nitric oxide (NO), an important modulator of vascular tone and blood pressure control. Long-term treatment of the patients with the nitrovasodilators leads to the development of tolerance characterized by the rapid loss of vasodilator effects. It is believed that the tolerance process is a multifactorial process and it involves increased production of vascular reactive oxygen species (ROS), decreased activity of soluble guanylyl-cyclase (sGC) and increased expression and activity of phosphodiesterases. Therefore, we have hypothesized that sodium nitrite ($NaNO_2$) would induce tolerance in intact or denuded endothelium rat aorta. The aim of the present study was to investigate whether pre-exposure to $NaNO_2$ of rat aortic rings with or without endothelium, induces tolerance to this compound. **Methods**: Male Wistar rats (200-250g) were killed under anesthesia and the thoracic aorta was quickly cut into rings of 4mm in length that were placed between two stainless-steel stirrups and connected to an isometric force transducer to measure the tension. The rings were placed in an organ chamber with Krebs solution maintained at pH 7.4 and gassed with carbogen at 37°C. Endothelium-intact (E+) and endothelium-denuded (E-) tissues were contracted with phenylephrine (EC_{50}: 100 µmol/L). After reaching a stable and maintained contraction, $NaNO_2$ (10 nmol/L–1 µmol/L) was cumulatively added to the organ bath. Experiments were conducted after 5 or 10min incubation (tolerance) with $NaNO_2$ (EC_{100}:1 µmol/L) followed by 20 min of wash-out, or in the absence of $NaNO_2$ (control). The parameters of maximum effect (ME) and Potency (pD_2) were analyzed. Results are expressed as mean ± SEM. Statistical significance was determined by using the Student's *t* test. In all cases, probability levels of less than 0.05 ($P<0.05$) were taken to indicate statistical significance. All pharmacological studies were performed in accordance with the Ethical Animal Committee of the University of São Paulo (2012.1.134.53.12). **Results**: The $NaNO_2$ induced concentration-dependent relaxation in aortas E+ (pD_2:4.76 ± 0.20; ME: 102.9 ± 1.8%, n=7) and E- (pD_2:4.75 ± 0.13; ME: 104.9 ± 1,5%, n=8). It was observed that the incubation with $NaNO_2$ EC_{100}, for 5 min followed by 20 min of washing did not affect the maximum relaxation or potency induced by the compound in E+ aorta (pD_2:5.07 ± 0.46; ME: 100.6 ± 1.0%, n=6) and in E- arteries incubated with RuBPY (pD_2:4.32 ± 0.14, ME: 101.5 ± 2.0%, n=9), compared with the respective control. Similarly, after 10 min of incubation with $NaNO_2$ (EC_{100}) followed by 20 min of washing, no alterations in the maximum relaxation or potency were observed in E+ aorta (pD_2:4.21 ± 0.33; ME: 101.5 ± 1.2%, n=5) or in E- arteries (pD_2:4.47 ± 0.13, ME: 100.6 ± 0.3%, n=6) compared with the respective control Discussion: Our data demonstrate that the pre-exposure for 5 min or 10 min with $NaNO_2$ (EC_{100}) does not induce tolerance in rat aorta with or without endothelium. Supported by FAPESP and CNPq.

06.014 Mitochondrial reactive oxygen species mediate the modulation of vascular contraction by periaortic adipose tissue. Costa RM[1], Filgueira FP[2], Carvalho MHC[2], Akamine EH[2], Lobato NS[1] [1]UFG – Biological Sciences, [2]ICB-USP

Introduction: Several studies have shown the endocrine role of the substances released by the white adipose tissue in the development of cardiovascular diseases without considering the paracrine role of the adipose tissue surrounding the vasculature (perivascular adipose tissue–PVAT). PVAT from the thoracic aorta influences vascular function and susceptibility to pathogenesis in obesity and the metabolic syndrome. Surprisingly, this tissue has been reported to have characteristics of brown adipose tissue. Considering the role of reactive oxygen species (ROS) mediating PVAT effects on vascular reactivity and given the immense amount of mitochondria in the periaortic adipose tissue, we hypothesized that mitochondrial ROS from this tissue mediate the modulation of the contractile response exerted by PVAT. **Methods**: The investigation was approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences, University of Sao Paulo (Protocol nº 75/2010). Male, 12-16 week-old Wistar rats were used. Vascular function was evaluated in thoracic aortic rings, using an organ bath system. Concentration-response curves for phenylephrine (PE) were performed in vessels with or without endothelium and PVAT. To assess the involvement of mitochondrial ROS on PVAT effects, a mitochondrial uncoupler (2,4-dinitrophenol-DNP, 10^{-5}M) or a superoxide anion scavenger (superoxide dismutase-SOD, 1500 U/mL) was used. **Results**: PVAT reduced the contractile response to PE in endothelium denuded vessels (Emax in g=3.5±0.2* vs. Control=4.0±0.1) and the presence of the endothelium inhibited the effects promoted by this tissue (Emax in g, Control=2.1±0.2; PVAT=1.9±0.1). In endothelium intact vessels, DNP incubation decreased the response to PE in both control and PVAT vessels. However, the response of PVAT vessels became reduced when compared to the control vessels in the presence of the uncoupler (Emax in g, Control DNP=1.0±0.1; PVAT=0.9±0.1). In endothelium denuded vessels, DNP incubation decreased the sensitivity to PE in the control vessels (PD2=7.3±0.1* vs. Control=8.3±0.1) and the maximal response in PVAT vessels (Emax in g=2.7±0.6). The concomitant incubation with DNP and SOD potentiated the contractile responses in control vessels both in the presence (Emax in g=0.5±0.1) and in the absence of the endothelium (Emax in g=0.1±2.6). However, in PVAT vessels, SOD did not get effective participation in decreasing the responsiveness of the vessels to PE either in the presence or in the absence of the endothelium. **Discussion**: In the absence of PVAT, ROS derived from endothelial mitochondria as well as O_2^- from other vascular sources, contribute to the modulation of vascular contraction. The presence of periaortic fat makes this tissue an additional source of mitochondrial ROS. In vessels with PVAT, ROS derived exclusively from the mitochondria modulate vascular contraction, indicating that the periaortic adipose tissue participates in the modulation of the contractile response of aorta by producing mitochondrial ROS that add to the relaxing and contractile factors generated by the vascular system. Emax, maximum effect; g, grams; *, $p<0,05$. Financial Support: FAPEG, CNPq, FAPESP.

06.015 Nitroglycerin but not the new nitric oxide donor RuBPY phosphorylates eNOS-Ser1177. Paulo M, Grando MD, Vercesi JA, da Silva RS, Bendhack LM FCFRP-USP – Física e Química

Introduction: Nitric oxide (NO) donors like nitroglycerin (GTN) are widely used as pharmacological tool to understand the physiological effect of NO, and to the treatment of cardiovascular diseases. The major therapeutic limitation of GTN is the tolerance that is characterized by rapid loss of its effects in long-term administration, or cross-tolerance to other vasodilator, which mechanism is still not clear. It is endothelium-dependent and the uncoupling endothelial NO-synthase (eNOS) could contribute to the tolerance. The major clinical benefit of organic nitrates, including the GTN has been attributed to their potent venodilator effect. **Aim:** This study aimed to verify if the new NO donor synthesized in our laboratory (RuBPY) induces *in vitro* tolerance in cava vein and to identify the cellular mechanisms involved. We compared the effects of RuBPY with GTN in relation to the maximum effect (ME) and potency (pD_2). **Methods:***In vitro* tolerance was induced by incubation for 10, 30 or 60 min with RuBPY (2 μM or 10 μM) or GTN (4 μM or 0.1mM). We have investigated the contribution of NO produced by eNOS with the inhibitor L-NAME (100 μM) and Tetrahydropterin (BH_4, 100 μM). We also investigated the role of the anion superoxide (O_2^-) by using the scavenger tiron (100 μM). The phosphorylation of eNOS in the activation site (Ser^{1177}) and in the inactivation site (Thr^{495}) was accessed by Western Blotting. The cytosolic concentration of NO ([NO]c) was measured by confocal microscopy. All these procedures were in accordance with the guidelines of the Animal Ethics Committee, University of São Paulo, Brazil (11.828.532). **Results:** Our results demonstrated that RuBPY induced greater relaxation (ME: 92.8 ± 4.4%; n=7, P<0.05) than GTN (ME: 75.3 ± 3.7%, n=6). Both NO donors increased [NO]c in vascular smooth muscle cells. Previous exposure for 10 min to RuBPY (2 μM or 10 μM) or GTN (4 μM or 0.1mM) did not induce tolerance. However, 30 min pre-exposure did not change the relaxation to RuBPY, but it reduced the relaxation to GTN (with 4 μM, ME: 45.4 ± 2.2%, n= 6, P<0.05, and 0.1 mM, ME: 39.2 ± 1.4%, n= 6, P<0.05). Pre-exposure for 60 min with RuBPY reduced the relaxation in the concentrations of 2 μM (ME: 48.0 ± 2.3%, n= 7, P<0.05) and to 10 μM (ME: 30.1±1.2%, n= 7, P<0.05). Tiron increased the Emax induced by GTN (60.0 ± 4.1%, n=6 P<0.05) and RuBPY (73.0 ± 3.4%, n=6, P<0.05) in tolerant vessels. BH_4 increased the Emax induced by GTN (67.4 ± 3.3%, n=7, P<0.05) and RuBPY (65.0 ± 2.3%, n=6, P<0.05) in tolerant vessels. L-NAME increased the Emax induced by GTN (70.3 ± 3.7%, n=6, P<0.05) but had no effect on RuBPY tolerant vessels. Whereas RuBPY and GTN had phosphorylated eNOS in Thr^{495}, only GTN phosphorylated eNOS-Ser^{1177}. **Conclusion**: Our data demonstrated that GTN and RuBPY induce tolerance in both concentrations only after exposure for 60 min. The tolerance induced by NO donors involves eNOS and O_2^-. GTN but not RuBPY interferes with the activation site of eNOs. Supported by FAPESP and CNPq.

06.016 Evaluation of cardioprotection ipriflavone in rats submitted to the left coronary ligature. Castro QJT[1], Albuquerque K[1], Carneiro CM[1], Guimarães HN[2], Leite R[1], Mosqueira VCF[1], Grabe-Guimarães A[1] [1]CiPharma-UFOP, [2]DEE-UFMG – Engenharia

Introduction: Ipriflavone (7-isopropoxy-3-phenyl-4H-1-benzopyran- 4-one) is a semi-synthetic soy derivative, used in several countries for prevention and treatment of osteoporosis. Its cardioprotective effect administered orally was demonstrated in isolated heart of rabbits (Feuer *et al*, 1981). Self-emulsifying drug delivery systems (SEDDS) have gained great importance as a promising technology to improve the bioavailability of poorly water-soluble drugs like ipriflavone. **Objective:**Evaluate the potential cardioprotective effect of SEDDS containing ipriflavone in rats submitted to the left coronary ligature. **Methods**: All the procedures were approved by The CEUA/UFOP (11/2009). Female Wistar rats (220 to 250 g) received vehicle or ipriflavone (240 mg/kg) in SEDDS by oral route. Sham non treated animals were used as control group. Under anesthesia (pentobarbital, 60 mg/kg), the lead II ECG signal was obtained before and 30 minutes after left coronary ligature. The hearts were arrested after euthanasia and submitted to histological analysis. **Results:** The PR interval of ECG increased after the coronary ligature (67.1 ± 4.27) compared to the Sham group (54.1 ± 1.01) and ipriflavone was not able to prevent this increase (68.3 ± 4.07). The QT interval and QTc parameters were increased after the ligature in animals without treatment, but QTc increase was not significant due to the large influence of bradycardia. The QT and QTc increases after the ligature were significantly reduced in ipriflavone treated animals compared to the vehicle treated animals. The values for the Sham group, untreated and treated animals are respectively for QT 61.69 ± 1.62 ms, 76.42 ± 5.78 ms, 65.82 ± 4.47 ms, and for QTc 107.5 ± 5.31, 117.9 ± 5.71, 108.0 ± 8.28. The histopathological changes observed in hearts subjected to coronary ligature regardless of the treatment were: hyaline degeneration, edema, signs of inflammation and congestion. The administration of a single dose of ipriflavone in SEDDS (240 mg/kg) caused a reduction of these changes. **Conclusion:** The most important finding of the present study is the cardioprotective effect of SEDDS containing ipriflavone by inhibiting the increases of the QT interval caused by coronary ligature in rats. **Funding agencies:** FAPEMIG (Rede Nanobiomg) and UFOP. **Acknowledgments:** FAPEMIG; CNPq; UFOP; CAPES

06.017 Evaluation of the mechanism of action of Riparin I, II and III of relaxation in mice mesenteric artery. Garcia DCG[1], Barbosa-Filho JM[2], Lemos VS[3], Cortes SF[1] [1]UFMG – Pharmacology, [2]UFPB – Pharmaceutical Technology, [3]UFMG – Physiology and Biophysics

Riparin I ((O-methyl)-N-benzoyltyramine), riparian II ((O-methyl)-N-(2-hydroxybenzoyl)-tyramine) and riparian III ((O-methyl) - N-(2,6-dihydroxibenzoyl)-tyramine) are alkamides alkaloids found in plants from Lauraceae family. Previous studies have shown that these compounds, isolated from the fruits from *Aniba riparia,* present cardiovascular effects, such as hypotension and bradycardia. These effects indicate that these drugs may be useful for the treatment of hypertension, reducing the risk of cardiovascular complications. As the resistance vessels are essential for the control of blood pressure, our study aimed to investigate the effects evoked by riparin I, II and III in the mesenteric artery from mice, verifying the structure-activity relationship of their vascular vasodilator effect in vitro. The experimental procedures were submitted to the ethics committee of the UFMG (CETEA protocol number 263/2013). Male Swiss mice were sacrificed by decapitation, and the small intestine with mesenteric vascular bed was dissected. Then, 2 mm rings from second ramification of the superior mesenteric artery were mounted on a myograph chamber with Krebs-Henseleit solution, aerated by a carbogenic mixture (95% O2 and 5% CO2). The endothelium was considered functional in rings of mesenteric artery pre-contracted with phenylephrine (3 μM), where the vasodilatation induced by acetylcholine (10 μM) was superior to 80 %. The absence of a functional endothelium was confirmed by acetylcholine-induced vasodilatation lower than 10%. Cumulative concentration-response curves to riparin-I (1 μM to 100 μM), II and III (0.1 μM to 100 μM) were obtained in vessels pre-contracted with phenylephrine (3 μM). These substances were able to induce relaxation in vessels with and without functional endothelium in a concentration-dependent manner. The values of the -log of the concentration inhibiting 50% of the sustained contraction (pIC_{50}) for riparin I, II and III in arteries with functional endothelium, were 4.68 ± 0.09, 5.29 ± 0.11 and 5.72 ± 0.11, respectively. Riparin I was less potent ($P < 0.001$) than II and III, while riparin III showed higher potency, suggesting that the addition of hydroxyl groups enhances the potency of these drugs. Tetraethylammonium (10 mM), a non-selective inhibitor of potassium channels and glibenclamide (1 μM) a selective K_{ATP} inhibitor, did not affect the concentration-effect curve of riparin. In vessels pre-contracted with KCl (50 mM), riparin I, II and III also induced concentration-dependent vasodilator effects, with pIC_{50} of 4.48 ± 0.03, 5.61 ± 0.03 and 5.50 ± 0.07, respectively. Selective inhibitors of protein kinase A (PKA) and G (PKG), H-89 (1 μM) and Rp-8-Br-PET-cGMP (3 μM) respectively, did not modify the concentration-effect curves to riparins. The present results led us to conclude that riparin I, II and III induce concentration-dependent vasodilator effects independently on activation of potassium channels or cyclic nucleotide-dependent protein kinases. The structure activity relationship study suggests that the increased number of hydroxyl radicals potentiates the vasodilator effect of riparin. **Support**: CAPES e FAPEMIG

06.018 Characterization of cardiovascular function and anthropometric parameters with aging in rats. Marques EB, Barros RB, Rocha NN, Scaramello CBV MFL-LAFE-UFF

Introduction: Aging poses the largest risk factor for cardiovascular (CV) disease (North &Sinclair. Circ Res,110:1097, 2012). In clinical practice and epidemiological surveys, anthropometric measurements represent an important component of nutritional assessment and it is known that body composition changes during aging (Perissinotto *et al*. Br J Nutr, 87:177, 2002). Many studies relate anthropometric measures and CV disease risk (Chen *et al*. Eur J Cardiovasc Prev Rehabil, 14:740, 2007; Oshaug *et al*. Int Arch Occup Environ Health, 67:359, 1995). A fundamental understanding of age-associated changes in CV structure and function ranging in scope from animals to molecules is required for effective and efficient prevention and treatment of CV disease in the eldery (Lakatta. Heart Fail Rev,7(1):29, 2002). **Methods:** Female rats were caged with male rats at a proportion of 2:1. After birth all litters were adjusted to 6 males per day (Fishbeck & Rasmussen. J Nutr, 117: 1967, 1987). After weaning, offspring body weight and food intake were monitored until 12 months-old. Body length, abdominal and thoracic circumferences as well as Body Mass Index (BMI) were also determined at 1, 3, 5 and 12 months-old. Feed efficiency ratio was calculated from the ratio between weight gain and food consumption (Novelli *et al*. Laboratory Animals, 41: 111, 2007). Echocardiographic studies were performed (Lang *et al*. J Am Soc Echocardiogr, 18: 1440, 2005) being measured ejection fraction (EF), relative wall thickness (RWT), left ventricular mass (LVM), interventricular septal wall thickness (IVS), posterior wall thickness (LVPW), left ventricle internal dimension (LVID), left atrium/aorta (LA/Ao) ratio and mitral deceleration time (DT). Maximal effort ergometer test was also performed (Brooks & White. J Appl Physiol, 45:1009, 1978). Data were presented as mean and standard error of the mean (at least 4 observations), analyzed by One Way ANOVA test and considered statistically different if $P<0.05$. The use of animals was according to Ethics Committee (CEPA/UFF00123-09). **Results:** Body weight, food intake, body length and BMI increased with age. As feed efficiency is measured from ratio between weight gain and food consumption, as body weight gain diminishes while food consumption raises along aging, feed efficiency ratio decreases. No differences were observed among groups about abdominal and thoracic circumferences ratio but the maximum developed speed decreased along aging in rats. In echocardiographic study, EF diminished but the mean values were superior to 50%; LVM, IVS, LVPW, LVID, LA/Ao ratio and mitral DT increased while RWT remained unchanged with age. **Discussion:** Rat obesity may be easily estimated through BMI and alterations in this index, as observed in this work due to aging, are associated with obesity, dyslipidemic profile, oxidative stress (Novelli *et al*., Lab Anim. 41:111, 2007) and suggest an increase of CV risk (Stevens *et al*. N Engl J Med, 338:1, 1998). Data obtained through maximal effort ergometer test shows that exercise tolerance decreased. Eccentric LV hypertrophy was observed, as well as signs of diastolic dysfunction, despite of the preserved EF (Sherif *et al*. Eur J Echo, 10: 165, 2009). It was found a ventricular dilation paralleled to a progressive prolongation of DT that could reflect an attempt to normalize the filling pressure (Temporelli *et al*. J Am Col Cardiol, 43: 1646, 2004). **Financial Support:** CNPq, CAPES, PROPPI/UFF.

06.019 Involvement of calcium on the positive inotropic effect produced by ATP and UTP right atria in hypertensive rats. Rodrigues JQD, Silva-Junior ED, Câmara H, Miranda-Ferreira R, Galvão KM, Caricati-Neto A, Jurkiewicz NH, Jurkiewicz A Unifesp – Pharmacology

Aims: The P1 and P2 purinergic receptors are expressed in cardiac tissue and are activated by Adenosine-5'-triphosphate (ATP) and Uridine-5'-triphosphate (UTP) released by sympathetic neurons. On right atria (RA), ATP and UTP induced a negative inotropic effect (NIE) followed by a positive inotropic effect (PIE). Knowing that purinergic receptors signaling are linked to voltage-gated calcium channels (VDCC) and calcium homeostasis is disturbed in hypertension, we decided to evaluate the role of extracellular and intracellular calcium in the PIE of ATP and UTP on RA of spontaneously hypertensive rats (SHR). **Material and Methods:** RA of NWR (normotensive Wistar rats) and SHR male rats (4-6 months) were isolated and mounted in organ bath. The RA presenting spontaneous beatings and frequency between 180 and 420 bpm were considered in the present study.To study the influence of extracellular calcium on PIE induced by ATP (300 mM) and UTP (300 mM), we used nifedipine (100 nM/ pre-incubated for 20 min) an L-type VDCC blocker or rianodine (3 nM/ pre-incubated for 20 min) a blocker of calcium from sarcoplasmatic reticulum (SR). The results were expressed by means ± SEM and analyzed by unpaired t test and one-way ANOVA. In accordance to the UNIFESP ethic committee (n° 0778/11). **Results:** ATP produced a NIE with amplitude of 40.7 ± 1.1% in NWR and 27.7 ± 2.4% in SHR ($p<0.05$), in relation to the baseline value. After this time, atrial inotropism gradually increased, reaching the plateau at 180-200s, showing a PIE of 33.8 ± 2.5% in NWR and 49.6 ± 1.3% in SHR ($p<0.05$), in relation to the final value of the NIE. In the presence of nifedipine, the PIE effect induced by ATP was reduced by 27% (33.8 ± 2.5% to 24.7 ± 0.9%, $p <0.05$) in NWR and 44% (49.6 ± 1.3% to 27.6 ± 1.1%, $p <0.05$) in SHR. In the presence of rianodine, the PIE effect induced by ATP was reduced by 45% (34.7 ± 1.7% to 19.0 ± 1.1%, $p <0.05$) in NWR and 68% (46.0 ± 2.3% to 19.5 ± 1.0%, $p <0.05$) in SHR. UTP produced an initial NIE with an amplitude of 16.8 ± 0.8% in NWR and 13.2 ± 1% in SHR ($p<0.05$) compared to baseline. After this time, atrial inotropism increased gradually, reaching the plateau at 150-180 min, with a valor of 27.3 ± 1.1% in NWR and 32.6 ± 1.0% in SHR ($p<0.05$). In the presence of nifedipine, PIE induced by UTP was reduced in 25% (27.3 ± 1.1% to 20.6 ± 0.8%, $p <0.05$) to NWR and 33% (32.6 ± 1.0% to 21.8 ± 0.6%, $p <0.05$) to SHR. In the presence of rianodine, the PIE effect induced by UTP was reduced by 61% (25.9 ± 1.6% to 10.1 ± 2.1%, $p <0.05$) in NWR and 63% (35.3 ± 1.9% to 13.0 ± 2.1%, $p <0.05$) in SHR. **Conclusion:** The PIE responses to ATP and UTP are higher in SHR, compared with NWR atria. The pharmacological interference with Ca^{2+} handling suggests the existence of an augmented SR Ca^{2+} store, with respect to NWR atria. These data add evidence to the hypotheses of a dysfunction of purinergic neurotransmission together with an enhanced sympathetic activity, as contributor factors in the pathogenesis of hypertension. **Financial support:** CAPES, CNPq and FAPESP.

06.020 New anti-inflammatory prototypes present anti-atherosclerotic effects through NF-kB inhibition. Vieira TBQ[1], Motta NAV[1], Fumian MM[1], Barreiro EJ[2], Maia RC[2], Kummerle AE[3], Brito FCF[1] [1]LAFE-FF-UFF – Fisiologia e Farmacologia, [2]LASSBio-UFRJ, [3]UFRRJ – Química

Introduction: Atherosclerosis is a major cause of cardiovascular diseases and is characterized by progressive deposition of lipid and fiber in arteries. Atherosclerosis was considered a consequence of dyslipidemia, but recent investigations have revealed that chronic inflammatory processes associated with dyslipidemia and endothelial dysfunction are also important contributors to its development (ZHANG *et al.,*Trends Pharmacol., 28: 286, 2008). Previously, our research group has described anti-inflammatory and antiplatelet activities to LASSBio-788 and LASSBio-1425 (Fumian *et al*., SBFTE 2012; Motta *et al*., SBFTE 2012). In this work we describe a relevant anti-atherosclerotic activity associated to these compounds and the elucidation of their mechanism of action. **Methods:** The animal protocols were approved by the Ethics Committee for Experimental Research of the Federal Fluminense University (CEPA/ UFF 00116/09). Adult male Wistar rats (150-200g) were randomly divided into four groups (n= 10, for each group): C (control group) has received a normal rat chow for 45 days. Atherosclerosis group (AT), LASSBio-788 group and LASSBio-1425 group have received a hypercholesterolemic diet (HCD) for 45 days. The vehicle (tween, etanol and water, 1:1:8) (0.05 ml/kg) was administered to C and AT, whereas LASSBio-1425 (100 µmol/kg) or LASSBio-788 (100 µmol/kg) were administered to the treated groups. The treatments were orally given, once a day, for 15 days. The animals were euthanized by cervical dislocation and decapitated under anesthesia. Blood samples were collected, the thoracic aorta, and liver were excised. Data were analyzed using Student's t-test ($p < 0.05$). **Results:** A high cholesterol diet administered for 45 days caused a dramatically significant increase in all lipid parameters. The treatment of hypercholesterolemic rats with LASSBio-788 or LASSBio-1425 for 15 days significantly decreased the total levels of cholesterol (76,6% and 43,0%), tryglicerides (74,1% and 44,3%), LDLc (82,4% and 65,3%) and VLDLc (78,8% and 52,4%), respectively, when compared to the AT group. In the vascular reactivity, LASSBio-788 group (CE_{50} $3.1x10^{-7}$M) and LASSBio-1425 group (CE_{50} = $6.9x10^{-7}$M) showed a decrease in the potency of phenylephrine dependent contraction when compared with AT group (CE_{50} $7.4x10^{-8}$M), as well as they have promoted an improvement in the potency of endothelium-dependent vasorelaxant response (CE_{50} : LASSBio-788 group= $3.2x10^{-8}$M; LASSBio-1425 group= $1.5x10^{-7}$M; AT group= $1.3x10^{-6}$M). The treatment with these compounds showed a significant decrease in the production of anti-inflammatory cytokine TNF-α(LASSBio-788 group= 60% and LASSBio-1425= 30%, respectively). In western blot assay, both compounds increased the expression of endothelial nitric oxide synthase and diminished the expression of NF-kB, when compared with AT group. **Discussion** – Our results show that the chronic treatment with LASSBio-788 or LASSBio-1425 promotes anti-atherogenic effects *in vivo*, presenting lipid-lowering, antioxidant, vasorelaxant and anti-inflammatory properties. The possible mechanism of action of these compounds is associated with inhibition of TNF-α production and NF-kB expression, resulting in decreased inflammatory response. These effects lead to a decrease of inflammatory markers production and an improvement of the vasodilatory response. LASSBio-788 and LASSBio-1425 represent new multi-targeted drugs candidates for atherosclerosis treatment. **Financial Agencies** – CAPES, PROPPI-UFF, FAPERJ.

06.021 Immunological tolerance to cardiac antigens improves healing after myocardial infarction. Ramos ERB, Ramos GC, Rezende Junior E, Bicca MA, Assreuy F UFSC – Farmacologia

It has been demonstrated that during a myocardial infarction (MI) necrotic cardiomyocytes release antigens that trigger autoimmunity against cardiac tissues. In a previous work we found that the development of immunological tolerance to such cardiac antigens may benefit heart healing and function after MI. Thus, the main objective of the present study was to further characterize those observations. Infarction-like myocardial lesions were induced in male Wistar rats (MI group) through the injection of high doses of isoproterenol (150 mg/kg, two consecutive days). In another group of animals, immunological tolerance to cardiac antigens was developed by means of oral exposure to cardiac antigens 7 days before isoproterenol-induced myocardial lesions (TOL+MI group). Naïve animals were used as non-infarcted control (CTRL group). Cardiac function was evaluated by means of a pressure-volume conductance catheter placed in the left ventricle and also through isolated and perfused heart preparations (Langendorff). In addition, migrated leukocytes were phenotyped through immunohistochemical analysis of paraffin-embedded myocardial sections (Institutional Ethics Committee License PP00305/CEUA/PRPE/2009). Analysis of left ventricular pressure-volume relation curves revealed that MI animals presented a significant impairment of systolic (e.g. ejection fraction) and diastolic indices (e.g. dP/dt min) at 3 and 15 days after isoproterenol-induced cardiac damage, when compared to CTRL animals. In contrast, TOL+ISO animals presented preserved ejection fraction at 3 days (CTRL 73.4 ± 2.8; MI 44.6 ± 6.3; TOL + MI 74.3 ± 4.4; $P < 0.05$ CTRL versus MI) and 15 days (CTRL 73.4 ± 2.8; MI 53.6 ± 8.8; TOL + MI 66.6 ± 4.1; $P < 0.05$ CTRL versus MI) after MI. Similar results were observed also for stroke work, Tau and dP/dt min. Immunohistochemical analysis has shown that myocardial sections from TOL+MI animals presented a decreased influx of pro-inflammatory substances (NOS-2^+, phospho NF-kB, p65^+ and MMP9^+) from leukocytes, when compared with MI group at 3 days. In addition, TOL+MI animals presented an increased early influx of anti-inflammatory cells (IL-10^+, but not arginase-I^+) into the damaged myocardium. Most strikingly, the influx of NOS-2^+ cells in myocardium was inversely correlated with the ejection fraction measured *in vivo* ($r^2 = 0.74$). In summary, these findings suggest that animals turned tolerant to cardiac antigens displayed an earlier resolution of myocardial inflammation after isoproterenol-induced MI and that correlated with an improved cardiac functionality.

06.022 Vasodilatory activity of fractions from the ethanolic extract of leaves of *Kielmeyera membranacea casar* (Calophyllaceae). Paes BM, Carneiro LC, Faria PP, Ferreira LLDM, Konno TUP, Leal ICR, Muzitano MF, Guimarães DO, Raimundo JM UFRJ

Introduction: The Restinga of Jurubatiba National Park (PARNA Jurubatiba), located in the northern Rio de Janeiro state, protects a coastal region of great biodiversity where we can find *Kielmeyera membranacea* (CALOPHYLLACEAE). We have previously shown that the ethanolic extract of leaves of *K. membranacea* produces intense endothelium-dependent vasodilation of rat aorta[1]. Therefore, the objective of this study was to investigate the vasodilatoy activity of different fractions from the ethanolic extract of *K. membranacea* leaves. **Methods:** The ethanolic extract of *K. membranacea* leaves was submitted to a multi-step liquid–liquid fractionation with solvents of increasing polarity leading to four fractions: hexanic (HKM), dichloromethane (DKM), ethyl acetate (EAKM) and butanolic (BKM). Vascular effects of the different fractions were assessed in endothelium-intact aortic rings from male Wistar rats (220–280 g), which were prepared for isometric tension recording. Aortic rings were placed in vertical chambers filled with Krebs-Henseilet solution and were stabilized under 1 g resting tension for 90 min. Then, the contractile response to phenylephrine (Phe, 10 µM) was measured before (control) and after exposure to increasing concentrations of fractions (1-100 µg/ml). Concentration-response curves were analyzed by non-linear regression and the concentration necessary to reduce Phe-induced contraction of aorta by 50% (IC_{50}) was determined. One-way analysis of variance (ANOVA) followed by Dunnett's test was used to compare the experimental and control groups (GraphPad Prism 5.0). All protocols were approved by the Animal Care and Use Committee under license Macaé01. **Results:** DKM, EAKM and BKM relaxed pre-contracted endothelium-intact aortic rings in a concentration-dependent manner. At 30 µg/ml, DKM, EAKM and BKM-induced vasodilation was 7.5 ± 1.9, 79.1 ± 4.9 and 83.9 ± 1.4 %, respectively ($P<0.05$ compared to control; n=4-5). HKM had no significant effect on vascular smooth muscle. The IC_{50} of DKM, EAKM and BKM in endothelium-intact aorta was 67.3 ± 0.7, 11.3 ± 2.2 and 1.7 ± 0.4 µg/ml, respectively. **Discussion:** EAKM and BKM induced intense vasodilation. BKM was more potent than the other fractions tested and also than the crude ethanolic extract (IC_{50}= 3.2 ± 0.2 µg/ml) in reducing Phe-induced aorta contraction. Thus, bioactive compounds present in BKM seem to be the main responsible for the vasodilatory activity of the ethanolic extract of *K. membranacea* leaves. **Reference:** [1]Paes BM. 44th Congress of Pharmacology and Experimental Therapeutics, 2012.**Financial support:** FAPERJ, FUNEMAC, UFRJ.

06.023 P-Type ATPases modulation goes along with cardiac dysfunction observed in rats fed with a high fat diet. Silva RM, Marques, EB, Oliveira GF, Rocha NN, Scaramello CBV UFF

Introduction: Previous data of our group (Silva *et al*., SBFTE2012, panel 06.035) suggest that rats submitted to high-fat diet (HFD) presented an inferior energy expenditure, a superior body mass index and a higher abdominal/thoracic circumferences ratio compared to rats fed with commercial chow. Many studies relate anthropometric measures and cardiovascular disease risk (Chen *et al*. Eur J Cardiovasc Prev Rehabil, 14:740, 2007; Oshaug *et al*. Int Arch Occup Environ Health, 67:359, 1995). Our group also found that HFD fed rats presented a smallest heart rate of hypertrophy which may decrease cardiac output and contractility (Fioretto *et al*., Am J Physiol Heart Circ Physiol 282:H1327,2002; Alden *et al*. Am J Physiol. 253:H380, 1987). Echocardiographic data suggested diastolic dysfunction with a reduced mitral deceleration time being the degree of left ventricle dilation related to the severity of left ventricle filling impairment. So the aim of this work was to characterize molecular mechanisms underlying the diastolic dysfunction observed in this malnutrition model. **Methods:** The use of animals was according to Ethics Committee (CEPA/UFF00099/2011). After weaning male Wistar rats were randomly divided and submitted to 30 or 60 days of experiment: G1- fed with commercial chow (Nuvilabò: 56% carbohydrate, 19% protein, 3.5% lipids, 4.5% fibers, 5% vitamins/minerals=4.1kcal/g); G2- fed with HFD (39.4% carbohydrate,17% protein, 17% lipids, 3% fibers, 4% vitamins/minerals=4.3kcal/g) prepared using commercial chow, butter and egg yolk. After 30 or 60 days of diet, rats were euthanized, the hearts were removed and cardiac homogenates were obtained (Bambrick *et al*., J Pharmacol Meth, 20: 313, 1988). Homogenates' protein concentration ($mg.mL^{-1}$) and P-type ATPases activity ($nmolPi.mg^{-1}$ in 1h) were determined (Lowry *et al*. J Biol Chem, 193:265,1951; Fiske & Subbarow. J Biol Chem, 66: 375, 1925). Data are presented as mean and standard error of the mean (at least 3 observations), analyzed by Student *t* test and considered statistically different if $P<0.05$(*).Tendency studies (Cohen. Statistical power analysis for behavioral sciences, 1977) were also applied and the intervention effect size seems to be great if $d>0.8$(^{ddd}), moderate if $d>0.5$(^{dd}) and small if $d>0.20$(^{d}). No effect is expected if $d<0.19$. **Results:** It was observed a significant decrease of cardiac Na^+/K^+ATPase activity after 30 days (G1=2880±274 vs G2=1356±563*) and 60 days of HFD (G1=3443±569 vs G2=1676±1121*). No differences were observed about Na^+ATPase activity after 30 days (G1=3360±900 vs G2=4345±923^{dd}) neither 60 days of HFD (G1=2894±644 vs G2=2474±1056^{d}). **Discussion:** Evidences indicate that Na^+/K^+ATPase activity are reduced in the failing human heart (Barwe *et al*. J Mol Cell Cardiol, 47: 552, 2009). Consequently Ca^{2+} overload may occur, affecting myocytes relaxation and conducting to a diastolic dysfunction (McDonough *et al.* Basic Res Cardiol, 97:I19,2002), as observed in this study. Changes in Na^+ ATPase activity are expected to equalize intracellular Na^+ level while Na^+/K^+ATPase activity is decreased (Reyes *et al*. Physiol Res, 58:693,2009). More assays need to be perform to ensure the nonexistent Na^+ATPase activity compensatory modulation. **Financial support:** CNPq, PROPPi/UFF

06.024 Activation of AT1-receptor by Angiotensin II modulates the release of nitric oxide in thoracic aorta of hamsters in the early stages of hypercholesterolemia. Pereira PC[1], Pernomian L[2], Franco JJ[2], Gomes MS[2], Uyemura SA[2], de Oliveira AM [1]FMRP-USP, [2]FCFRP-USP

Introduction: There is increasing evidence of cross-talk between hypercholesterolemia and renin-angiotensin system in atherogenesis The aims from the present study were to investigate the temporal consequences on vascular reactivity to angiotensin II (Ang II) and to evaluate the role of nitric oxide in this response during the early stages the development of hypercholesterolemia. **Methods:** Male Golden Syrian hamsters, 45 days old, were fed a standard diet + 1% cholesterol for 5, 10, 15, 20, 25 and 30 days. Was determined the serum lipid profile of the animals by automated analyzer employing the ABBOTT VP Super System. Cumulative concentration-response curves to angiotensin II (Ang II) (10^{-11} to 10^{-6} mol/L) were obtained in thoracic aorta with intact endothelium (E +) and in its absence the endothelium (E-) in all the different periods of treatment. Cumulative concentration-response curves to Ang II in thoracic aortic the hamsters control (GC) and hamsters fed cholesterolemic diet for 15 (G15) and 30 days (G30) were obtained in the absence or the presence of L-NAME (100 µmol/L), non-selective nitric oxide synthase (NOS) inhibitor; L-NNA (100 µmol/L), selective endothelial NOS inhibitor; L-NPA (50 nmol/L), selective neuronal NOS inhibitor and 1400W (10 nmol/L), NOS inducible inhibitor. The parameters used to evaluate vascular reactivity were maximal response (Emax) and potency (pD2). All procedures were approved by the Ethics Committee on Animal Experiments of FMRP (nº 141/2011). **Results:** In aortic rings (E+), Ang II produced a decrease in the response of the contraction for the periods 10, 15 and 20 days of treatment with cholesterolemic diet. The lower amplitude Emax was observed during 15 days, 2.86 ± 0.11 g/mg, in relation the CG, 3.60 ± 0.15 g/mg. Likewise, in rings (E-), the Emax for Ang II in the group G15 (Emax: 3.71 ± 0.12 g/mg), was smallest in relation to the CG (Emax: 4.65 ± 0.21 g/mg). In G15, the presence of L-NAME (Emax: 5.03 ± 0.24 g/mg) and L-NNA (Emax: 3.61 ± 0.12 g/mg) in the aorta (E+) induced increase of Emax for Ang II, compared to the absence of these inhibitors (Emax: 2.86 ± 0.11 g/mg). In aortic rings (E-), was observed an increase the Emax in the presence all the inhibitors; L-NAME (Emax: 5.06 ± 0.25 g/mg), L-NNA (Emax: 4.77 ± 0.33 g/mg), L-NPA (Emax: 4.97 ± 0.25 g/mg) and 1400W (Emax: 5.12 ± 0.45 g/mg) in relation to the absence of these inhibitors (Emax: 3.71 ± 0.12 g/mg). At 30 days, presence of the inhibitor L-NNA in aortic rings (E+) induced a reduction of Emax for Ang II (Emax: 2.04 ± 0.39 g/mg) compared to absence of inhibitor (Emax: 3.56 ± 0.22 g/mg). No significant difference was observed in values of pD2 (E+) and (E-) in the different study protocols. **Discussion:** Our results suggest that cholesterolemic diet in hamsters induces an increase in production and/or release of nitric oxide derivate of NOS endothelial, neuronal and inducible, present in muscle layer, only during the initial stage of the development of hypercholesterolemia. **Financial Support:** CNPq, FAPESP and NAP-DIN.

06.025 Nitric Oxide as a target for the hypotensive and vasorelaxing effects induced by (z)-ethyl 12-nitrooxy-octadec-9-enoate in rats. Machado NT[1], Marciel PMP[1], Alustau MC[1], Queiroz TM[1], Furtado FF[2], Silva TAF[1], Vasconcelos WP[1], Santos PC[1], Oliveira-Filho AA[1], Veras RC[1], Araújo IGA[1], Athayde-Filho PF[1], Medeiros IA[1] [1]CCS-UFPB, [2]CFP-ETSC-UFCG

Introduction: The organic nitrates are classified as drugs donors of nitric oxide (NO) that might be useful in the treatment of cardiovascular diseases, mainly to mimick endogenous NO. A new-found organic nitrate, the (z)-ethyl 12-nitrooxy-octadec-9-enoate (NCOE), synthesized from ricinoleic acid from castor oil, was studied in order to evaluate its cardiovascular effects. **Methods**: All protocols were approved by CEUA/UFPB (0304/12). For *in vivo* experiments, wistar rats were anesthetized. Abdominal aorta and inferior vena cava were cannulated for pressure recordings and administration of drugs, respectively. For *in vitro* experiments, isolated rat superior mesenteric rings were suspended by cotton threads for isometric tension recordings in a Tyrode's solution at 37 ºC, gassed with 95% O_2 and 5% CO_2; and primary cultures of rat thoracic aortic smooth muscle cells were obtained by the explant method. **Results and Discussion:** Intravenous injection of NCOE (10, 20, 30, 40 e 60 mg/kg, randomly) produced a dose-dependent hypotension (-2.5±0.9; -3.9±0.7; -31.0±6.6; -40.6±3.9 and -50.4±3.5%) and bradycardia (-5.6±0.9; -8.9±1.0; -57.2±8.9; -70.9±5.3 and -77.9±2.7%). In mesenteric artery rings pre-contracted with phenylephrine (1 µM), NCOE (10^{-10}-10^{-3} M) induced a concentration-dependent vasorelaxation in presence (MR=107.3±4.43%; pD_2=5.59±0.06) or absence (MR=118.0±3.5%; pD_2=5.90±0.05) of endothelium. The pre-incubation with PTIO (300 µM), a free radical form of NO ($NO^{\bullet}$) scavenger, attenuated the NCOE vasorelaxation potency (pD_2=5.10±0.05). However, in the presence of L-cysteine (3 mM), a reduced form of NO (NO^{-}) scavenger, NCOE response was potentiated (pD_2=6.34±0.03). The NCOE effect was not changed in the presence of proadifen (10 µM), an inhibitor of cytochrome P450 (MR=119.0±7.64%) and was attenuated in the presence of cyanamide (1 mM), a mitochondrial aldehyde dehydrogenase inhibitor (mtALDH) (MR=94.3±6.26%). The vasodilation was also reduced in the presence of ODQ (10 µM), a soluble guanylyl cyclase inhibitor (sGC) (MR=55.2±3.60%); and TEA (3 mM), a non-selective K^+ channel blocker (MR=107.1±7.09). In addition, the pre-incubation of iberotoxin (100 nM, MR=106.2±1.49%), a BK_{Ca} blocker and glibenclamide (10 µM, pD_2=5.49±0.04), a K_{ATP} blocker, reduced NCOE effects. However, the effect was not modified by 4-aminopyridine (1 mM), a K_V blocker (MR=116.5±4.63%). Furthermore, NCOE increased NO levels in rat aortic smooth muscle cells, detected by NO-sensitive probe DAF-2T by flow cytometry. These results together suggest that NCOE induces short-lasting hypotension and bradycardia, and promotes vasorelaxation due to $NO^{\bullet}$ release through the compound metabolism via mtALDH and consequent sGC, K_{ATP} and KB_{Ca} activation. **Keywords**: Organic nitrate, nitric oxide, mesenteric artery, vasorelaxation. **Financial support:** CNPq and CAPES

06.026 ***In vivo*** **effects of spironolactone and eplerenone on the cardiac ischemia of rats**.
Amancio GCS[1], Alvarenga AC, Guimarães AG[2], Isoldi MC[1] [1]UFOP – Ciências Biológicas, [2]Cipharma-UFOP

The increasing plasma levels of aldosterona after myocardial infarction (MI) is related to its prognostic aggravation and contributes to the cardiac failure pathogenesis. The MI treatment with the mineralocorticoid receptors (MR) antagonists, spironolactone and eplerenone, can reduce the left ventricular remodeling and sudden death occurrence. However, clinical and experimental evidences demonstrated that this cardioprotection could be in part independent of the aldosterone inhibition. The mechanisms activated by these two drugs in the heart remain unknown. Spironolactone also presents glucocorticoid receptors (GR) affinity, which also shows cardioprotective properties. The objective of the present work was to evaluate the cardioprotective effects of spironolactone and eplerenone in a model of acute cardiac ischemia in rats. All the procedures were approved by the CEUA/UFOP (nº 2011/88).Male Wistar rats were first subjected to adrenalectomy and received by oral route 20 mg/kg of spironolactone or 10 mg/kg eplerenone in the presence and in the absence of mifepristone (20 mg/kg), a GR receptor antagonist. The ECG signal was obtained in anaesthetized rats before and after the left coronary ligature, during 1 hour. The area under the curve of the ST segment of ECG, a parameter that indicates myocardial ischemia, was significantly smaller for the spironolactone (53.9.3±13.07) and eplerenone (31.3±10.08) treated animals compared to the control animals (179.4 ± 32.3). Mifepristone was not able to alter the effects of spironolactone. These results suggest that low doses of spironolactone and eplerenone are cardioprotective even when the aldosterone levels are not augmented and that this effect is not dependent of GR receptors activation. Keywords: Espironolactone, Eplerenone, cardioprotection, adrenalectomy. Apoio Financeiro: FAPEMIG, UFOP

06.027 Involvement of nitric oxide and oxidative stress in the modulation of blood pressure observed in late pregnancy of spontaneously hypertensive rats (SHR). Zancheta D[1], Souza GDS[2], Alves GA[1], Costa TCP[1], Antoniali C[1] [1]FOA-UNESP-Araçatuba – Basic Sciences, [2]FMRP-USP – Physiology

A decrease in blood pressure associated with reduced sympathetic perivascular modulation and *in vivo* responses to vasoconstrictor agonists were observed at the end of pregnancy in rats. These effects have been associated to the greater modulation of nitric oxide (NO) on vascular reactivity. Oxidative stress (OS) is taken a central role in the pathophysiology of hypertensive complications by direct action on the vascular smooth muscle or even by reducing the bioavailability of NO. Therefore, the objective of our study was to evaluate the role of NO and OS on the blood pressure of pregnant hypertensive rats (SHR) and on the cardiovascular responses to *in vivo* administration of vasoconstrictor and vasodilator agonists. Cannulas were inserted into the abdominal aorta of estrus (E) and pregnant (P, 20th day of pregnancy) rats, through the right femoral artery for recording of mean arterial pressure (MAP) and femoral vein for administration drugs. MAP and HF(heart frequency) were recorded before (basal) and after intravenous administration of Tempol (30mg/kg), an SOD mimetic and L-NAME (10 mg/kg), a nitric oxide synthase (eNOS) inhibitor. The *in vivo* effects of phenylephrine (PHE) - (8 mg / kg), SNP (30 mg / kg) and acetylcholine (ACh 10mg / kg) were calculated as the delta of change in MAP. The results (mean±SEM) were compared between groups (Student's t test, $p < 0,05$). Our results showed that the basal MAP values of PSHR (111.8 ± 1.3mmHg) was lower than ESHR (160.1 ± 2.9 mmHg, n=5). Tempol reduced the MAP in ESHR (137.19 ± 1.41 mmHg, n=5), however in PSHR, Tempol did not alter MAP (100.6 ± 2.7mmHg, n=5). There was an increase in MAP after L-NAME in ESHR (188.2 ± 1.08 mmHg, n=5), as well as in PSHR (152.5 ± 3.3mmHg, n=5). PSHR was more sensitive to the L-NAME effect. HF was reduced at the end of pregnancy in SHR (ESHR: 388.6±3.6x PSHR: 375.2±1.6bpm, n=5). There was an increased response to Phe in PSHR (34.1±1.6 mmHg) than in ESHR(25±1.1 mmHg) but, the HF was not different between the groups (ESHR:-50±0.9 x PSHR: -54±1.6bpm). We observed reduced vasodilator response to SNP in PSHR (-48.4±1.6 mmHg, 109±1.6bpm) than in ESHR (-85.2 ± 5.3 mmHg; 120±1.05 bpm).The vasodilator response to ACh was also reduced in PSHR (-54.3±2.3mmHg; 61.02±1.4bpm) than in ESHR (-98.6±2.4 mmHg; 100±1.1bpm). In conclusion, our results demonstrate that pregnancy blunted MAP and HF in SHR. These effects are associated to a reduced OS and to an increased NO production in P SHR. However, the vasodilators responses to the endothelial-dependent and independent drugs were reduced and the Phe response was increased in P SHR. These results suggest that in pregnant SHR, the cardiovascular mechanisms involved in the control of MAP after drugs administrations were not influenced by the increased NO production. Approved by the local Ethics Committee (CEEA-FOA - 1495/2012.). **Financial Support**: CAPES, FAPESP (Proc. 2012/20398-6)

06.028 Hydrogen peroxide activates the endothelial enzymes NO-synthase (eNOS) and cyclooxigenase (COX) in renal hypertensive rat aorta. Silva BR[1], Grando MD[2], Bendhack LM[2] [1]FMRP-USP – Pharmacology, [2]FCFRP-USP – Physic and Chemistry

The endothelium plays an important role on the vascular tone control. In cardiovascular diseases such as hypertension, the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) induced by contractile agonists is increased in blood vessels, mainly in the endothelial cells. This study aimed to evaluate the role of H_2O_2 on the endothelial enzymes eNOS and COX to the contraction induced by phenylephrine (PE) in isolated aorta from renal hypertensive rat (2K-1C) as compared to normotensive sham-operated rat (2K). Concentration-effect curves for PE were constructed in denuded rat aorta (E-) and in intact endothelium rat aorta (E+), in the absence or in the presence of the non-selective inhibitors to eNOS (L-NAME, 100 μM) or COX (Indomethacin, INDO 10 μM). The potency (pD_2) and efficacy (ME) of PE in inducing contraction were evaluated. Concentration-effect curves for H_2O_2 were performed in E+ and in E- aortas contracted with 0.1 μM PE in absence or presence of L-NAME or INDO. In organ chamber, aortas (E+) and (E-) were stimulated or not (basal) with 0.1 μM PE and H_2O_2 production was measured using Amplex red H_2O_2 assay kit. The expression of COX isoforms and Ser^{1177} phosphorylated eNOS were evaluated by Western Blotting. The production of prostanoids thromboxane A_2 and prostacyclin stimulated with PE was measured by enzyme immuneassay. This study was approved by the Ethics Committee of the University of São Paulo (156/2009). The contractile response induced by PE was not different between aortas E- from 2K and 2K-1C. However, the contractile response induced by PE was less potent in aortas E+ than in E-, in both 2K and 2K-1C. In aortas E+, the ME was decreased only in 2K-1C (2K: 2.2±0.1g; pD_2:7.44±0.03; n=5 e 2K-1C: 1.2±0.2g, n=7; $p<0.001$). L-NAME abolished the decreased contraction induced by PE in 2K-1C E+. INDO decreased the ME only in aortas E+ 2R (1.4±0.3g, n=4; $p<0.001$). In both 2K and 2K-1C, the basal H_2O_2 production was higher in aortas E+ than in aortas E-. However, PE stimulated higher H_2O_2 production in 2K-1C (E+ or E-) as compared with 2K (E+ or E-), respectively. In low concentration (< 1 μM), exogenous H_2O_2 induces relaxation in endothelium- dependent way only in 2K, which response is abolished by L-NAME. In high concentration (>1 μM), exogenous H_2O_2 induces higher endothelium- dependent contraction in 2K-1C than in 2K that was abolished by INDO. COX isoforms (COX1 and COX2) are more expressed in 2K-1C than in 2K aortas. PE induces higher Ser^{1177} phosphorylation of eNOS and prostanoid production in 2K-1C than in 2K aorta. Our results indicate that although PE stimulates high production of contractile prostanoids, the reduced contraction induced by PE in intact endothelium aorta from 2K-1C is due to high H_2O_2 production in the endothelial cells and eNOS-Ser^{1177} phosphorylation. Supported by FAPESP and CNPq.

06.029 Use of ruthenium compounds as a Nitric Oxide scavenger in rat aortic rings: **Restoration of vascular tone**. Moura AL[1], Roveda Jr AC[2], Franco DW[2], Tfouni E[3], Cespedes IC[1], Spadari RC[1] [1]Unifesp, [2]IQSC-UNESP, [3]IQRP-UNESP

Introduction: The overproduction of nitric oxide (NO) that occurs in some circumstances (for example, septicemia) is implicated in lowering of the blood pressure that usually is less responsive to conventional therapy with adrenaline and vasopressin. Thus, new therapeutic approaches such as the use of specific, high affinity NO scavengers are of great relevance. Accordingly, some new compounds with this putative property have been developed. **Objectives:** To evaluate the NO scavenging activity of a series of newly synthesized ruthenium compounds. **Methods:** Aorta rings (3 - 4 mm each) were obtained from the thoracic segment of the aorta of adult male Wistar rats and suspended under 60% of maximal tension in an organ bath (Radnoti, Monrovia, CA, USA) containing 15 mL Krebs-Henseleit solution of the following mM composition: NaCl 115.0, KCl 4.6, $CaCl_2.2H_2O$ 2.5, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 2.5, $NaHCO_3$ 25.0, glucose 11.0 and ascorbic acid 0.1; at 36.5 ± 0.1ºC, pH 7.2-7.4; continuously gassed with 95% O_2 and 5% CO_2. The isometric tension was recorded by an Acquisition System controlled by Power Lab Software (ADInstruments do Brasil, São Paulo, SP). After a 60 min stabilization period, maximum contraction of the aortic rings was obtained in response to noradrenaline. After that, acetylcholine was added in order to obtain maximal relaxation. Then, cumulative concentration-response curves were obtained to the following ruthenium compounds: trans-$[Ru(NH_3)_4(SO_4)(4\text{-picoline})](BF_4)$ **(Ru-pic)**; trans-$[Ru(NH_3)_4(SO_4)(\text{nicotinamida})](BF_4)$ **(Ru-ina)**; trans-$[Ru(NH_3)_4(SO_4)$ (isonicotina mide)] (BF_4) **(Ru-isn)**; $[Ru(NH_3)_5Cl]Cl_2$ (Ru-Cl); [Ru(edta)Cl] **(Ru-EDTA)**; cis-$[Ru(NH_3)_4\text{oxalato}](TFMS)_3$**(Ru-cis-Ox)**; cis-$[Ru(NH_3)_4(H_2O)_2(TFMS)_3$**(Ru-cis-aquo)**and trans$[Ru(NH_3)_5(H_2O)(TFMS)_3$ **(Ru-trans-aquo)**. The same procedure was performed in the presence of L-NAME, an inhibitor of nitric oxide synthase. Control rings were not exposed to any of the compounds to be tested. The protocols were approved by the "Comitê de Ética em Pesquisa" of UNIFESP (CEP 079/10). **Results:** The mean ± sem of the tension developed by the tissues (gf) following exposition to noradrenaline was: 1.94 ± 0.17. Acetylcholine reduced the developed tension to 0.17 ± 0.04. In the presence of the compounds the developed tensions were: 1.68 ± 0.19 (Ru-pic; n=5); 1.43 ± 0.15 (Ru-isn; n=5); 1.47 ± 0.16 (Ru-ina; n=5); 1.74 ± 0.13 (Ru-EDTA; n=5); 2.93 ± 0.24 (Ru-Cl; n=5); 2.85 ± 0.07 (Ru-cis-Ox; n=5); 3.08 ± 0.15 (Ru-cis-aquo; n=5); 3.12 ± 0.12 (Ru-trans-aquo; n=5); [$p<0.05$ compared to control]. All the analyzed compounds were able to cancel the relaxation effect of acetylcholine and had similar potencies (pD_2; mean ± sem): 5.60 ± 0.18 (Ru-pic); 5.34 ± 0.25 (Ru-isn); 5.40 ± 0.27 (Ru-ina); 5.48 ± 0.23 (Ru-Cl); 6.45 ± 0.39 (Ru-EDTA); 5.19 ± 0.30 (Ru-cis-Ox); 5.59 ± 0.24 (Ru-cis-aquo); 5.03 ± 0.37 (Ru-trans-aquo). None of the effects of acetylcholine or the compounds was observed when they were tested in the presence of L-NAME. **Conclusion**: It is concluded that all tested compounds were able to scavenge NO in rat aortic rings and to restore the vascular tone. **Financial support**: FAPESP and CNPq

06.030 Atorvastatin downregulates vascular Nox expression and ameliorates oxidative stress-associated inflammatory process in type 2 diabetic db/db mice. Bruder-Nascimento T[1], Callera G[2], Montezano A[3], He Y[2], Antunes T[2], Cat AND[3], Tostes RC[1], Touyz RM[3] [1]FMRP-USP – Pharmacology, [2]University of Ottawa, [3]University of Glasgow – Cardiovascular and Medical Sciences

Introduction: Nox-derived reactive oxygen species generation plays a role in endothelial dysfunction and vascular inflammation, which underline vascular damage observed in diabetes. Increasing evidence indicates that the beneficial effects of atorvastatin are associated with antioxidant mechanisms. We tested the hypothesis that atorvastatin influences Nox isoforms expression and ameliorates diabetes-associated vascular inflammation and redox signaling. **Methods:** The present study was approved by the COBEA (nº 062/2012). Diabetic mice (db/db model of obesity and diabetes type II) and their control counterparts (db/+) were treated with atorvastatin (10 mg/kg/day, p.o., 2 weeks). **Results:** No differences were observed in BP among the groups. Improved oral glucose tolerance was observed in atorvastatin-treated db/db group (area under curve, db/db: 2858 ± 81 vs db/db atorvastatin: 2251 ± 158). Atorvastatin lowered plasma thiobarbituric acid-reacting substances levels in db/db mice (db/+: 4,7 ± 0,7 ;db/db: 6,5 ± 1,1 vs db/db atorvastatin: 4,9 ± 0,5). Increased expression of Nox1, 2 and 4 (1 to 3 fold increase vs db/+) and the associated enhance of ROS generation in the vasculature of db/db mice (db/+: 4312 ± 874; db/db: 27828 ± 8215 vs db/db atorvastatin: 8742 ± 2393 RLU) were abrogated by atorvastatin. The increase of vascular p47phox and RAC1/2 membrane translocation observed in db/db mice was also inhibited by atorvastatin. Diabetes-associated increase of VCAM-1 expression, NFkB p65 phosphorylation, and vascular monocyte adhesion were reduced by aotrvastatin treatment. Higher MAP kinase phophorylation levels observed in db/db mice were blunted by atorvastatin. Impaired vascular responses to acetylcholine (maxium effect %; db/+: 83 ± 5,1 ;db/db: 48 ± 6,4 vs db/db atorvastatin: 75 ± 5,2) and insulin (maxium effect %; db/+: 103 ± 7,5 ;db/db: 47 ± 2,0 vs db/db atorvastatin: 95 ± 7,1) in db/db mice were normalized by atorvastatin treatment. **Discussion:** Our results demontrate that atorvastatin ameliorates oxidative stress and inflammatory process in db/db mice. Findings from this study identify NADPH oxidase as a target for atorvastatin. We describe novel mechanisms whereby statins may improve vascular function in obesity-associated diabetes. Financial support: FAPESP (10/52214-6; 2011/01785-6; 2011/22035-5); AUF (550738)

06.031 Acute modulatory effect of atorvastatin on nNOS/H_2O_2 pathway in aorta from normolipidemic mice. Mota GPC[1], Pelaez JMN[1], Lemos VS[2], Cortes SF[1], Capettini LSA[1] [1]ICB-UFMG – Pharmacology, [2]ICB-UFMG – Physiology and Biophysics

Introduction: Statins are among the most prescribed drugs in clinical practice, they are commonly used for the treatment of hypercholesterolemia as they reduce the cholesterol biosynthesis by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Several pleiotropic effects have been attributed to atorvastatin, specifically to the increase synthesis and/or bioavailability of the endothelium-derived nitric oxide (NO). NO is essential for regulating vascular tone and blood pressure. Classically, NO is produced by vascular endothelial NO synthase (eNOS). However, our research group recently demonstrated that hydrogen peroxide (H_2O_2) produced by neuronal NOS (nNOS) in endothelial cells is a major endothelium-derived relaxing factor. The purpose of this study is to evaluate the acute effect of atorvastatin on the nNOS/ H_2O_2 pathway in normolipidemic mouse aorta. **Methods:** Studies of vascular reactivity were performed using aorta thoracic rings of C57BL/6J male mice, 12 weeks old (approval CETEA-UFMG 0076/12). In a serie of experiments, aortic rings were pre-incubated with atorvastatin (1 and 10 μM) for 2 hours and then curves were performed with phenylephrine or acetylcholine (1nM to 100 μM) in the presence or absence of N^w -nitro-L-arginine methyl ester (L-NAME, 300 μM; non-selective inhibitor of NOS), 1 - [2 - (trifluoromethyl) phenyl] imidazole (TRIM, 300 μM; selective inhibitor of nNOS) or the enzyme Catalase (2400U/ml, which degrades H_2O_2). By Flow Cytometry, using endothelial cell (Eahy.296) was measured nitric oxide and hydrogen peroxide through the probes DCF and DAF. **Results and Discussion:** The incubation for 2 hours with atorvastatin had no effect in the relaxation induced by acetylcholine (E_{max}: 85.6±3.7; 76.5±4.7; 81.8±2.5 mN/mm; for control, atorvastatin 1 and 10μM, respectively). However, 2 h incubation with 1 and 10 μM, significantly reduced phenylephrine-induced constriction (E_{max}: 5.4±0.1; 3.6±0.1; 3.2±0.1 mN/mm; for control, atorvastatin 1 and 10μM, respectively), but not in the absence of endothelium. The non-selective inhibitor of NOS, L-NAME, restored the contraction induced by phenylephrine in vessels pretreated with atorvastatin, at all concentrations used (E_{max}: 6.7±0.3; 6.8±0.3; 7.0±0.2 mN/mm; for control, atorvastatin 1 and 10μM, respectively). Selective inhibition of nNOS with TRIM also restored the contraction induced by phenylephrine in vessels pretreated with atorvastatin (E_{max}: 5.3±0.2; 5.0± 0.2; 4.7±0.2 mN/mm; for control, atorvastatin 1 and 10μM, respectively) suggesting that atorvastatin could activate nNOS. Finally, reduction of H_2O_2bioavailability with Catalase also restored the contraction induced by phenylephrine in vessels pretreated with atorvastatin (E_{max}: 5.0±0.2; 4.8±0.3; 4.5±0.2 mN/mm; for control, atorvastatin 1 and 10μM, respectively), suggesting that atorvastatin increases the synthesis of H_2O_2. Flow Cytometry experiments showed that pretreatment (2h) of Eahy.296 cells with atorvastatin directly increased the production of NO and H_2O_2. In the present study, we have demonstrated that incubation with atorvastatin can directly modulate the contractility of mouse aorta via nNOS-dependent activation. **Financial Support:** Fapemig, CNPq, PRPq/UFMG, Capes.

06.032 Effects of taurine supplementation upon adiposity, glucose homeostasis and vascular reactivity in MSG rats. Leão VF, Faria PP, Ferreira LLDM, Raimundo JM, Ribeiro RA
UFRJ

Introduction: Taurine (Tau) is an amino acid presentin high levels in mammalian tissues, which regulates cardiovascular function[1]. Ours studies demonstrated that Tau improves liver glucose control[2]and ameliorates plasma lipid profile in obese rodents[3]. Here, we evaluated the glucose homeostasis and vascular reactivity in monosodium glutamate (MSG)-obese rats supplemented with Tau. **Methods:** Male Wistar rats received subcutaneous injections of MSG [4g/kg body weight (BW), MSG group] or saline [1.25 g/kg BW, control (CTL)] during the first 5 days of life. At 21 days, rats were distributed into the groups: CTL, MSG and MSG supplemented with 2.5% Tau in their drinking water (MTAU). Glucose tolerance test (GTT) and blood biochemistry assays were evaluated at 90 and 150 days of life. At 150 days of age, rats were weighted and euthanized. Adipose tissue was removed and weighted. Thoracic aorta rings were isolated and prepared for isometric tension recording. Concentration-response curves to phenylephrine (Phe; 10^{-9} to 10^{-5}M) and to acetylcholine (ACh; 10^{-9} to 10^{-5} M) were obtained. All experiments were approved by the UFRJ's Committee on Ethics in Animal Experimentation (license number MACAÉ05). **Results:** MSG rats developed obesity, with increased retroperitoneal (39.0±8.0 mg/g BW) and perigonadal fat pads (31.0 ± 6.0 mg/g BW), and scapular brown adipose tissue (2.4±0.3 mg/g BW) compared to CTL (12.7±1.4, 7.2±1.5 and 1.0±0.1 mg/g BW, respectively). Tau supplementation reduced by 39 and 22% the retroperitoneal and perigonadal fat deposition in MTAU (23.9±1.6 and 23.8±2.8 mg/g BW, respectively), when compared to MSG. In the GTT, total glycemia did not differ between MSG (19268±293.6mg/dL.min^{-1}) and CTL rats (21103±4124mg/dL.min^{-1}). In addition, at 90 days, fed MSG rats showed hypertriglyceridemia (145.0±14.0 mg/dL) and hyperinsulinemia (4.6±0.7ng/mL) compared to CTL (96.0±7.0 mg/dL and 1.2±0.3 ng/mL; $P<0.01$). Tau treatment normalized plasma triglycerides and partially reduced insulinemia(97±9 mg/dL and 3.2±0.9 ng/mL, respectively). The vasoconstrictor response to Phe was similar in all groups (At 10 mMPhe: CTL1.0±0.1, MSG 1.1±0.1 andMTAU 1.1 ± 0.3 g; $P>0.05$). However, the maximal vasodilatory response to Ach (10 mM) was reduced in aortas from MSG (40.6±7.8%) when compared to CTL group(77.2±3.5%; $P<0.05$). Tau supplementation restored the reduced vascular response to Ach observed in MSG, with maximal relaxation to Ach of 75.1±3.0%. **Discussion:** Tau had beneficial effects on MSG rats improving the lipid profile, reducing the fat deposition and partially correcting the hyperinsulinemia. Also, Tau treatment corrected the lower response of aorta to Ach. Similar results have been observed in SHR rats and in cholesterol-fed and streptozotocin-induced diabetic rodents[1,4]. The improvement in vascular function induced by Tau may be associated to a reduction of oxidative stress due to its antioxidant activity and to its direct vasodilator effect with increased nitric oxide release[4,5]. **References:** [1]Azuma J. AdvExp Med Biol 643:37, 2009; [2]Batista TM. Mol NutrFood Res 57:423, 2013. [3]Nardelli TR. Amino Acids 41:901, 2011. [4]Abebe W. Am J CardiovascDis 1:293, 2011.[5]Niu L. Eur J Pharmacol 580:169, 2008. **Financial Support:** FAPERJ, CNPq and PIBIC-UFRJ.

06.033 High-salt (4%) plus high-fructose (6%) diet reduces the vascular reactivity to vasocontrictor *in vitro* but increases the systemic arterial pressure in rats. da Silva RCVAF[1], Souza P[1], da Silva-Santos JE[2] [1]UFPR, [2]UFSC

Introduction: Hypertension is a leading cause of mortality and morbidity in the world. Genetic and lifestyle factors are involved in this pathology. Nowadays diet is one of the major lifestyle factors that contribute to the development of hypertension. The purpose of this study was evaluate how the excessive intake of both sodium and fructose can modulate the cardiovascular function and contributes to the development of cardiovascular diseases. **Methods:** All procedures were approved by the Institutional Ethics Committee of UFPR (protocol number 601). Weaned (21 days old) male *Wistar* rats were divided in 4 groups: i) control group (CT), feed with regular chow; ii) high-salt group (HS), feed with chow containing 4% NaCl; iii) high-fructose group (HF), feed with chow containing 6% fructose, and iv) high-salt-fructose group (HS-F), feed with chow containing both 4% NaCl and 6% fructose. After six weeks under these treatments, the animals were anesthetized and prepared for direct measurement of systemic arterial pressure. In addition, the reactivity of isolated aortic rings and perfused mesenteric vascular bed (MBV) to vasoactive agents was evaluated. Parameters as food ingestion and water consumption, as well as urinary output, were accompanied weekly during the period of treatment. **Results and Discussion:** No differences were observed in weight gain and food intake between the experimental groups. However, both the ingestion of water and the diuresis were significantly higher in HS and HS-F groups than in CT rats. The HS-F group presented increased mean arterial pressure (MAP), when compared with the CT group (117.6 ± 4.2 mm Hg and 91.6 ± 3.3 mm Hg, respectively; $p < 0.05$). The vascular reactivity to vasopressin (AVP) was reduced in both aortic rings and perfused MVB obtained from HS, HF, and HS-F groups, when compared with the CT group. Indeed, AVP (30 and 100 pmol) had its effects in the perfusion pressure of the MVB reduced by 37 and 62%, respectively, in the HS-F group, when compared with CT. Resembling the reactivity to AVP, the MVB from both HF and HS-F (but not HS) groups presented reduced responses (~60%) to phenylephrine (PE, 0,3, 1 and 30 µmol). In addition, when compared with the CT group, the high-fructose diet reduced about 48% the maximum contraction induced by PE in endothelium-intact aortic rings, an effect fully reversed by L-NAME (a nitric oxide synthase inhibitor, 100 µM). PE-induced contraction was not impaired in aortic rings obtained from HS and HS-F groups. The present study indicates that the association of high amounts of sodium and fructose in the diet can raise the systemic arterial pressure, in spite of the reduced vascular reactivity to vasoconstrictors found in isolated vascular preparations, such as the aorta and the mesenteric vascular bed. Additional experiments are being carried out in order to clarify the mechanisms involved in our findings, as well as their role in the genesis of cardiovascular disorders. This study is supported by CAPES, with a scholarship grant to RCVAFS.

06.034 Endothelium abolishes the vasoconstriction induced by the calcium Ionophore A23187 in renal hypertensive (2K-1C) rat aorta. Feitoza PR[1], Silva BR[2], Bendhack LM[1]
[1]FCFRP-USP – Físico-Química, [2]FMRP-USP – Farmacologia

The vascular endothelium plays physiological role in the control of vascular tone by releasing endothelium-derived relaxing factors (EDRFs) and endothelium-derived contractile factors (EDCFs). Endothelial dysfunction that is characterized by reduced production and bioavailability of nitric oxide (NO) is present in several cardiovascular diseases such as hypertension. The cation-specific ionophore (A23187) is a useful tool to study the role of intracellular Ca^{+2}. The plasma membrane is normally highly impermeable to Ca^{+2}. The ionophore A23187 acts on Ca^{+2} ions forming a soluble complex that dissociates into the cytosol, increasing the intracellular concentration of Ca^{+2}. This study aimed to verify the effect of the influx of Ca^{+2} induced by A23187, on contraction and relaxation endothelium-dependent in aorta of renal hypertensive rats and to evaluate the effect of A23187 on cytosolic concentration of NO ([NO]c) in endothelial cells isolated. We performed concentration-effect curves for the ionophore A23187 (0.1 nM to 10 μM) in aortic rings with (E+) and without endothelium (E-) hypertensive rats (2K-1C) and their respective control normotensive (2K) under previous contraction with phenylephrine (EC_{50}) or under basal tension to verify the vasorelaxation and vasoconstriction. We analyzed the efficacy values (ME, g tension). It was quantified [NO]c in endothelial cells isolated from rat aorta in the presence and absence of A23187. The experimental procedures were approved by the Ethics Committee (CEUA 2012.1.120.53.0). The compound A23187 induced vascular contraction at the concentration of 10 μM in the aortas of 2K rats, which was endothelium-independent (E+ ME: 0.42±0.18g, n=6 and E- ME: 0.45±0.19g, n=5;), but in the aortas of rats 2K-1C the contraction was abolished in the presence of endothelium (E- ME: 0.54±0.06g, n=3 and E+ ME: 0.05±0.04g, n=3, $P<0.05$). The A23187 was able to promote vascular relaxation in a concentration dependent manner in rat aortas 2K (E+: 58.3±18.7g, n=5) and 2K-1C (E+/ME: 61.4 ± 6.1g, n=5). This effect was reduced in aortas of 2K-1C (E- ME: 30.8 ± 8.5g, n=3, $P<0.05$) and 2K (E- /ME: 19.8 ± 16.2g, n=5, $P<0.05$) when compared to E+ aortas. In aortas E+ 2K, ME relaxation was followed by a contractile response in the higher concentrations, probably due to the influx of Ca^{2+} into smooth muscle cells, that was not reproduced in aortic E+ 2K-1C, where vascular relaxation was maintained in all concentrations. The fluorescence intensity to DAF-2DA of endothelial cells was slightly increased when stimulated with A23187, indicating that there was an increase in NO production. Our results demonstrate that the Ca^{2+} ionophore (A23187) induced endothelium-dependent vascular relaxation by NO production. At high concentrations, A23187 induced relaxation and vasoconstriction in the aortas of rats 2K but not 2K-1C. Supported by FAPESP and CNPq.

06.035

USE OF NEW SELECTIVE ORAI AND TRPC3 BLOCKERS FOR RE-INVESTIGATING THE CALCIUM PERMEABLE CHANNELS INVOLVED IN ENDOTHELIUM-DEPENDENT RELAXATIONS.

Silva JDP[1], GONÇAVES MS[1], Alves Filho FC[1], GROSCHNER K[2], Ballejo G[1] - [1]FMRP-USP - Pharmacology, [2]MedUniv Graz, Austria - Institute of Biophysics

Introduction: Calcium influx in endothelial cells (ECs) is required for the production of endothelium-dependent relaxations (EDRs). However, the channel (s) involved in this influx and whether distinct extracellular Ca^{2+}-dependent stimuli of ECs involve the same or a different set of channels remain unknown. The aim of this study was to re-investigate this phenomenon by utilizing the recently described *pyrazole* derivatives which cause selective blockade of the Ca^{2+} permeable channels ORAI (BTP2; Pyr6) and TRPC3 (Pyr3; Pyr10).

Methods: Rat, rabbit and guinea-pig thoracic aortic rings (*AoR*) and right porcine coronary artery (PCA) were suspended in an isolated organ bath for recording isometric contractions evoked by phenylephrine (PE, 100nM, aortic rings) or U46619 (15-60nM, PCA). Procedures were approved by CETEA-USP (084/2011).

Results: In **rat** *AoR*, acetylcholine (ACh, 1μM), thapsigargin (TG, 30nM) and GSK101 (GSK, 3nM; TRPV4 agonist) caused relaxations strictly endothelium dependent; the onset of these responses was rapid for ACh/TG (<30s) and slower for GSK (>2min); and they reached a steady state within 4-6min which persisted for at least 10min. In contrast, in nominally calcium-free medium, ACh/TG caused transient relaxations of reduced magnitude, whereas the effect of GSK was abolished. Ruthenium red (**RR**, 10μM, a non-selective TRP blocker) reverted the relaxations caused by ACh (*dT/dt*=1.46±0.06mg/s, n=3) slower than those caused by GSK (*dT/dt*=8.6mg/s, n=2). The magnitude of ACh-elicited relaxation was reduced in rat *AoR* pre-incubated with RR (3-30μM, 5min), but it was not altered by HC-067047 (3μM, selective TRPV4 blocker). **BTP2** and **Pyr6** (both at 1-3μM) completely reverted the ACh- (n=7) or TG- (n=5) induced relaxations; **Pyr3** (0.3-3μM) reverted the relaxations evoked by ACh faster (*dT/dt*=5.1±0.1mg/s; n=5) than those evoked by TG (*dT/dt*=3.1±0.26mg/s; n=3). Similar results were observed in rabbit *AoR*. Furthermore, in rat (n=5) and rabbit (n=4) *AoR* pre-incubated (5min) with BTP2 (3μM) or Pyr6 (1μM), ACh and TG produced transient relaxations of reduced magnitude. In addition, BTP2 or Pyr3 (1-3μM) fully reverted the EDRs caused by A23187 (100nM) in rat and guinea-pig *AoR*. The effect of GSK was unaffected by BTP2, Pyr3 or Pyr6 in any artery. In **PCA**, endothelium- and calcium-dependent relaxation elicited by bradykinin (BK, 100nM) was reverted by Pyr3 (1-3μM) or Pyr6 (3μM) and became transient after 5min pre-incubation with Pyr6 (3μM). Intriguingly, the selective TRPC3 blocker **Pyr10** (1-3μM) did not affect the relaxations caused by any of the agents in any of the arteries studied. Finally, BTP2, Pyr3 or Pyr6 (1-3μM) affected neither the PE/U46619-induced contractions nor relaxations caused by sodium nitroprusside (100nM).

Discussion: These observations indicate that BTP2, Pyr6 and Pyr3 (ORAI and TRPC other than TRPC3) sensitive Ca^{2+}-permeable channels are involved in the calcium influx elicited by ACh, TG, A23187 or BK required for the production and release of relaxing factors by ECs. Furthermore, these channels do not appear to be involved in smooth muscle contraction stimulated by PE/U46619. Finally, they indicate that BTP2, Pyr6 and Pyr3 do not affect endothelial TRPV4 channels.

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06.036

HIGH SALT INTAKE IMPAIRS THE REPONSIVENESS TO DIURETIC DRUGS IN RATS

Crestani S[1], Souza P[2], Silva RCVAF[2], Marques MCA[2], Gasparotto Junior A[3], Cosmo MLA[3], Victório JC[3], Santos JES[1] - [1]Universidade Federal de Santa Catarina - Farmacologia, [2]Universidade Federal do Paraná - Farmacologia, [3]Universidade Paranaense - Farmacologia

Introduction: The cardiovascular diseases, mainly hypertension, constitute one of the major causes of death worldwide (WHO, 2013). Clinical strategies to achieve a lowering of blood pressure include the use of diuretics drugs (Williams et al 2004). High salt intake is putatively associated with hypertension and cardiovascular diseases (Orlov and Mogin 2007). This study aimed to evaluate the effects of diuretic drugs in rats exposed to high amounts of salt. **Methods:** Male Wistar rats (21 days old) were subjected to chow containing NaCl at 8%, while the control received regular food. Evaluation of diuresis was done weekly and in all experiments the animals received a single administration of physiological saline (0.9% NaCl; 5 mL/100 g) by oral route to impose a uniform water and salt load (Benjumea et al., 2005), and were immediately placed in metabolic cages. The urine was collected in a graduated cylinder and the volume was recorded at intervals of 2 h during 8 h. The urinary output was calculated and expressed as ml/100 g. Electrolyte contents (Na^+, Cl^-, K^+ and HCO_3), pH, density, and conductivity, were estimated at the end of the experiment (8 h). After 6 weeks under the high salt diet, the rats were treated with the following diuretic drugs (v.o.): furosemide (10 mg/kg), spironolactone (50 mg/kg), hydrochlorothiazide (10 mg/kg), amiloride (20 mg/kg), or acetozolamide (10 mg/kg), and all previously described parameters were evaluated. All procedures were approved by the Institutional Ethics Committee of Universidade Federal do Paraná (n. 345). **Results:** The high-salt intake for 6 weeks did not result in any change in the urinary volume, K^+, Cl^- and Na^+excretion, density and conductivity. However, we found a significantly augmented pH and reduced excretion of bicarbonate in the urine of high salt rats. The diuretic effect of furosemide was increased from 4.5 ± 0.2 mL in control to 7.3 ± 0.2 mL in animals subjected to the high salt diet. Similarly, amiloride presented augmented effects in the high salt group (about 30% higher), when compared with control. On the other hand, the diuretic effects of both hydrochlorothiazide and acetazolamide were reduced by 23 and 22%, respectively, in rats exposed to the high salt chow. The administration of acetazolamide normalized the levels of bicarbonate in the urine, while amiloride reduced it (~ 11%). With exception of acetazolamide, all diuretic drugs tested were able to restore the urinary pH. The K^+ and Cl^- excretion in high salt diet rats were increased by acetazolamide. In addition acetazolamide and hydrochlorothiazide elevated the levels of sodium levels by 47 and 40%, respectively, in the urine collect from high salt rats, when compared with control rats. **Discussion**: Our study reveals that long-term exposition to high salt amounts impairs the renal balance, as well as the effects of diuretic drugs. If applied for humans, these results can explain, at least in part, why some patients suffering hypertension do not respond to diuretic agents. Additional experiments are being carried out to investigate the mechanisms involved in these effects.

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06.037

Evaluation of artemether cardiac toxicity

Grabe-Guimarães A[1], Karam S[2], Meschin P[2], Thireau J[2], Mosqueira VCF[1], Richard S[2] - [1]Universidade Federal de Ouro Preto - CiPharma, [2]INSERM U1046, France

Artemether, a drug used in artemisinin-based combination therapy (ATC) is recommended by WHO (2010) to treat uncomplicated *P. falciparum* malaria. It is co-administered with lumefantrine. Artemether has well defined neurotoxicity but its cardiotoxicity is controversial. QT interval prolongation, considered as a marker surrogate of potential arrhythmic events, has been observed experimentally in rats and humans. Considering that Ca^{2+}-dependent arrhythmias can be triggered independently of QT prolongation, calcium (Ca^{2+}) handling is critical to evaluate drugs toxicity. The objective was to verify the effect of artemether on QT interval *in vivo* and to investigate potential effect on Ca^{2+} sparks arising from abnormal ryanodine receptors activity frequently involved in arrhythmias. Methods: The experimental procedures were approved under number UFOP 03/2011. The ECG lead II was obtained before and after artemether single dose treatment (80 mg/kg, I.P.) in conscious and unrestrained moving Swiss mice using a telemetric system, three days after the sensor implantation surgery under anesthesia. Six, 12 or 24 hours or one week after treatment, the mice had the heart rapidly excised via a thoracotomy and submitted to liberase action using a langendorff perfusion system in order to obtain single left ventricular myocytes, suspended with Tyrode solution supplemented with 1 mM $CaCl_2$. Myocytes were then incubated for 17 minutes with fluorescent Ca^{2+} indicator Fluo-4 AM (5 µmol/l). Changes in intracellular Ca^{2+} and spontaneous Ca^{2+} sparks were recorded in line-scan mode with an LSM510 Meta Zeiss confocal microscope (63X water-immersion objective; NA: 1.2). Data were analyzed using ImageJ 1.41 coupled with SparkMaster. Artemether induced significant reduction of mice heart rate (Control = 526 ± 28.0; treated = 383 ± 48.6 bpm) and QT (control = 45 ± 1.4; treated = 65 ± 9.3 ms) and QTc (control = 92 ± 2.9; treated = 118 ± 13.5 ms) intervals, mainly during the first hour following administration (n = 6 per group). In quiescent myocytes, the frequency, number and amplitude of Ca^{2+} sparks were not different between the groups, and this similarity was maintained at all the times evaluated. Conclusion: artemether induced QT prolongation but had no abnormal spontaneous Ca^{2+} release through ryanodine receptors. The involvement of transmembrane ion channels and/or the influence of the autonomic nervous system on the QT prolongation remains to be studied.

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06.038

CHRONIC TREATMENT WITH FLUOXETINE INDUCES OXIDATIVE STRESS AND ALTERS AORTIC CONTRACTION IN RATS

Simplicio JA[1], Resstel LBM[1], Tirapelli CR[2] - [1]Faculty of Medicine of Ribeirão Preto - Department of Pharmacology, [2]College of Nursing of Ribeirão Preto - Laboratory of Pharmacology

Introduction: The selective serotonin reuptake inhibitors (SSRIs) is the class of antidepressive drugs most used in the treatment of depression. Recently, chronic treatment with fluoxetine was shown to increase blood pressure in rats. However, the mechanisms underlying such response are not completely elucidated. **Objectives:** To investigate the effect of chronic administration of fluoxetine on the vascular oxidative stress and reactivity in isolated rat aortas. **Methods:** Male Wistar rats (200-250 g) were divided into four groups: chronic vehicle - CV (21 days of vehicle treatment - saline + 0.2% Tween-80, 1 ml / kg ip), chronic fluoxetine - CF (21 days with fluoxetine treatment - 10 mg / kg ip); acute vehicle - AV (single injection of vehicle ip); acute fluoxetine - AF (single injection of fluoxetine ip). The thoracic aorta was isolated for vascular reactivity assays; evaluation of oxidative stress by lucigenin chemiluminescence and real time PCR analysis of NOS isoforms. All experimental protocols were approved by the Ethical Animal Committee from the Campus of Ribeirão Preto – USP (Protocol: 11.1.1593.53.9). **Results:** Chronic treatment with fluoxetine increased the contraction (grams) induced by phenylephrine (PhE) (1.76 ± 0.11, n=7) compared to CV (1.26 ± 0.12, n=7), AV (1.36 ± 0.08, n= 6) and AF (1.40 ± 0.10, n=7) in aortic rings with intact endothelium (E+). Chronic treatment with fluoxetine also increased the serotonin(5-HT)-induced contraction (grams) in E+ rings (1.67 ± 0.10, n=8) when compared to the other groups: CV (1.21 ± 0.07; n=5), AV (1.39 ± 0.04, n= 6) and AF (1.08 ± 0.08, n=7). There was an increase in KCl-induced contraction in E+ aortic rings from animals chronically treated with fluoxetine. In rings without endothelium (E-), chronic treatment with fluoxetine did not alter the contraction induced by PhE, 5-HT or KCl. There was no change in the relaxation induced by acetylcholine, sodium nitroprusside or L-arginine after treatment with fluoxetine. Incubation of E+ rings with indomethacin (nonselective COX inhibitor) and tiron (superoxide anion scavenger) reversed fluoxetine-induced increase in the contraction of 5-HT and PhE. The chronictreatment with fluoxetine increased aortic oxidative stress (326.02 ± 29.04 RLU/mg protein, n = 6) when compared to CV (229.44 ± 31.41 RLU/mg protein, n=7). There were no changes in the mRNA levels of iNOS, eNOS or nNOS after chronic treatment with fluoxetine. **Conclusions:** Chronic treatment with fluoxetine alters vascular function and increases oxidative stress in the rat aorta. The increased vascular contraction is endothelium-dependent and appears to involve vasoconstrictor prostanoids, superoxide anion generation and alteration of the NO-cGMP pathway. **Financial support and acknowledgments:** Capes, Fapesp.

06.039

REACTIVE OXYGEN SPECIES ARE INVOLVED IN THE VASCULAR RELAXATION INDUCED BY NITRIC OXIDE DONOR IN MESENTERIC RESISTANCE ARTERY FROM 2K-1C RATS

Andrade FA[1], Silva RS[2], Bendhack LM[2] - [1]School of Medicine of Ribeirão Preto - University of São Paulo - Pharmacology, [2]Faculty of Pharmaceutical Sciences of Ribeirão Preto - University of São Paulo - Physics and Chemistry

Introduction: Nitric oxide (NO) is a physiological modulator that plays an important role in the control of vascular tone and blood pressure. Many studies have highlighted the development of compounds that may serve as vehicle to NO release in biological systems, mainly when the endogenous NO bioavailability is impaired such as in hypertension. In this case, the increased production of reactive oxygen species (ROS) is important factor to modulate the vasodilator effect of NO. We hypothesized that the NO donor *cis*-[Ru $(bpy)_2(py)(NO_2)](PF_6)$ (RuBPY) induces relaxation of isolated mesenteric resistance artery from normotensive (2K) and renal hypertensive (2K-1C) rats and this effect may be modulated by ROS. This work aimed to study the vasodilator effect of RuBPY in isolated mesenteric resistance artery from 2K and 2K-1C rats, the time required to promote the maximum relaxation (ME) and to investigate whether the nitrite of the compound is converted to NO. It was also evaluated the involvement of superoxide (O_2^-) and other ROS in this effect. **Methods:** This study was approved by the Ethics Committee of USP (CETEA-Protocol n° 044/2008). We performed amperometric measurements of NO in mesenteric arteries using an electrode sensitive to NO during the study of the temporal effect of RuBPY to promote the ME. Concentration-effect curves for RuBPY (0.01nM-10 μM) were constructed in mesenteric rings contracted with phenylephrine without or with the antioxidant/NADPH oxidase inhibitor Apocynin (100 μmol/L), the O_2^- scavenger Tiron (0.1 mmol/L), superoxide dismutase inhibitor DDC (100 μmol/L) or the intracellular catalase mimetic PEG-catalase (300 u/ml), incubated for 30 min. The maximum effect (ME) was analyzed. The data were analyzed by Student's *t* test. **Results:** RuBPY released NO with similar profile in 2K and 2K-1C arteries. The time required to promote the ME was similar in both arteries (80 min). Relaxation induced by RuBPY was similar in 2K (77.6±8.9%, n=7) and 2K-1C arteries (76.9±4.5%, n=13). Apocynin reduced the relaxant effect in 2K-1C arteries (45.7±3.9%, n=6). However, tiron, DDC and PEG-catalase had no effect in RuBPY relaxation in both groups. **Discussion:** These findings suggest that the compound RuBPY induces relaxation of mesenteric resistance arteries from 2K and 2K-1C rats. The results indicate that RuBPY similarly releases NO in both rat artery groups. However, in arteries isolated from 2K-1C rats, the vasodilator effect induced by RuBPY may be positively modulated by ROS possibly other than superoxide anion and hydrogen peroxide. **Financial Support:** FAPESP and CNPq

06.040

REGULATION OF ANGIOTENSIN-II POTENCY BY METABOLITES DERIVED FROM CYCLOOXYGENASES IN CAROTID ARTERY FROM RAT EXPOSED TO REPEATED RESTRAIN STRESS

CÔCO H[1], Gomes MS[2], Pernomian L[2], de Oliveira AM[2] - [1]Faculdade de Medicina de Ribeirão Preto - USP - Farmacologia, [2]Faculdade de Ciências Farmacêuticas de Ribeirão Preto - USP - Farmacologia

Introduction: This work was aimed to study the consequences of repeated restrain stress on the contraction evoked by angiotensin II (Ang II) in the rat carotid arteries, and to investigate the participation of metabolites derived from cyclooxygenase (COX)-1 and COX-2 in the modulation of this response. **Methods:** This study was approved by ***Ethical Commission from Faculty of Medicine from Ribeirão Preto/University of São Paulo (nº 12.1.343.53.0)***. Wistar rats (60 days) were exposed to 3h restraint stress for 5 days, using a plastic tube and experiments were performed on the 5th day. Concentration-response curves for Ang II (10^{-11} to 10^{-6} mol/L) were obtained in endothelium-intact or -denuded carotid rings from control (C) or exposed to restrain stress (RS) rats, in the absence or presence of the selective COX-1 inhibitor, SC-560 (9 nmol/L) or the selective COX-2 inhibitor, SC-236 (10 nmol/L). **Results:** Ang II potency of contraction (pD_2) was augmented in RS rat endothelium-intact carotid (8,19±0,08) when compared to control rat endothelium-intact carotid (7,82±0,06), without changes Emax values (C=0,61±0,05 g/mg; RS=0,69±0,06 g/mg). In RS rat endothelium-denuded carotid, pD_2 for Ang II was increased (8,65±0,15) in relation to control rat endothelium-denuded carotid (8,22±0,07). No changes was observed in Emax for Ang II (C=1,10±0,05 g/mg; RS=1,11±0,08 g/mg). The removal of endothelium increased Emax and pD_2 values for Ang II in control and RS group when compared to endothelium-intact arteries. SC-560 increased pD_2 value from control rat endothelium-intact (8,14±0,03) and denuded carotid (8,58±0,08), without changes in Emax values (endothelium-intact carotid: 0,69±0,05 g/mg; endothelium-denuded carotid: 1,20±0,07 g/mg), in relation to the absence of the inhibitor. In RS rat endothelium-intact carotid, SC-560 did not alter the pD_2 for Ang II (8,00±0,05), but it reduced the Emax value (0,40±0,04 g/mg), when compared to the absence of the inhibitor. SC-560 did not alter the Emax (1,06±0,07 g/mg) nor pD_2 (8,40 ± 0,05) for Ang II in RS rat endothelium-denuded carotid when compared to absence of the inhibitor. SC-236 did not alter the Emax (0,73±0,03 g/mg) nor pD_2 (8,03±0,05) for Ang II in control rat endothelium-intact carotid, but it enhaced the Emax value for Ang II (1,40±0,05 g/mg) in control rat endothelium-denuded carotid, without changes in pD_2 value (8,55±0,07) in relation to the absence of the inhibitor. In RS rat endothelium-intact carotid, SC-236 reduced the pD_2 (7,97±0,02) and the Emax values (0,43±0,05 g/mg) for Ang II when compared to absence of the drug. The addition of SC-236 in RS rat endothelium-denuded carotid did not alter the Emax (1,17±0,08 g/mg) nor pD_2 (8,43±0,05) for Ang II. **Discussion:** In carotid arteries from control rat, it was observed the participation of metabolites derived from COX-1 and COX-2 with vasorelaxant properties and presented in muscle layer. On the other hand, in RS rat carotid arteries, it appears that the participation of endothelium-dependent vasocontraction metabolites derived from COX-1 and COX-2 have lead role. **Financial support:** CAPES, CNPq, FAPESP (2012/00640-7 and 2012/09019-3) and NAP-DIN (11.1.21625.01.0).

06.041

Molecular mechanisms associated with reversion of proteinuria in hypertensive rats treated with RAS inhibitors

Corrêa JWN[1], Girardi ACC[2], Boaro KR[2], Salles T[2], Yogi A[3], Callera GE[3], Touyz RM[3], Bendhack LM[4], Krieger JE[2] - [1]Universidade Federal do Amazonas - Lab. de Farmacologia - Departamento de Ciências Fisiológicas - ICB, [2]Instituto do Coração - HC-FM-USP, [3]Kidney Research Centre - University of Ottawa, [4]Universidade de São Paulo - Faculdade de Ciências Farmacêuticas de Ribeirão Preto

Introduction: Leakage of albumin from the plasma into urine is the first sign of renal injury. Many pathological conditions such as hypertension can lead to proteinuria. This work aimed to evaluate the effects of the blockade of renin angiontensin system (RAS) enalapril and/or losartan on the proteinuria in 2K-1C hypertensive rats. **Methods:** 2K-1C and sham-operated (2K) were induced by surgery in male Wistar rats. After six weeks, rats were treated with losartan (30mg/kg/d), enalapril (20mg/kg/d), losartan+enalapril (20+30mg/kg/d) or saline by gavage for 14 days. The rats were housed in metabolic cages for urine collection and determination of renal function parameters. Plasma and cortical kidney samples were collected in the day of the sacrifice to determine renal function parameters and perform histology and western blott analysis. All the experimental procedures are in accordance to Ethics Committee (CEUA-USP-RP 07.1.607.53.1). **Results:** Systolic blood pressure (SBP, mmHg) was higher in 2K-1C (247±5) than in 2K (129±2, P<0.0001). Losartan (197±6, P<0.05) and enalapril (205±7, n=8, P<0.05) progressively reduced 2K-1C SBP, with an additional hypotensive effect in the group treated with both drugs (173±16mmHg, P<0.001). The kidney hypertrophy index (kidney/tibia lenght) was higher in the right kidney of 2K-1C compared to 2K (0.61±0.04 vs 0.41±0.01, P<0.001) and it was not changed by the treatments. The glomerular filtration rate (GFR, L/kg/day), was reduced in 2K-1C control rats as compared with 2K control rats (8.94±1.86 n=6 vs. 15.21±3.15, n=6, P<0.05). The values of GFR were not changed by the treatments. Plasma levels of sodium, potassium, creatinine, Ang II and TBARS were similar in 2K and 2K-1C rats and were not changed by the treatments. Total proteinuria (mg/mL/kg/24h), together with microalbuminuria (protein/creatinin ratio), were highly increased in 2K-1C control rats when compared to 2K control rats (15.47±3.81 n=6 vs. 0.96±0.09, n=6, P<0.01 and 151,.50±27.57 n=7 vs. 10.97±2.76, n=6, P<0.001, respectively). Treatment of 2K-1C rats with losartan, enalapril or combined therapy similarly reduced the total protein and albumin excretion observed when compared to the hypertensive control group. Lucigenin-derived chemiluminescence was not different in the right and left kidneys of 2K and 2K-1C rats. Treatments had no effect upon NADPH oxidase activity. Silver stained urinary gels revealed a glomerular and probably tubular proteinuria in 2K-1C, that was completely abolished by the treatments. 2K-1C treated with losartan and/or enalapril. Western blott analysis showed reduction in the expression of nefrin (45%) and podocin (52%), markers of glomerular damage, and a 25% reduction of megalin expression, involved in tubular protein reabsortion in the renal cortex of 2K-1C compared to 2K. Treatment with enalapril alone or in combination with losartan evoked a recovery of the expression of the glomerular proteins. **Discussion:** 2K-1C hypertensive rats presented proteinuria which is minimized by the treatment of losartan or enalapril together with blood pressure. Combined therapy does not seems to induce additional effects on proteinuria, but cause a significant additional reduction of blood pressure. The mechanism of proteinuria reduction induced by RAS inhibitors in 2K-1C rats observed in this study does not involve inhibition of ROS, but are associated with normalization of the molecular expression of glomerular proteins involved in the protein filtration process. Supported by FAPESP, CAPES, CNPq, CIHR and Heart and Stroke Foundation of Canada.

06.042

THE NITRIC OXIDE DONOR [Ru(terpy)(bdq)NO+]3+ (TERPY) DOES NOT ALTER THE EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) IN SHR AORTAS.

Potje SR[1], Perassa LA[1], Graton ME[1], da Silva RS[2], Bendhack LM[2], Antoniali C[1] - [1]Faculty of Dentistry of Araçatuba, UNESP-SP - Department of Basic Sciences, [2]Faculty of Pharmaceutical Sciences of Ribeirão Preto, USP-SP. - Department of Physics and Chemistry

In aortas of rats, the nitric oxide (NO) donor [Ru (terpy) (bdq) $NO^{+}]^{3+}$ (TERPY) can uncoupling indirectly eNOS. Endothelial removal or L-NAME incubation increased the vasodilator effect of TERPY in aortas of Wistar or 2K-1C rats. However, in SHR aorta, the absence of endothelium or NOSe inhibition reduced TERPY´s effect. In a previous study, we observed that TERPY (10^{-5}M) increased the [NO] (DAF-2DA, 10µmol/L) in endothelial cells (EC) of SHR, but not in Wistar rats EC. Incubation of L-NAME (10^{-6}M) + TERPY decreases the [NO] in EC of SHR, but not of Wistar rats. Our hypothesis is that TERPY do not uncouple eNOS in SHR aortas. In this study we analyzed the eNOS expression (Western Blott) in aortas before and after TERPY (10^{-5}M) stimulation. The aortas were homogenized (RIPA buffer+ protease inhibitor), centrifuged (15.000 rpm, 10 minutes, 5°C) and the supernatant was separated. The proteins were measured by Lowry method. One hundred micrograms (100 µg) of protein were subjected to electrophoresis (8% gel-poliacrilamide) and transferred to nitrocellulose membrane. The membranes were incubated with primary anti-eNOS antibody (1:2500) overnight at 4°C and then incubated with anti-rabbit secondary antibody (1:1000) for 1h at environment temperature. The results were normalized by β-actin and compared between groups (Student´s t test). The results showed lower ($p < 0.05$) eNOS expression (0.88 ± 0.02, n = 5) in the aorta of SHR rats than in Wistar rats (1.27 ± 0:08, n = 5). After stimulation with TERPY, there was a decrease in the expression of eNOS in aortas of Wistar rats (0.81 ± 0.02, n = 5), but in SHR, the expression was not altered (0.95 ± 0:06, n = 5). This data suggests that, differently from the results observed in vascular smooth muscle of normotensive and hypertensive 2K-1C rats, in SHR aorta, TERPY did not uncouple eNOS. This results could be corroborated by the observation that the eNOS activity contribute to the greater effect of TERPY observed in SHR intact than in aortas without endothelium.

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06.043

NAD(P)H OXIDASE INHIBITION WITH APOCYNIN PREVENTS THE INCREASE IN BLOOD PRESSURE INDUCED BY CHRONIC ETHANOL CONSUMPTION

Marchi KC[1], Tirapelli CR[2] - [1]Faculdade de Medicina de Ribeirão Preto - USP - Departamento de Farmacologia, [2]Escola de Enfermagem de Ribeirão Preto - USP - Laboratório de Farmacologia

The first step for cardiovascular dysfunction associated with chronic ethanol consumption involves the generation of reactive oxygen species (ROS) and reduced nitric oxide (NO) bioavailability. These processes are mediated by the enzyme NAD(P)H oxidase. The aim of our study was to evaluate the participation of this enzyme in cardiovascular effects induced by chronic ethanol consumption through its inhibition by apocynin (APO). All protocols were approved by the local Ethics Committee (11.1.1648.53.8). Male Wistar rats (200-250g) were divided into 4 groups: Control group (C): 0.9% saline intraperitoneal (ip) injection; Control + APO group (AC): APO injection (10mg/kg/day, ip); Ethanol group (E): ethanol 20% (v/v) for 2 weeks and 0.9% saline ip injection; Ethanol + APO group (AE): ethanol 20% and APO (10mg/kg/day, ip). Arterial blood pressure measurement was assessed by tail cuff plethysmography. Aortic superoxide anions levels were detected by lucigenin chemiluminescence and TBARS assay was made to evaluate lipid peroxidation in plasma and aorta. Total antioxidant activity was evaluated by spectrophotometry. Protein expression of Nox1 and MAPKs (phosphorylated and total) were assessed by western blotting, and the values were normalized by corresponding β-actin. Results are expressed as mean ± SEM. ANOVA followed by Newman-Keuls post-test were performed to detect differences between values. $P < 0.05$ was considered significant. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) (mmHg) increased after chronic ethanol treatment (SBP=139.8 ±2.4, n=9; DBP =101.3±3.1 n=9; MAP=114.1±2.8, n=9) compared to Control group (SBP=124.4±1.2, n=10; DBP=78.7±1.3, n=10; MAP=93.9±1.0, n=10). APO treatment prevented the increase in blood pressure induced by ethanol consumption (SBP=125.8±2.10, n=10; DBP=83.8±1.96, n=10; MAP=97.8±1.85, n=10). No changes were observed in the heart rate among groups during treatment. Superoxide anion levels in the aorta (RLU/mg protein) were higher in ethanol group (244.8±13.4, n=6) when compared to control (120.7±20.5, n=7). APO prevented the increase in superoxide anion levels induced by ethanol (157.1±36.3, n=5) ($P<0.05$, ANOVA). Antioxidant capacity (µmol/L) was increased after treatment with ethanol (5.87±0.2, n=9) compared to control (4.46 ±0.2, n=10). Treatment with APO increased the antioxidant capacity in both control and ethanol-treated rats (AC=7.43 ±0.2, n=10; AE=9.66 ±0.5, n=9). Chronic ethanol consumption increased Nox1 (arbitrary units) (E = 1.66 ± 0.16, n = 4) and JNK (total) (E = 1.17 ± 0.25, n = 4) expression when compared to the control group (Nox1 = 0.63 ± 0.10, n = 4; JNK = 0.49 ± 0.08, n = 5). Treatment with APO prevented these responses (Nox1 = 0.64 ± 0.32, n = 4; JNK = 0.58 ± 0.13, n = 5). Ethanol, Control+APO and Ethanol+APO groups showed a decrease in ERK1/2 phosphorylation (E =0.36 ± 0.03; CA = 0.40 ± 0.08; EA = 0.31 ±0.07, n=4) compared to Control group (C = 0.67 ± 0.05, n=4). Chronic ethanol consumption induced vascular oxidative stress, increased blood pressure and the expression of Nox1 and JNK in aortic tissue,being these responses mediated by NAD(P)H oxidase.

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06.044

CHRONIC TREATMENT WITH APOCYNIN REDUCES ARTERIAL PRESSURE AND ANGIOTENSIN II*IN VIVO* EFFECT IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

Graton ME[1], Perassa LA[1], Potje SR[1], Lima MS[1], Antoniali C[1] - [1]School of Dentistry of Araçatuba, UNESP - State University of São Paulo - Department of Basic Sciences

It has been suggested the involvement of NOX/ NADPH oxidase enzyme on pathogenesis of many cardiovascular diseases, once the expression of this enzyme is increased in experimental models of hypertension as Spontaneously Hypertensive Rats (SHR). NOX inhibitors, such as apocynin, were developed and their antihypertensive effects have been studied. This study evaluated the chronic treatment with apocynin, a NOX/ NADPH oxidase inhibitor, on Systolic Arterial Pressure (SAP) e Diastolic Arterial Pressure (DAP) and on pressor response to angiotensin II (ANG II) in SHR and normotensive Wistar Rats. Wistar and SHR Rats (n= 5 to 7) were treated or not, from the 4th the 10th week of life with apocynin (30 mg/kg, in drinking water). Abdominal aorta and femoral vein were cannulated for direct registry of arterial pressure (AP) or for the drug administration. The ANG II effect was calculated as arterial pressure variation after administration of the doses 2.5 and 10ng/kg. The results (mean±SEM) were compared between the groups (ANOVA, $p<0.05$). The AP was decreased in SHR treated with apocynin (DAP: 100.1 ± 0.8; SAP: 148.2 ± 1.0 mmHg) if compared to SHR non-treated (DAP: 132.9 ± 0.9; SAP: 180.6 ± 2.4 mmHg). In Wistar, the treatment did not alter the AP (DAP: 87.7 ± 1.0 mmHg; SAP: 121.4 ± 1.4 mmHg) as compared to Wistar non-treated (DAP: 85.9 ± 1.3 mmHg; SAP: 123.7 ± 1.2 mmHg). The responses to ANG II were increased in SHR (2.5ng/kg: 24.9 ± 0.9 mmHg; 10ng/kg 37.1 ± 1.3 mmHg, n=5) when compared to Wistar (2.5ng/kg 15.9 ± 0.3 mmHg; 10ng/kg: 28.3 ± 0.7; mmHg, n=5). In SHR treated, ANG II was less effective (2.5ng/kg: 16.6 ± 0.5 mmHg; 27.6 ± 0.4 mmHg, n=5) than in SHR non-treated. These data demonstrated that the chronic treatment with apocynin decreased arterial pressure in SHR, but not in Wistar rats. These results suggest that the inhibitory effect of apocynin can be observed only when the activity of NOX is increased. As ANG II activates NOX in vascular smooth muscle, our results suggest that in SHR treated with apocynin, the reduced effect of ANGII could be associated to the NOX inhibition. The mechanisms involved on these effects will be studied.

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06.045

INHIBITION OF NO-SYNTHASE DOES NOT NORMALIZE THE CONTRACTILE RESPONSE TO NOREPINEPHRINE AND EPINEPHRINE IB RENAL HYPERTENSIVE RAT AORTAS.

Bocalon AC[1], Silva BR[2], Bendhack LM[1] - [1]Faculty of Pharmaceutical Sciences of Ribeirão Preto - University of São Paulo - Dept. Physics and Chemistry, [2]School of Medicine of Ribeirão Preto - University of São Paulo - Dept. Pharmacology

The vascular endothelium has great importance on the vascular responses mediated by adrenergic agonists. Hypertension is associated with endothelial dysfunction, and is observed less relaxing response and less contractile vascular response. The contractile response induced by adrenergic agonists norepinephrine (NE) and epinephrine (EP) is reduced in renal hypertensive rats aorta than normotensive rats aorta. Therefore the hypotheses this work is that NO-Synthase (NOS) could be involved in this reduced contraction. This study aimed to evaluate the effect of NOS inhibition on the contractile response to NE and EP in intact endothelium aortas (E+) from 2K and 2K-1C rats. Male rats were submitted to median laparotomy and implantation of a silver clip on the left renal artery to induce renovascular hypertension (2K-1C). Control animals (2K) were submitted only to laparotomy. 2K-1C animals were considered hypertension with systolic blood pressure < 160 mmHg. The thoracic aorta was removed, cut into 4-mm rings and mounted in isolated organ bath. Concentration-effect curves were developed for NE and EP in 2K and 2K-1C E+ aortas in the absence (Control) or after incubation for 30 min, with the non-selective inhibitor of NOS (L-NAME 100 µmol/L). This study was approved by the Ethics Committee (N°. 2012.1.1419.53.0). The maximal effect (ME, in grams of tension) and the potency (pD_2) were evaluated. We found that L-NAME increased the ME of contractile response to NE in 2K-1C E+ aortas from 1,5 ± 0,2g; n=9 to 2,1 ± 0,2g n=3 and did not change pD_2. It did not change neither ME non pD_2 to 2K (ME: 2,6 ± 0,1g; pD_2: 7,44 ± 0,11; n=7/Control, ME: 2,5 ± 0,2; pD_2: 7,20 ± 0,14; n=10). For contractile response to EP, L-NAME also increased the ME of in 2K-1C E+ aortas from 1,4 ± 0,1g; n=9 to 2,0 ± 0,2g; n=5 ($p<0.05$), but did not change pD_2. And it did not change the contractile response to EP in 2K (ME: 2,9 ± 0,2g; pD_2: 7,33 ± 0,13; n=6/Control, Emax: 2,6 ± 0,1g; pD_2: 7,13 ± 0,11; n=11). In the control preparations the ME was lower ($P<0.01$) and the potency was higher ($P<0.01$) in 2K-1C (ME: 1.5 ± 0.2g; pD_2: 7.73 ± 0.11, n = 9) than in 2K (ME: 2.5 ± 0.2g; pD_2: 7.20 ± 0.14, n=10). In a similar way, after incubation with L-NAME the ME was lower ($P<0.05$) and the potency was higher ($P<0.05$) in 2K-1C (ME: 2.1 ± 0.2g; pD_2: 7.90 ± 0.16; n= 3) than in 2K (ME: 2.6 ± 0.1g; pD_2: 7.44 ± 0.11, n= 7). The contractile response induced by EP presented lower ME ($p<0.001$) in 2K-1C (ME: 1.4 ± 0.1g; pD_2: 7.37 ± 0.16, n= 9) than in 2K (ME: 2.6 ± 0.1g; pD_2: 7.13 ± 0.11, n= 11). After incubation with L-NAME, the ME was lower ($P<0.05$) in 2K-1C (ME: 2.0 ± 0.2g; pD_2: 7.71 ± 0.18, n = 5) than in 2K (ME: 2.9 ± 0.2g; pD_2: 7.33 ± 0.13, n= 6). Our results suggest that inhibition of NOS increases the contractile response induced by NE and EP only in 2K-1C rat aortas. However L-NAME did not reduce the difference in the contractile response to NE and EP between of 2K-1C and 2K rats E+ aortas. Then, NO produced by NOS should not be the only factor involved in the reduced contractile response induced by NE and EP in 2K-1C intact endothelium rat aorta.

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06.046

PARTICIPATION OF ANGIOTENSIN II IN OXIDATIVE STRESS, ALTERATION OF VASCULAR REACTIVITY AND NEUROHUMORAL INDUCED BY CHRONIC ETHANOL CONSUMPTION.

PASSAGLIA P[1], Antunes-Rodrigues J[2], COELHO EB[3], Tirapelli CR[4] - [1]USP - TOXICOLOGIA, [2]USP - FISIOLOGIA, [3]USP - CLÍNICA MÉDICA, [4]USP - FARMACOLOGIA

Introduction: The cardiovascular dysfunction induced by chronic ethanol consumption is associated with the formation of reactive oxygen species (ROS). Angiotensin II (ANG II) via AT_1 receptors, is a major maker of ROS in the cardiovascular system. **Objective:** To evaluate the role of AT_1 receptors in the cardiovascular dysfunction induced by chronic ethanol consumption. **Methods:** Male *Wistar* rats were divided into four groups: Control (C) received water "ad libitum"; Ethanol (E) received ethanol solution 20% (vol./vol.), Control + Losartan (C+L) received water "ad libitum" and losartan (10 mg / kg) daily by gavage; Losartan + Ethanol (E+F) received 20% ethanol solution and losartan. Measurements were performed to determine plasma and tissue (aorta and mesenteric bed) levels of angiotensin (Ang) I and Ang II, plasma renin activity, and plasma levels of oxytocin (OT), atrial natriuretic peptide (ANP), osmolality, sodium and TBARS. The thoracic aorta was isolated to concentration-response curves for phenylephrine, acetylcholine and sodium nitroprusside (SNP). ROS production was measured in the aorta and mesenteric bed by chemiluminescence of lucigenin. The protocols were approved by CEUA Campus USP, Ribeirão Preto (Protocol: 11.1.1103.53.1). **Results:** The ethanol treatment induced an increase in plasma activity (pg/mL/hr), renin (C: 5.89±1.0, n=6, E: 14.25±1.5, n=7), and plasma levels (pg / ml) of ANGI (C: 358±32.8, n=14, E: 2.959±0.3, n=6) and ANG II (C: 40.1±6.5, n=13, E: 437.2 ± 60.1, n=8), and these effects were prevented by administration of losartan. There was no change in tissue levels of ANGI and ANGII in aorta or mesenteric bed of the rat. Ethanol promoted increased plasma levels (nmol/mL) of TBARS (C: 16.5±1.1, n=9, E: 30.1±3.7, n=9) and treatment with losartan prevented this response. The chronic consumption of ethanol increased the vasoconstrictor response induced by phenylephrine in aortic rings with (C: 1.0 ± 0.1g, n=5 E: 1.4±0.1g, n=5) and without endothelium (C: 1.4±0.2g, n=9, E: 2.4 ± 0.1g, n=8). The losartan treatment prevented the increase in contractile response to phenylephrine induced by ethanol. Ethanol consumption induced increase in ROS production in aorta and mesenteric bed and this response was prevented by losartan. The treatment with ethanol did not alter the relaxation response induced by acetylcholine (C: 93.9±8.7%, n=5; I: 80.7 ± 10.4%, n=6) or SNP (C: 119.6 ± 2.2%, n = 12, E: 126.8 ± 14.4%, n=6) in rat aorta. The treatment with ethanol did not alter the osmolarity (C: 286.9±1.3 mOsm, n=9, E: 287.2±3.3mOsm, n=8) or sodium levels (C: 150.9 ± 3.9mEq/L, n=9, E: 146.4 ± 3.9mEq/L, n=9). In animals treated chronically with ethanol the plasma OT was decreased (C: 1.6 ± 0.2pg/mL, n=9, E: 0.90 ± 0.1pg/mL, n=8) and plasma level of ANP was increased (C: 22.6 ± 2.7pg/mL, n=6, E: 51.9±6.1pg/mL, n=8). Losartan did not prevent these effects induced by ethanol. Conclusion: Chronic ethanol consumption activates the systemic RAS, induces systemic oxidative stress and changes in vascular reactivity. The change in the vascular reactivity and the increased systemic oxidative stress are ANG II-mediated effects since treatment with losartan prevented these responses. **Financial Support:** FAPESP.

06.047

NOREPINEPHRINE-INDUCED CONTRACTION OF RAT RENAL AND FEMORAL VEINS INVOLVES BOTH α1 AND α2-ADRENOCEPTORS

Rossignoli PS[1], Pereira OCM[2], Chies AB[3] - [1]FAMEMA/UNIMAR - Laboratory of Pharmacology, [2]IBB/UNESP - Department of Pharmacology, [3]FAMEMA - Laboratory of Pharmacology

Introduction: It has been suggested that the stimulation of beta-adrenergic receptors may induce contractile responses in some veins, which contrasts with the arterial bed where these receptors normally mediates vasodilation. (Kaiser et al., J Pharmacol exp Ther, 144, 156, 1964; Müller-Ruchholtz et al., Pflugers Arch, 370, 247, 1977). We recently observed that norepinephrine (NOR), a non-selective agonist of adrenergic receptors, induces a bigger vasoconstriction in rat renal veins than phenylephrine, a selective agonist of alpha-adrenergic receptors. The objective was to investigate the involvement of α_1 and α_2-adrenoceptors in the constriction of rat renal and femoral veins induced by NOR.

Methods: The study was approved by CEUA-FAMEMA (protocol 236/11). Male Wistar rats (300-350g) were sacrificed in CO_2 chamber and exsanguined. Rings (4–5mm) of renal and femoral veins were removed and set up in organ bath containing Krebs-Henseleit solution, 37°C, pH 7.4, gassed with 95% O_2/5% CO_2. These preparations were stimulated by NOR in the absence and presence of atenolol (10^{-6}M), ICI 118551 (ICI; 10^{-6}M), yohimbine (10^{-8}, 10^{-7}, 10^{-6}M) and prazosin (10^{-8}, 10^{-7}, 10^{-6}M). The contractile responses were registered by isometric transducers and expressed as concentration-response curves. Log of EC_{50} (pEC_{50}) and maximal response (R_{max}) obtained from these curves (n=8) were compared by Student “t” test or one way ANOVA/Bonferroni. Differences were considered significant when $P<0.05$.

Results: Neither atenolol nor ICI modified the responses of NOR in both renal and femoral veins. In contrast, the NOR R_{max} in renal veins was reduced by yohimbine 10^{-8}M (from 0.35±0.03 to 0.18±0.04), 10^{-7}M (to 0.13±0.03) or 10^{-6}M (to 0.14±0.07). Similarly, the NOR pEC_{50} in femoral veins was decreased by yohimbine 10^{-8}M (from 5.30±0.07 to 5.05±0.05), 10^{-7}M (to 4.97±0.08) or 10^{-6}M (to 4.70±0.07). Moreover, the NOR R_{max} in renal veins was decreased by prazosin 10^{-7}M (from 0.32±0.06 to 0.01±0.01) or 10^{-6}M (to 0.03±0.02) and in the femoral veins by prazosin 10^{-8}M (from 0.99±0.15 to 0.49±0.12), 10^{-7}M (to 0.40±0.09) or 10^{-6}M (to 0.37±0.10).

Discussion: The results suggest that β-adrenoceptors are not involved in noradrenaline-induced contraction of rat renal and femoral veins. This find is in accordance with literature that shows different agonists effects in venous bed (Imms et al., Br J Pharmacol, 60, 107, 1977; Imai et al., Circ Res, 43, 553, 1978; Abdelrahman et al., Eur J Pharmacol, 190, 321, 1990). However, noradrenaline-induced contraction of rat renal and femoral veins involves stimulation of both α_1 and α_2-adrenoceptors.

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06.048

A DUAL-NOX ACTIVATION INDUCED BY ANGIOTENSIN II MODULATES VASCULAR SMOOTH MUSCLE CELLS FUNCTIONS: ROLE OF ALPHA1BETA1 INTEGRIN AND ILK

Moraes JA[1], Frony AC[1], Dias AM[1], Renovato-Martins M[1], Rodrigues G[1], Marcinkiewicz C[2], Assreuy J[3], Barja-Fidalgo C[1] - [1]UERJ - Biologia Celular, [2]Temple University, [3]UFSC - Farmacologia

Introduction

The atherogenesis is a process initiated by a local inflammatory response, characterized by the release of inflammatory mediators which amplifies this process and can induce vascular smooth muscle cells (VSMC) activation. Among the inflammatory molecules in circulation during a vascular injury, Angiotensin II (AngII) highlights, being able to induce different effects in VSMC, via the activation of NOX1 and integrin-mediated signaling pathways. This study was carried out to elucidate the molecular mechanisms underlying AngII effect on VSMC functions, focusing on the role of alpha1beta1 integrin and ILK pathway.

Methods

Primary VSMC were isolated from rat aorta and VSMC cell lineage A7r5 were obtained from ATCC. The disintegrin Obtustatin (OBT) was used to block alpha1beta1 (a1b1) integrin; Chemotaxis (4h) was performed in Boyden chamber; Intracellular ROS production quantified by DCF; P47 distribution analyzed by immunocytochemistry (1h). Actin, AKT, ILK, NOX1 and p21 expression evaluated by immunoblotting; siRNA assay performed with validated siRNAs (Ambion) in Lipofectamin; Cell proliferation measured by cell cycle analysis (PI), [3H] Thymidine incorportation and MTT assay.

Results: AngII induced VSMC migration and proliferation, which parallels with ROS production. Both effects were impaired by DPI, a pan-inhibitor of NOX. The inhibition of NOX2 by apocynin, a p47 inhibitor, only partially repressed ROS production, although had abolished migration. Similar effect was obtained by a1b1 integrin blockage with OBT, which inhibited NOX2-p47 subunit translocation to cell membrane, and diminished ROS production induced by AngII. OBT also inhibited FAK phosphorylation and impaired chemotaxis, confirming the involvement of a1b1 integrin activation in the effects induced by AngII on VSMC. Furthermore, the activation of FAK by AngII leads to its dissociation from ILK, and OBT blocked this effect and also reduced the expression of ILK and the activation of its molecular targets: AKT (phosphorylation) and Bad (degradation). Using a small interfering RNA (siRNA) for ILK, we observe that it did not affect FAK activation, but impaired VSMC migration and partially inhibited ROS production, p47 activation and AKT phosphorylation. On the other hand, siRNA for NOX1, a NADPH oxidase associated to AngII effects on VSMC, abolished ROS production, inhibited FAK phosphorylation, AKT activation, and impaired migration and proliferation induced by AngII.

Discussion: Taken together the results suggest that AngII triggers NOX-1 activation, and the ROS produced would modulate the inside-out activation of a1b1 integrin, leading to FAK activation and its consequent dissociation from ILK. Activated ILK triggers signaling pathways associated to cell migration and proliferation, and induce p47-subunit translocation to cell membrane, activating NOX2, which sustained ROS production to maintain the process. The blockage of a1b1 integrin activation by OBT, completely inhibited the signaling path, impairing chemotactic/proliferative effect of AngII, suggesting that integrin may be a key target for developmentt of more effective therapeutic interventions in atherosclerosis.

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06.049

CARDIOPROTECTIVE EFFECT OF ORLISTAT ON ARRYTHMIAS CARDIAC AND LETHAL ARISING FROM RATS ISCHEMIA AND REPERFUSION.

Rodrigues FSM[1], Tavares JGP[1], Lima EPS[1,1], Miranda-Ferreira R[1], Jurkiewicz NH[2], Jurkiewicz A[1], Caricati-Neto A[1] - [1]UNIFESP - DEPARTAMENTO DE FARMACOLOGIA, [2]UNIFESP - DEPARTAMENTO FARMACOLOGIA

Introduction: Acute myocardial infarction (AMI) is characterized by ischemic lesions that severely compromise cardiac structure and function, and even the survival of mammals. Although conventional therapy based on the cardiac reperfusion, this procedure increases cardiac damage caused by ischemia. The increasing prevalence of AMI, especially in the last decade, aroused the interest of pharmacologists in the search for compounds capable of alleviating the cardiac damage by ischemia and reperfusion (I/R), classified as cardioprotective drugs. In this study, we adopt a model *in vivo* I/R heart to evaluate the possible cardioprotective effects of agents used in the pharmacotherapy of dyslipidemia, such as orlistat (ORL, pancreatic lipase inhibitor). **Methods:** Adult male Wistar rats (250 - 350g) anesthetized (urethane 1.25 g/kg, i.p.) were placed on mechanical ventilation (60 cycles / min) and underwent surgery to induce cardiac I/R by obstructing left descending coronary artery (10 min) followed by reperfusion (75 min). Following protocol I/R, the animals were connected to electrocardiogram system (ECG) to record cardiac electrical activity during I/R. The rats subjected to I/R were treated for 10 days with 0.9% saline (I/R + SS) or ORL 53 mg/kg, p.o. (I/R + ORL). At the end of reperfusion, blood samples were collected for determination of serum biochemical markers of cardiac damage (creatine kinase or CK). This study was approved by the Research Ethics of UNIFESP (CEP 0065/12). **Results:** In I/R + SS group (n= 7), we observed a high incidence of ventricular arrhythmias or AV (85%), atrioventricular block or AVB (79%) and lethality or LET (70%), a typical response of cardiac damage caused by I/R. These damages in I/R + SS group were confirmed by high serum levels of CK (4883 U/L, n=7). Compared to I/R + SS, the incidence of AV was reduced to 21 - 28% and LET was reduced to 17% in I/R + ORL group (n=7), indicating cardioprotective effect of the ORL. This ORL effect was confirmed by reduction to 43% of serum levels of CK (2771 U/L, n=7), in relation to I/R + SS group (4883 U/L, n=7). **CONCLUSION:** These results suggest that ORL produced cardioprotective effect against cardiac damage caused by I/R.

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06.050

CONSEQUENCES ELICITED BY STRESS RESTRICTION ON THE AT_1 RECEPTOR ACTIVATION IN DIABETIC RAT CAROTID

Moreira JD[1], Pernomian L[2], de Oliveira AM[2] - [1]USP/FMRP, [2]USP/FCFRP

Introduction: Diabetes Mellitus and psychological stress contribute significantly to the pathogenesis of cardiovascular disease. During diabetes, vascular dysfunction occurs with exacerbation of Ang II induced contraction in rat carotid by reactive oxygen species (ROS)-dependent mechanism. In parallel, acute stress by restriction induces overexpression of AT_1 and increases the formation of renin and Ang II. The hypothesis of the project is that acute stress exacerbating diabetic changes on the vascular system angiotensinergic, favoring the hyperreactivity diabetic from AT_1 receptor in rat carotid. **Objective:** determine the effects of acute stress on the activation of Ang II on AT_1 from angiotensinergic system diabetic rat and verify the possible involvement of oxidative stress on this activation. **Methods:** This study was approved by CEUA/USP-RP (protocol nº13.1.441.53.2). Wistar rats underwent to a single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg). After 28 days of treatment with STZ the animal undergoes to acute stress by restriction for 3 hours. Then the animals are sacrificed to remove common carotid arteries and submitted to the study of reactivity mounted in organ bath chambers with Krebs solution for cumulative concentration-response curves for Ang II (10^{-11} – 10^{-7} mol/L) in carotid arteries from diabetic rats, in endothelium-intact (E^+) or endothelium-denuded (E^-) carotid rings, in the absence or presence of L-NAME (L-nitro-arginine methyl ester, 100µmol/L) a non-selective inhibitor of nitric oxide synthase (NOS), added 30 minutes prior Ang II. **Results:** The value of maximum Ang II-induced contraction (Emax) increased in carotid vascular reactivity of stressed diabetic rats with endothelium-intact (0.85±0.05) and was exacerbated in endothelium-denuded (1.37±0.06) when compared with control E^+(0.52±0.04) E^-(0.87±0,04); stressed control E^+(0.50±0.05) E^-(0,86±0.04) and diabetic E^+(0.45±0.06) E^-(1.05±0.05) and increase in potency of contraction (pD_2) to Ang II from diabetic group E^-(9.00±0.20) in relative to stressed control E-(8.52±0.06). In endothelium-intact rat carotid rings pre-treated with L-NAME, a non-selective NOS inhibitor, the Emax values of the control+L-NAME E^+(0.90±0.07); stressed control+L-NAME E^+(0.91±0.14) and diabetic+L-NAME E^+(0,93±0.06) approached the values the stressed diabetic group E^+(0.89±0.12) in the absence of inhibitor. And the Emax value of stressed diabetic group +L-NAME E^+(1.19±0.09) increased when compared with the diabetic stressed E^+(0.85 ± 0.12). **Discussion:** The findings suggested that stress associated with diabetes leads to uncoupling of some isoform of the NOS and/or participation of oxidative stress by increased ROS that reducing the bioavailability of nitric oxide, impairs the negative modulation of the endothelium. And also involvement of other isoform of NOS induced by stress in diabetes in which there was an increased response to Ang II in the stressed diabetic group with endothelium-intact when pre-treated with NOS inhibitor. Financial support: CNPq (470142/2012), FAPESP (2012/00640-7 and 2012/09019-3), NAP-DIN (11.1.21625.01.0) and CAPES.

06.051

PARTICIPATION OF NOX1/NADPH OXIDASE IN THE PRODUCTION O^-_2 IN THORACIC AORTA OF MICE YOUNG UNDERGOING CHOLESTEROL DIET (1%)

Moreira RP[1], Pernomian L[1], Gomes MS[1], de Oliveira AM[1] - [1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto - USP - Física e Química

Introduction: The importance of dyslipidemia in adults was well established by its association with the development of atherosclerosis. Current knowledge, however indicate that the dyslipidemia initiated in childhood can lead to an disease inflammatory vascular. The dyslipidemic process promotes increased production of reactive oxygen species (ROS) by the action of NADPH oxidase, decreasing the availability of nitric oxide (NO), leading to oxidative stress and triggering a process of endothelial dysfunction. However some studies show that eating foods high in fat, although not lead to changes in the lipid profile, is enough for the development initial of endothelial dysfunction. The aim of this study was to evaluate the effects of high cholesterol diet (1%) on the contraction of angiotensin II (Ang II), and the involvement of NADPH oxidase in this response. **Methods**: Mice young C57BL/6 males (25 days old) weighing 12g were used in this study in accordance with the Animal Ethics Committee of the Campus of Ribeirão Preto - University of São Paulo (Protocol: 12.1.976.53.2). The animals were divided into control group (GC) fed with standard diet, and group (GT) fed with standard diet + 1% cholesterol for 3 months. Concentration-effect curves to angiotensin II were obtained (10^{-10} - 10^{-7}) in the thoracic aorta of male C57BL/6 Young with intact endothelium (E^+) and in its absence the endothelium (E^-) in the presence or absence of the Tiron, scavenger of superoxide anion, and specific inhibitor of Nox1 (ML171). Was evaluated potency (*p*D2) and maximal response (E_{max}) from the concentration-effect curve to angiotensin II. Were analyzed the lipid profile and the enzymes of liver AST and ALT. **Results:**The lipidic profile as well as the liver enzymes of the GT remained the same the GC. In the analysis of reactivity Ang II, there was an increase in the E_{max} of GT rings (E^+) (E_{max}: 0.195 ± 0.004 n = 9) or rings (E^-) (E_{max}: 0.219 ± 0.004 n = 9) compared with the GC rings (E+) (E_{max}: 0.071 ± 0.005 n = 9) or rings (E^-) (E_{max}: 0.158 ± 0.002 n = 9). There were no change in potency (*p*D2) the CG and GT. In the presence of ML171, the E_{max} before exacerbated in the GT, was restored in both rings (E^+) (E_{max}: 0.056 ± 0.006 n = 9) and in rings (E^-) (E_{max}: 0.134 ± 0.017 n = 9). In the GC no differences were seen in E_{max} in the presence of ML171. In the presence of Tiron, the E_{max} before exacerbated in the GT, was restored in both rings (E^+) (E_{max}: 0.076± 0.006 n=9) and in rings (E^-) (E_{max}: 0.122 ± 0.007 n=9). The GC was not significant differences in E_{max} in the presence of Tiron. There were no difference in potency (*p*D2) the CG and GT in the presence of Tiron and ML171. **Discussion:** The data show the involvement of NADPH oxidase in the specific Nox1 in response to exacerbation of Ang II. While there is no change in lipid profile, there is an increase in the contraction for Ang II in the thoracic aorta of male young C57BL/6 fed a to high-cholesterol diet (1%). The hyperresponsiveness is due in part by production superoxide anion and the variation was endothelium-independent. **Financial Support:** CAPES, FAPESP and NAP-DIN

06.052

EFFECT OF N-ACETYLCYSTEINE ON THE PURINERGIC AND NORADRENERGIC NEUROTRANSMISSION IN SMOOTH MUSCLE OF RAT VAS DEFERENS

Lima EPS[1], Rodrigues FSM[1], Simões ACB[1], Sousa GL[1], Jurkiewicz NH[1], Jurkiewicz A[1], Caricati-Neto A[1] - [1]Unifesp - Farmacologia

Introduction: Recent studies showed that N-Acetylcysteine (NAC) produce anti-hypertensive effects in experimental and human hypertension (Pechanova et al, Clin Sci, 2006; Krug et al, Clin Exp Pharmacol Physiol, 2008). However, involvement of autonomic mechanisms in the anti-hypertensive effect of NAC remains unknown. Using a richly innervated (sympathetic nerves) smooth muscle (vas deferens, VD) as model for study of autonomic transmission, we investigate if NAC modifies purinergic and noradrenergic components of sympathetic transmission. **Methods:** VD of adult Wistar rats (16 to 20 weeks) were isolated, cleaned of adjacent tissues, mounted in 10 ml organ bath containing Tyrode solution and placed between two parallel platinum electrodes coupled to electrical stimulator (pulses of 10 Hz, 3ms, 60 V) to evoked contractions produced purinergic (ATP) and noradrenergic (Noradrenaline) transmitters released from sympathetic ending nerves. These neurogenic contractions were recorded by isometric transducers coupled to analogical-digital system (AD Instruments). The effects of NAC (1 to 10 mM) on these contractions were studied in the absence or presence of voltage-operated K^+ channel (Kv) 4-Aminopiridine (4-AP, 2 mM), oxide nitric (NO) inhibitor N^G-Methyl-L-arginine acetate (LNMMA, 100 µM), NO substrate biosynthesis L-Arginine (LARG, 1 mM) and guanylyl cyclase (GC) inhibitor 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 30 µM). All procedures were approved by Ethical Comitte in Research of UNIFESP (CEP Nº 0092/12). **Results:** NAC (9 and 10 mM) significantly increased purinergic contractions (47 to 51%, n=8-10, $P<0.05$) and reduced noradrenergic contractions (59 to 69%, n=8-10, $P<0.05$). Increase of purinergic contractions produced by NAC was significantly potentiated by 4-AP, LNMMA, LARG and ODQ (50 to 70%, n=8-10, $P<0.05$). Reduction of noradrenergic contractions induced by NAC was significantly attenuated by 4-AP and ODQ (85 to 90%, n=8-10, $P<0.05$), but not by LNMMA and LARG. **CONCLUSION:** The increase of purinergic contractions and reduction of noradrenergic contractions produced by NAC involve multiple mechanisms, especially activation of Kv and NO-GC-cGMP signaling pathway.

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06.053

Evaluation of cavernous smooth muscle reactivity in rats with chronic heart failure: Model of chronic volume overload.

Tartarotti PS[1], Silva FH[2], Rojas-Moscoso JA[2], Priviero FBM[1], Nucci G[2], Antunes E[2], Claudino MA[1] - [1]Universidade São Francisco - Laboratório Multidisciplinar de Pesquisa, [2]Universidade Estadual de Campinas - Departamento de Farmacologia

Introduction: Chronic heart failure (CHF) affects million people in the world making it a challenge to public health. Studies have shown a strong association of CHF with the development of erectile dysfunction. Epidemiological studies have shown that 58-85% of CHF patients reported an episode of erectile dysfunction. Since approximately 75% of these patients described impaired libido and 30% has complete absence of sexual activity. The CHF may causes changes in the normal balance of vasoconstriction mechanisms and reduction in the NO bioavailability in erectile tissue. Thus the aim of present study was evaluated the corpus cavernosum relaxation and contractile mechanisms in CHF rats. **Methods:** The experimental protocols were approved by the Ethics Committee for Experimental Research of the Sao Francisco University. Heart failure was induced by surgical creation of an arteriovenous fistula between the abdominal aorta and inferior vena cava, distal to the origin of the renal arteries. After 4 weeks to validate the heart failure were measure the total cardiac mass and left ventricular mass. Concentration-response curves to acethylcholine (ACh), sodium nitroprusside (SNP) and phenylephrine (PE) were obtained in rats CC. The values of potency (pEC_{50}) and maximal responses (E_{max}) were calculated. Nitrergic and neurogenic responses induced by electrical-field stimulation (EFS) were also obtained in all groups. **Results:** After 4 weeks, the total cardiac mass and left ventricular mass were significantly higher ($P<0.05$; 82% and 76%, respectively) when compared to control rats. The endothelium-dependent NO-mediated relaxing responses to ACh induced concentration-dependent CC relaxations, and both pEC_{50} (5.12 ± 0.03) and E_{max} (35 ± 3%) did not changes in CHF animals, compared with control animals (5.07 ± 0.04 and 39 ± 3%, respectively). The endothelium-independent NO-mediated relaxing responses to SNP produced concentration-dependent relaxation in isolated rats CC, and both pEC_{50} (5.55 ± 0.12) and E_{max} (82 ± 4%) did not changes in the CHF animals when compared to the control group (5.35 ± 0.05 and 87 ± 7%, respectively). However, nitrergic relaxation response induced by EFS was significantly reduced in the CHF rats ($P<0.05$) in the higher frequencies (8, 16 and 32Hz) when compared to control animals. In addition, cumulative addition of the PE produced concentration-dependent contractile responses in the rat cavernosal tissues. The pEC_{50} of PE did not change in CHFR (5.45 ± 0.04), when compared to control rats (5.38 ± 0.04), however, the E_{max} was significantly increased (2.5 ± 0.1mN/mg of tissue; $P<0.05$) compared with control rats (1.8 ± 0.1mN/mg of tissue). Moreover, neurogenic contractile response was significantly increased in the higher frequency (32 Hz), when compared to control animals. **Conclusion:** CHF rats exhibit an impaired nitrergic relaxant response and an increase of contractile mechanism in the isolated corpus cavernosal smooth muscle. Thus, our preliminary results may contribute to understand the development of erectile dysfunction associated with CHF. *Financial support*: FAPESP/CNPq.

06.054

Oxidative stress impaired the vasorelaxation effect of nitric oxide-releasing indomethacin derived (NCX2121) in hypertensive rats aorta

Paula T D[1], Silva BR[2], Bendhack LM[1] - [1]Faculdade de Ciêcias Farmacêuticas de Ribeirão Preto USP - Física e Química, [2]Faculdade de Medicina de Ribeirão Preto USP - Física e Química

In some cardiovascular diseases such as hypertension the mechanisms of balance between endothelium constrictor factors (EDCFs) and endhotelium relaxing factors (EDRFs) produced by the endothelium are impaired. The increased of oxidative stress with augmented levels of reactive oxygen species (ROS) can reduced the nitric oxide (NO) bioavailability, the main EDRF, while the increased of ciclooxigenase (COX) activity increased the prostanoids production like prostaglandins (PGs) and thromboxane (TXs), major EDCFs. Alternatively, the NO donor simultaneously to COX activity inhibition can improve the balance between EDCF and EDRF. NCX 2121 is a NO-releasing indomethacin derived of the new class of non-steroidal anti-inflammatory drugs. It is used due to minor side effects such as upper gastrointestinal tract ulceration. Some studies had been show that this drug can reduce the blood pressure and produce vasorelaxation. The present study aimed to characterize the role of oxygen reactive species in vasorelaxant effect of NCX 2121 in renal hypertensive (2K-1C) and normotensive (2K) rats aorta. **Methods:** Renovascular hypertension was induced in anesthetized rats with tribromoethanol. All the procedures were approved by the Ethics Committee (CEUA No. 12.1.120.53.0). Sham-operated rats were the control group (2K). After 6 weeks the SAP was measured and rats were considered hypertensive with SAP ≥ 160 mmHg. The rats were killed under anesthesia and aortic rings were removed and mounted in organ chambers for isometric tension measurements. The integrity of the endothelium was tested with acetylcholine (1mM). Cumulative concentration-effect curves were constructed for NCX2121 (1pM-100 mM) in aortic rings with endothelium (E+) and without endothelium (E-) and in presence of the inhibitor of soluble guanylyl-cyclase (ODQ 10 mM) and O_2^- scavenger (Tiron 1 mM). The data are show as mean ± S.E. and statistical analysis was performed by student *t* test, point by point, including the maximum effect (ME). **Results:** NCX 2121 produced relaxation in a concentration-dependent way. There is no differences between 2K-1C and 2K rats aorta. The endothelium increased the ME in 2K (89.9 ± 4.4% (E+) *vs* 70.1 ± 6.9% (E-) $p<0.05$) and 2K-1C (78.5 ± 3.9% (E+) *vs* 54.1 ± 5.5 (E-) $p<0.01$). ODQ abolished the relaxation in 2K (70.1 ± 6.9% (E-) *vs* 13.0 ± 4.6 % ODQ ($p<0.001$) and 2K-1C (54.1 ± 5.5% E- *vs* 13.4 ± 5.2% ODQ $p<0.001$). Tiron did not interfere with the effect of NCX 2121 in 2K aortas, however increased ME in 2K-1C (54. 1 ± 5.5 % E (-) *vs* 82.1 ± 6.1 % Tiron). **Conclusion:** The relaxation of NCX 2121 was due NO and the endothelium potentiated the relaxation. Moreover the increased of oxidative stress impaired the relaxation in 2K-1C. **Support**ed by FAPESP and CNPq.

06.055

Increase of contractile response in detrusor smooth muscle of rats with chronic heart failure: Evaluating the development of overactive bladder

Furquim SR[1], Silva FH[2], Rojas-Moscoso JA[2], Priviero FBM[1], de Nucci G[2], Antunes E[2], Claudino MA[1] - [1]Universidade São Francisco - Laboratório Multidisciplinar de Pesquisa, [2]Universidade Estadual de Campinas - Departamento de Farmacologia

Introduction: Chronic heart failure (CHF) is a major challenge for world public health. Studies have shown a strong association of CHF with the development lower urinary tract symptoms (LUTS). Epidemiological studies in patients with CHF showed that 32-34% of men and 41-62% of women reported episode of LUTS. Moreover, evidence suggests that LUTS and CHF failure share risk factors that are associated with autonomic nervous system dysfunction, which has also been implicated in development of overactive bladder. Thus the aim of present study was evaluated the contractile mechanism of detrusor smooth muscle in CHF rats. **Methods:** The experimental protocols were approved by the Ethics Committee for Experimental Research of the São Francisco University. Heart failure was induced by surgical creation of an arteriovenous fistula between the abdominal aorta and inferior vena cava, distal to the origin of the renal arteries. After 4 or 12 weeks to validate the heart failure were measure the total cardiac mass and left ventricular mass. Cumulative concentration-response curves to muscarinic agonist, charbacol (CCh; 1 nM - 100 µM) and hyperpolarizing solution, chloride potassium (KCl; 1-300mM) was obtained in rats detrusor smooth muscle (DSM). The values of potency (pEC_{50}) and maximal responses (E_{max}) were calculated. Neurogenic contractile responses induced by electrical-field stimulation (EFS) were also obtained in all groups. **Results:** Our results were divided in 4 and 12 weeks. After 4 weeks, the total cardiac mass and left ventricular mass were significantly higher (48% and 44%, respectively; $P<0.05$) when compared to control rats. Cumulative addition of the CCh produced concentration-dependent contractile responses in DSM, and the pEC_{50} (5.73 ± 0.13) did not change did not changes in CHF animals when compared with control animals (5.71 ± 0.09), whereas the E_{max} was significantly increased in CHF rats (1.23 ± 0.12 mN/mg of tissue; $P<0.05$), compared with control rats (0.86 ± 0.09 mN/mg of tissue). Cumulative addition of the KCl produced concentration-dependent contractile responses in DSM, and both the pEC_{50} (1.46 ± 0.15) and E_{max} (0.92 ± 0.13 mN/mg of tissue) did not change did not changes in CHF animals when compared with control animals (1.60 ± 0.19 and 1.14 ± 0.36, respectively). The neurogenic contractile response induced by EFS remained unaltered in all investigated groups. After 12 weeks, the total cardiac mass and left ventricular mass were significantly higher ($P<0.05$; 82% and 76%, respectively) when compared to control rats. The pEC_{50} of CCh and KCl (5.76 ± 0.15 and 1.34 ±0.06, respectively) did not change did not changes in CHF animals when compared with control animals (5.69 ± 0.11 and 1.05 ± 0.13, respectively), however the E_{max} to CCh and KCl were significantly increased in CHF rats (1.46 ± 0.13 and 1.28 ± 0.13 mN/mg of tissue, respectively; $P<0.05$), compared with control rats (1.09 ± 0.08 and 0.88 ± 0.05 mN/mg of tissue, respectively). Moreover, neurogenic contractile response was significantly increased in the all frequency (1-32 Hz; $p<0.05$), when compared to control animals. **Conclusion:** CHF rats exhibit an increase of contractile mechanism in the isolated detrusor smooth muscle. Thus, our preliminary results suggest that these alterations may contribute to understand the development of LUTS associated with CHF. Financial support: FAPESP/CNPq.

06.056

VASORELAXANT EFFECT OF WE011 A DERIVATIVE AMINOGUANIDINIC ON MESENTERIC ARTERY RINGS RATS

HERCULANO EA[1], SILVA JCG[2], CLEMENTINO-NETO J[2], Moura MTD[2], FRANÇA PHB[1], Araújo-Júnior JX[1], Ribeiro EAN[2], COSTA CDF[3] - [1]UFAL - IQB, [2]UFAL - ESENFAR, [3]UFAL - RENORBIO

Introduction: The hypertension is the most common cardiovascular disease and is a major public health issue (Nikam, J Pharma and Pharmacol, 60, 1501, 2008), affecting more than 10% of the worldwide population (Chen, Asia Pac J Clin Nutr, 16, 309,2007). Therefore, is important the development new therapeutic agents acting on cardiovascular system. The aminoguanidinics derivatives as well as clonidine guanabez and guanfacine have as main effect lowering blood pressure, hence in the present study, we examined the vasorelaxant effect of WE011 a derivative aminoguanidinic.

Methods: Superior mesenteric artery rings of male Wistar rats (2 - 4 mm) were maintained in Tyrode's physiologic solution at 37 ° C, and suspended by carbogênica mixture of cotton yarn for recording of isometric tension under a tension of 0.5 g. We studied the concentration-dependent relaxant effect of WE011 on endothelium-intact and endothelium-denuded mesenteric rings that were pre-contracted Phe (10^{-6}M) or 80 mM KCl. During the tonic phase of the contraction, WE011 ($3x10^{-8}$ - 10^{-5} M), cumulatively) was added to the organ bath. The relaxant effect was expressed as the percentage of Phe- or KCl-induced contraction. All results were expressed as the percentage reduction of contraction induced by Phe (10^{-6}M) or KCl 80 mM. The WE011 ($3x10^{-8}$ - 10^{-5} M). All values were expressed as mean ± S.E.M. The results were analysed with student's t-test. Probability values < 0.05 were considered to be significant. The pD2 values were obtained by nonlinear regression. All analysis was performed using GraphPad™ Prism software, version 5.0®.Protocol approved by the ethics committee for animal experimentation: 009481/2011-21.

Results AND **Discussion**: WE011 ($3x10^{-8}$ - 10^{-5} M) evoked concentration-dependent relaxation responses of endothelium-intact aortic rings, pre-contracted with Phe (10^{-6} M) (Emax = 55.04 ± 1.24% and pD2 = 4.37 ± 0,09), in endothelium-denuded rings, the relaxant effect induced was not changed (Emax = 60.03 ± 2.64% and pD2 = 4.46 ± 0,08), suggesting that the vasorelaxant effect of the extract is mediated endothelium independent mechanisms. To check the participation of K^+channels in the mechanism of relaxation of this substance, the mesenteric rings were contracted with KCl 80 mM and cumulative relaxation responses were obtained by adding of WE011. Contraction with KCl 80 mM significantly attenuated the maximal relaxations to WE011 in endothelium-intact mesenteric rings (Emax = 6.5 ± 2.4 %) as wells as endothelium-denude (Emax = 8.15 ± 1.44%), compared with that endothelium-intact and endothelium-denuded mesenteric rings that were pre-contracted Phe (10^{-6}M). This results confirms that the presences of endothelium is not essential for relaxant response expression and suggest the involvement of channels in effect.

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06.057

PARTICIPATION OF REACTIVE OXYGEN SPECIES AND METABOLITES DERIVED FROM CYCLOOXYGENASE IN THE CONTRACTILE RESPONSE INDUCED BY ENDOTHELIN-1 IN CORPUS CAVERNOSUM FROM ETHANOL-TREATED RAT

Leite LN[1], Tirapelli CR[2] - [1]FMRP-USP, [2]EERP-USP

Introduction: Endothelin-1 (ET-1) is a vasoconstrictor peptide that plays an important role in controlling the tone of the cavernosal smooth muscle (CSM). However, it has been demonstrated that this peptide is also involved in erectile dysfunction (ED) associated with diabetes mellitus and hypertension. The vascular damage caused by ethanol involves the generation of reactive oxygen species (ROS). This study aimed to investigate the cellular and functional consequences of chronic ethanol consumption on the endothelinergic system in penile circulation as well as the involvement of ROS in this response. **Methods**: The experimental protocols were approved by the Ethical Committee from USP (10.1.1084.53.6). Male Wistar rats were treated with ethanol (20% vol/vol) for 6 weeks. Reactivity experiments were performed on isolated CSM. Systemic oxidative stress was evaluated by measuring plasma thiobarbituric acid-reacting substances (TBARS) and superoxide anion levels in CSM homogenates evaluated by lucigenin-derived chemiluminescence assay. Superoxide dismutase (SOD) activity in the CSM was determined by spectrophotometry. **Results**: ET-1-induced contraction was significantly higher in cavernosal strips from ethanol-treated rats (32 ± 2.8% KCl 120mM; n=7) when compared to control (21.5 ± 1.5% KCl 120mM; n=6) ($P<0.05$, Student's t test). In presence of Tiron and polyethylene glycol (PEG)-catalase were observed increase in E_{max} values for ET-1 in CSM from control rats (Tiron E_{max}: 65.1±6.7% KCl 120mM; n=5; PEG-catalase E_{max}: 43.2±2.2% KCl 120mM; n=5). In addition, ET-1-induced contraction was significantly increase by Tiron (E_{max}: 53.2±4.5% KCl 120mM; n=5), DMTU (E_{max}: 54.2±1.8% KCl 120mM; n=5), SC-560 (E_{max}: 55.8±1.6% KCl 120mM; n=5) and SC-236 (E_{max}: 50.7±7.3% KCl 120mM; n=6) in CSM from ethanol-treated rats ($P<0.05$, ANOVA). Plasma TBARS levels were significantly increased in ethanol-treated rats compared with control rats. Lucigenin-derived luminescence and SOD activity were significantly higher in CSM from ethanol-treated rats ($P<0.05$, Student's t test). **Discussion:** Reactive oxygen species (ROS) and metabolites derived from cyclooxygenase (COX) modulate negatively ET-1-induced contraction and appear to be important mediators of ethanol-induced ET-1 hyper-reactivity in the isolated CSM. Our results show that chronic ethanol consumption increases ET-1 induced contraction in isolated CSM and that this response is mediated by an increase in ROS generation. **Financial Support:** CAPES, FAPESP.

06.058

MINERALOCORTICOID RECEPTOR AND G PROTEIN-COUPLED ESTROGEN RECEPTOR MEDIATE THE VASCULAR EFFECTS OF ALDOSTERONE IN FEMALE MICE WITH TYPE TWO DIABETES.

Ferreira NS[1], Cau SBA[2], Manzato CP[1], Silva MAB[1], Tostes RC[1] - [1]University of Sao Paulo - Department of Pharmacology, [2]Federal University of Juiz de Fora - Department Basic - Health Area

Introduction: Aldosterone excess aggravates endothelial dysfunction in diabetes. In addition to binding to mineralocorticoid receptor (MR), aldosterone also activates G protein-coupled estrogen receptor (GPER). The aim of this study was to evaluate the role of MR and GPER on aldosterone-induced changes in vascular reactivity. The project was approved by the local Ethics Committee (protocol: 012/2013-1). Methods: Second-order mesenteric arteries from control (C57BL6) and diabetic (db/db) female mice were incubated with 10 nM aldosterone and the effects on vascular reactivity to phenylephrine (Phe) were determined. The vascular effects of aldosterone were also determined in the presence of MR antagonist, eplerenone 10 μM and GPER antagonist, G15 1μM. The results are presented as Mean±SEM, N. Results and discussion: Aldosterone 10 nM did not alter Phe potency (EC_{50}) (p> 0.05), but increased Phe maximal response (Emax) in 12% (Emax: 114.9±4.2; 5 *vs*. 129.4±2.2; 5 p<0.05) in arteries from control mice. Aldosterone did not change Phe potency or maximum response in arteries from db/db mice (p>0.05). In vessels from control animals, the MR antagonist did not alter Phe potency or maximum response either in the absence or presence of aldosterone (p>0.05). Interestingly, in arteries from db/db mice, the MR antagonist decreased Phe potency both in the absence (EC_{50}:6.6±0.1;8 *vs*. 7.0±0.2;7 p<0.05) and presence (EC_{50}:6.6±0.1;7 *vs*. 7.0±0.2;7 p<0.05) of aldosterone. Eplerenone also decreased Phe maximum response by 23% when compared to the response in the presence of aldosterone (Emax: 134.7±9.4;7 *vs*. 103.7±9.2; 7 p<0.05). In db/db mice, eplerenone and aldosterona decreased Phe responses when compared with arteries incubated with only aldosterone (Emax: 134.7±9.3;8 *vs*. 101.1±8.8; 7 p<0.05). In arteries from control mice, the GPER antagonist G15 did not alter Phe potency, but abrogated the increase of the maximum response induced by aldosterone (Emax: 129.4±2.17; *vs*. 115.8±4.0;4 p<0.05), indicating that aldosterone-induced changes in vascular reactivity are mediated by the activation of GPER. In db/db mice, the GPER antagonist did not alter Phe potency, but reduced Phe maximum response both in vessels incubated with vehicle (Emax: 134.1±10.8;8 *vs*. 99.3±8.5;6 p<0.05) and aldosterone (Emax: 134.7±9.4;7 *vs*. 114.2±10.1;6 p<0.05), suggesting that aldosterone-induced changes in vascular function are mediated by both MR and GPER. Conclusions: Aldosterone acutely increases contractile responses to phenylephrine in mesenteric arteries from control animals by GPER-dependent mechanisms. In diabetic animals, both MR and GPER seem to contribute to aldosterone effects in the vasculature. Both MR and GPER seem to be activated, even in the absence of exogenous aldosterone in arteries from db/db mice, contributing to the increased contraction to phenylephrine. Financial Support: CAPES, CNPq.

06.059

CHRONIC TREATMENT WITH FLUOXETINE POTENTIATES SYMPATHETIC NEUROTRANSMISSION AND REDUCES ALPHA1-ADRENERGIC CONTRACTILE RESPONSES IN RAT MESENTERIC ARTERIAL BED

Pereira CA[1], Resstel LBM[1], Tostes RC[1] - [1]Universidade de São Paulo - Farmacologia

Introduction: Fluoxetine is a selective inhibitor of serotonin reuptake and has been widely used in the treatment of depression. Fluoxetine has several adverse effects on the cardiovascular system, altering the cardiac function and the vasomotor tone. Rats chronically treated with fluoxetine exhibit increased blood pressure and a reduction of the sympathetic component of the baroreflex, indicating a possible increase in the vasomotor tone or an imbalance in factors that control the vascular tone. **Objective**: The aim of this study was to evaluate whether vascular reactivity is increased after chronic treatment with fluoxetine. **Methods**: We used *Wistar* rats, 230-270 g, which were treated either with: (i) vehicle (water for 21 days) or (ii) fluoxetine (fluoxetine 10 mg/ kg/day for 21 days in the drinking water). At day 21 of treatment, rats were anesthetized and the mesenteric arterial bed was isolated. The preparation was placed in a chamber containing physiological Krebs solution and connected to a perfusion system and a pressure transducer for continuous recording of mesenteric perfusion pressure. The Krebs solution was bubbled (95% O2 and 5% CO2), warmed at 37ºC and the preparations were perfused at a constant flow (4 mL/min). After a stabilization period (30 minutes) dose-response curves to phenylephrine (0.0003 to 10 µmol) or KCl (30, 45 and 60 mM) in the absence and presence of Prasozin (10 nM) were performed. Frequency-response curves to periarterial nerve stimulation (PNS) (7-30 Hz, 5 ms, 34 V) were also performed. Data are presented as (mean ± standard error of mean, N). **Results**: Chronic treatment with fluoxetine reduced phenylephrine maximum response [Emax (mmHg) 83.4 ± 3.4 *vs* 60.3 ± 2.0] and potency [(pD_2) 7.8 ± 0.1 *vs* 6.9 ± 0.1] (n = 5, p <0.05). PNS-induced contractile responses were similar between the groups. KCl-induced vasoconstriction was augmented in rats treated with fluoxetine when compared to preparations from vehicle-treated rats [Emax, 30.2 ± 2.4 vs 44.9 ± 6.4 (n = 7; p <0.05)]. Similar contractile responses to KCl were observed when the concentration-effect curves were performed in the presence of prasozin. **Conclusion**: These results suggest that chronic treatment with fluoxetine enhances sympathetic neurotransmission and downregulates alpha1-adrenergic signaling. Financial Support: CAPES, CNPq.

Protocol of Animal Use Ethic Committee: 013/2013

06.060

CHEMERIN DECREASES INSULIN-INDUCED VASODILATION BY REDUCING PI3K-AKT AND MAPK SIGNALING.

Neves KB[1], Lobato NS[2], Lopes RAM[3], Mestriner FL[3], Oliveira AM[1], Tostes RC[3] - [1]FCFRP - USP - Pharmaceutical Sciences, [2]UFG - Biological Sciences, [3]FMRP - USP - Pharmacology

Introduction: The adipokine chemerin has been linked to key aspects of the metabolic syndrome. Proinflammatory adipokines are strongly related to insulin resistance. Although chemerin decreases insulin sensitivity and promotes vascular dysfunction, the effects of chemerin on vascular responsiveness to insulin have not been investigated. We hypothesized that the adipokine chemerin decreases the responsiveness of mesenteric resistance arteries to the actions of insulin by reducing PI3K/Akt signaling. **Methods:** Isometric force was recorded in endothelium-intact mesenteric arteries in a DMT wire myograph system. Vessels were incubated with chemerin (0.5 ng/mL; 1 hour) or vehicle (phosphate buffer saline [PBS] 0.1% bovine serum albumin [BSA]) and vasodilatation to insulin (0.1 **-** 3000 ng/mL) or acetylcholine (ACh,10^{-9}-3x10^{-5} mol/L) was determined. Vessels were also incubated for 30 minutes with the PI3K activator (YS-49, 10^{-6} mol/L), p38 inhibitor (SB203580, 10^{-6} mol/L), JNK inhibitor (SP600125, 10^{-6} mol/L), or ERK1/2 inhibitor (PD98059, 10^{-6} mol/L). PI3K and Akt protein expression was determined in vascular smooth muscle cells (VSMC) incubated with chemerin (0.5ng/mL, 1 hour) by western blotting. The measurement of glucose uptake was determined in serum-starved VSMC stimulated with vehicle or chemerin (0.5ng/mL, 1 hour) by measurement of insulin-induced [^{3}H]2-deoxy-glucose (2DG) uptake. **Results:** The adipokine chemerin decreases the relaxation responses to insulin (pD_2: vehicle= 23.60±0,10; chemerin 0.5 ng/mL= 94.50±0.13, Emax: vehicle= 100.20±4.36, chemerin 0.5 ng/mL= 90.83±7.24) and ACh (pD_2: vehicle= 6.61±0.06; chemerin 0.5 ng/mL= 5.23±0.08, Emax: vehicle= 93.66±3.57, chemerin 0.5 ng/mL= 68.83±2.11). Chemerin effects on insulin-induced vasodilatation is reverted by YS-49, a PI3K activator (pD_2: vehicle= 23.02±0.05; chemerin= 108.50±0.06, YS-49 + chemerin= 28.46±0.10), and inhibitors of p38 (pD_2: vehicle= 23.02±0.05; chemerin= 108.50±0.06, p38 inhibitor + chemerin= 27.10±0.08) and ERK1/2 (pD_2: vehicle= 24.56±0.07; chemerin= 112.05±0.07, ERK1/2 inhibitor + chemerin= 25.38±0.08) but not by JNK inhibitor (pD_2: vehicle= 24.56±0.07; chemerin= 112.05±0.07, JNK inhibitor + chemerin= 94.00±0.07). Chemerin also decreases PI3K (arbitrary units [a.u.]: vehicle= 1.11±0.08; chemerin= 0.79±0.09) and Akt (arbitrary units [a.u.]: vehicle= 1.24±0.11; chemerin= 0.81±0.10) phosphorylation in VSMC. Furthermore, chemerin pretreatment for 1 hour inhibits insulin-stimulated glucose uptake by VSMCs (counts per minute [cpm]: vehicle= 9923±662.7; chemerin= 5668±729.0). **Discussion:** In the present study, we demonstrated that chemerin decreases insulin-induced vasodilatation via a reduction of PI3K/Akt pathway and p38 and ERK1/2 signaling. In addition, chemerin inhibited insulin-induced glucose uptake by VSMCs. These results indicate a mechanism for chemerin-induced insulin resistance and suggest that chemerin may be involved in the pathogenesis of vascular insulin resistance. Our study may contribute to a better understanding of the role of factors released by the visceral adipose tissue on insulin vascular responsiveness and, consequently, on the vascular dysfunction in obesity and obesity-associated diseases. **Financial Support:** FAPESP (2012/13144-8). **Protocol of the Animal Use Ethic:** 12.1.1593.53.0.

06.061

Maternal fluoxetine treatment during pregnancy and lactation increased the endothelium modulation of vasoconstriction in female adult offspring

Silva MA[1], Gerardin DC[1], Moreira EG[1], Pelosi GG[1], Akamine EH[2], Carvalho MHC[2], Ceravolo GS[1] - [1]Universidade Estadual de Londrina - Departamento de Ciências Fisiológicas, [2]Universidade de São Paulo - Departamento de Farmacologia, Instituto de ciências biomédicas

Introduction: Fluoxetine (FLX) is commonly used to treat depressive disorders during pregnancy. Since FLX crosses the placenta and is excreted in milk, maternal treatment with this antidepressant may expose the fetus and neonate to increased levels of serotonin (5-HT). Prenatal exposure to FLX has been associated with a range of adverse neonatal outcomes, including cardiac malformations, persistent pulmonary hypertension and vascular hypertrophy. The aim of the present study was to evaluate if maternal exposure to FLX during pregnancy and lactation would induce aorta reactivity alteration in adult rats. **Methods:** Male and female Wistar rats (75 days old) whose progenitor had received by gavage: FLX (5mg/kg, FLX offspring) or water (control offspring - C) where anesthetized, aorta was removed, cut in two rings, one with (E+) and other without (E-) endothelium. Concentration-effect curves phenylephrine (Phe) were performed in aortic rings. The results were expressed as media±SEM of maximal response (Rmax:g) to Phe. Statistical analysis was carried out using one way ANOVA with Tukey's correction for multiple comparisons among the groups. Values were considered statistically significant when $P<0.05$. The procedures were approved by the ethics committee of animal use (CEUA nº 16166.2012.12). **Results:** The Phe induce contraction on E+ rings was reduced in FLX female rats, when compared to C female rats (Rmax: C: 2.5±0.1 vs. FLX: 1.9± 0.1; n=10/group) and the removal of endothelium abolished this difference (Rmax: C: 3.0± 0.2 vs. FLX: 3.3± 0.2, n=10). In male rats, the aortic response to Phe was similar between the FLX and C groups, in the presence or absence of endothelium (n=8/group). **Conclusion:** Our data suggested that the exposure to FLX and during development induced alteration in contractile response of female adult rats' aorta, probably by an endothelium-dependent mechanism.

Key words: Fluoxetine, aorta, vascular reactivity, pregnancy, lactation.

06.062

Effects of dichloromethane extract of Eugenia punicifolia and derivatives in smooth muscle

Teixeira RGS[1], Pascual R[2], Araújo KGL[1], Gandía L[2], Silva CLM[3], Santos WC[1] - [1]UNIVERSIDADE FEDERAL FLUMINENSE, [2]Universidad Autónoma de Madrid, [3]UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

Introduction: We have recently described the isolation and identification of the triterpene barbinervic acid (3α,19α,24 trihydroxyurs-12-en-28-oic acid) from dichloromethane extract of *E. Punicifolia*. Our previous results indicated that some molecules from *E. punicifolia* might have neuroprotective effects; considering that there is a correlation between neuroprotection and the nitrergic pathway and given that rat aorta and renal vasculature are biological preparations modulated by nitric oxide (NO) released from both nitrergic nerves or generated by autonomic stimulation, we therefore designed experiments to test dichloromethane extract and barbinervic acid in rat aorta and renal circulation.

Material and Methods: Plant was collected at Manaus, AM, by using *Centro de Instrução de Guerra na Selva (CIGS)* facilities. Male Wistar rats of nearby 300 g were killed by euthanasia, according *CEUA-UFF* recommendations and thoracic aorta was removed and cleaned up from tissue connections. Strips were dissected and mounted in 15 mL organ bath filled with 36ºC Krebs-Hepes solution, gassed with a mixture of 95% O_2 e 5% CO_2. After stabilization, 1 μM phenylephrine was added for tonus. Changes in isometric tension by chemicals were recorded by transducers and transferred to a data acquisition system. For experiments in renal circulation, male rat SD rats of nearby 300 g were anesthetized and renal artery and vena were canulated and perfunded with Krebs-Hepes solution. Vascular tonus was induced by 1 μM phenylephrine. After stabilization, drug cumulative concentration response curves on phenylephrine effect were constructed. All procedures described were reviewed and approved by the Ethics Committee for the Use of Animals of the Universidade Federal Fluminense (CEUA-UFF) (Project No. 364/2013).

Results and Discussion: In 1 μM phenylephrine pre contracted rat aorta, cumulative addition of *E. punicifolia* dichloromethane extract relaxed the aorta up to 77% (EC_{50} 0.1mg/mL). Addition of barbinervic acid, but not of the extract in renal circulation reduced renal pressure up to 10.7% of control (100 μM of barbinervic acid). 100 μM L-NAME previous added to the bath totally reverted effect of the triterpene. 10 μM ODQ also entirely reverted the effect.

Conclusion: The results pointed to a strong possibility of *Eugenia punicifolia* as a source of molecules with action on cardiovascular system, likely with interactions in the nitrergic pathway. However, to elucidate this action on cardiovascular system more data is necessary.

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06.063

Biophysical and pharmacological characterization of a new and efficient agonist of the high-conductance BK potassium channels.

Duarte BO[1], Suarez-Kurtz G[2], Nascimento JHM[3], Ponte CG[1] - [1]IFRJ - Núcleo de Ciências Químicas - NCQ - Campus Rio de Janeiro, [2]INCA - Programa de Farmacologia, [3]UFRJ - Laboratório de Eletrofisiologia Cardíaca

Potassium channels present high therapeutic potential since they regulate several cellular functions. Once activated, the BK-high conductance potassium channels lead to smooth muscle cells hyperpolarization that causes relaxation. Based on this property, BK channels have been considered to be suitable therapeutic targets for the treatment of diseases in which smooth muscle hyperactivity is involved, such as arterial hypertension (AH) and urinary incontinence (UI).

Recently, a new family of tetrahydroquinolines has been characterized as BK channels agonists, specially the “Z” (3aR,4S,9bS)-4-(Naphthalen-1-yl)-3a,4,5,9btetrahydro-3H-cyclopenta[c]quinoline-8-carboxylic Acid and the “Y” ((3aS,4R,9bR) -4-(Naphthalen-1-yl)-3a,4,5,9btetrahydro-3H-cyclopenta[c]quinoline-8-carboxylic Acidenantiomers[1]. Using the CHO-BK cell line and patch clamp, we determined the EC_{50} values for the activation of BK channels by compounds Z and Y (2.2 ± 0.12 and 31.6 ± 0.35 mM, respectively). When the BK channel activity was recorded using the *inside in* patch clamp configuration, compound Z did not affect the conductance and did alter the channel’s voltage sensitivity in the range around -90 mV. Using current clamp configuration, we observed that the CHO-BK resting membrane potential increased from -23.1 ± 3.6 mV in the absence to -58.2 ± 6.6 mV in the presence of compound Z (1 mM). Our results provide the first evidence of how these tetrahydroquinoline compounds regulate the BK potassium channel.

References: [1] - PONTE CG, Mol Pharmacol 81:567–577, 2012.

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06.064 Oxidation of DHA is responsible for its anti-arrhythmic effects on mouse ventricular myocytes. Roy J, Olivia TM, Roussel J, Oger C, Galano JM, Pinot E, Durand T, Le Guennec JY INSERM – Physiology cardiovascular

The cardioprotective effects through prevention of cardiac arrhythmias of long-chain polyunsaturated fatty acids of the n-3 series (PUFAs) have been demonstrated over the last 40 years. The main n-3 PUFAs are eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3, DHA) and both are highly peroxidable due to the presence of skipped dienes. The effects of n-3 PUFAs on cardiac function are controversial, notably due to lack of information on the mechanisms involved. Particularly, it is not well understood which is the active lipid: the PUFA or one of its oxygenated metabolites. A diet enriched in n-3 PUFAs (mainly fish-based), leads to enrichment in these fatty acids of cardiac cell membranes. Our hypothesis is that, after an infarct, the oxidative stress and the genesis of reactive oxygen species (ROS) cause an oxidation of membrane-bound PUFAs. Thus, the oxygenated metabolites generated could modulate the activity of ionic channels to exert anti-arrhythmic effects[4]. We thus decided to investigate the influence of the peroxidation of DHA on its potentially anti-arrhythmic properties. In this study, we applied DHA on freshly isolated mouse ventricular myocytes without or with α-tocopherol (Vitamin E, to prevent oxidation) or hydrogen peroxide (to enhance oxidation). We investigated, using a photometric system, calcium transients (using the ratiometric calcium fluorescent dye Indo-1) and cell shortening of electrically stimulated myocytes. By stimulating β-adrenergic pathways with 10 nM isoproterenol, it is possible to observe the occurence of arrhythmic events. We observed that DHA reduced the percentage of arrhythmic cells. The effects of DHA are correlated with the peroxidation of the fatty acid since α-tocopherol prevented the anti-arrhythmic effects while hydrogen peroxide enhanced them. In resting cells, single RyR channels spontaneously open with a very low frequency. An increased frequency of these events can cause propagating calcium waves that can lead to arrhythmias. The spontaneous calcium released events named sparks, can be observed by confocal microscopy. Here again, we observed that the frequency of calcium sparks is negatively correlated with the oxidative status of the DHA solution. These results suggest thus that rather than DHA itself, it is one or more non-enzymatic oxygenated metabolites derived from DHA that are potentially anti-arrhythmic such as neuroprostanes and/or neurofuranes[4]. 1 Bang H., Dyerberg J. & Nielsen A. (1971) Plasma lipid and lipoprotein pattern in Greenlandic west-coat eskimos. Lancet. 1: 1143-1145. 2 Jahn, U., Galano, J-M., Durand, T. (2008). Beyond prostaglandins chemistry and biology of cyclic oxygenated metabolites formed by free-radical pathways from polyunsaturated fatty. Angew Chem Int Ed Eng 47, 5894-5955 3 Saravanan P., Davidson N., Schmidt E. & Calder P. (2010). Cardiovascular effects of marine omega-3 fatty acids. Lancet 376, 540-550. 4 Judé, S. Bedut, S., Roger, S., Pinault, M., Champeroux, P., White, E., Le Guennec, JY. (2003). Peroxidation of docosahexaenoic acid is responsible for its effects on I_{TO} and I_{SS} in rat ventricular myocytes. Br. J. Pharmacol. 139, 816-822.