06. Cardiovascular and Renal Pharmacology

06.001 Renal effects and gender differences in aged Dahl salt sensitive rats. Costa PHS[1], Jorge ARC[2], Martins ICMT[2], Santos CF[2], Nascimento NRF[2], Monteiro HSA[1], Fonteles MC[1,2]
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Introduction: Dahl salt rats are a genetic model of salt sensitivity hypertension that affects the renal and cardiovascular systems. Some studies in Dahl salt sensitive (DS) rats have shown that blood pressure is higher in female than in male at similar ages after menopause. This observation suggests that loss of sex hormones contributes to the development and progression of renal disease. To evaluate possible renal differences of mature DS male and female, in the absence of neural and humoral mechanisms, we have used isolated perfused kidney method. **Methods:** Isolated kidneys from 18-month-old male (M) and female (F) DS rats weighing 300 to 360g (n=9) were perfused with Krebs-Henseleit solution containing 6g% of bovine serum albumin previously dialyzed for 24 hours and perfused for 120 minutes. The gender differences were studied on glomerular filtration rate (GFR), urinary flow (UF), perfusion pressure (PP), renal vascular resistance (RVR) and percentage of total transport of sodium (%TNa^+) and potassium (%TK^+) at 30, 60, 90 and 120 minutes of experiment. All data were analyzed by unpaired t test with level of significance of *p<0.05. The experimental protocols were approved by the State University of Ceará Animal Research Ethical Committee, license number of 11516758-7/61. **Results:** Females had increased PP at 90 and 120 minutes ($PP_{90}F$=156.00 ± 7.00 vs. PP90M=148.40 ± 2.30; $PP_{120}F$=157,80 ± 7.98 vs. $PP_{120}M$=149.60 ± 3.09 mmHg.$mL^{-1}.g^{-1}$). RVR was raised in females at all evaluated times ($RVR_{30}F$=7.89 ± 0,91 vs. $RVR_{30}M$=3.80 ± 0.46;$RVR_{60}F$=8.57 ± 1.023 vs. $RVR_{60}M$=3.41 ± 0,47; $RVR_{90}F$=8.99 ± 0.97 vs. $RVR_{90}M$= 3.51 ± 0.41; $RVR_{120}F$=10.02 ± 0.98 vs. $RVR_{120}M$=3.59 ± 0.46 mmHg/mL.$g^{-1}.min^{-1}$). In addition, female had increased %TNa^+ also at all evaluated times (%$TNa^+_{30}F$=77.67 ± 7.45 vs. %$TNa^+_{30}M$=69.75 ± 1.21; %$TNa^+_{60}F$=84.47 ± 5.81 vs. %$TNa^+_{60}M$=42.42 ± 3.34; %$TNa^+_{90}F$=77.20 ± 5.8 vs. %$TNa^+_{90}M$=44.86 ± 2.64; %$TNa^+_{120}F$= 65.67 ± 6.22 vs. %$TNa^+_{120}M$=41.14 ± 2.10). On the other hand, female rats had decreased UF at all the periods ($UF_{30}F$=0.06 ± $UF_{30}M$ vs. 0.11 ± 0.03; $UF_{60}F$=0.06 ± 0.01 vs. $UF_{60}M$= 0.12 ± 0.01; $UF_{90}F$=0.05 ± 0.01 vs. $UF_{90}M$=0.10 ± 0.01; $UF_{120}F$=0.01 ± 0.009 vs. $UF_{120}M$= 0,11 ± 0.006 mL.$g^{-1}.min^{-1}$). GFR was diminished at 30 minutes in female rats ($GFR_{30}F$=0.13 ± 0.02 vs. $GFR_{30}M$=0.37 ± 0.10 mL.g^{-1}.min-^{1}). Finally, female had decreased %TK^+ at all the times (%$TK^+_{30}F$=21.10 ± 13.55 vs. %$TK^+_{30}M$=74.75 ± 2.61; %$TK^+_{60}F$=2.93 ± 9.92 vs. $TK^+_{60}M$=55.61 ± 3.83; %$TK^+_{90}F$=26.00 ± 8.98 vs. %$TK^+_{90}M$=47.18 ± 2.87; %$TK^+_{120}F$=9.18 ± 11.75 vs. %$TK^+_{120}M$= 37.29 ± 4.52). **Conclusion:** In rats, menopause starts around 15 months of age. Because of the age of our animals, it is possible that both changes in glomerular and tubular functions in females are related to estradiol deficit. In addition, some studies have shown that renin-angiotensin-aldosterone system (RAAS) is upregulated in menopause. Thus, it is feasible that excess of RAAS components, mainly angiotensin II and aldosterone, may act by increase of RVR and changing %TNa^+ and %TK^+ in female DS rats. **Financial support:** CNPQ, CAPES and FUNCAP.

06.002 High fat diet (HFD)-induced mitochondrial oxidative stress in the PVAT promotes loss of its anticontractile effects by activation of RhoA/Rho kinase signaling. Costa RM, Fais RS, Dechandt CR, Alberici LC, Lobato NS, Tostes RC

Introduction: Obesity is associated with structural and functional changes in the PVAT, favoring the release of reactive oxygen species (ROS), vasoconstrictor and proinflammatory substances. Recent studies have shown that ROS caused the activation of RhoA/Rho kinase pathway. We tested the hypothesis that obesity-associated PVAT dysfunction is mediated by augmented mitochondrial ROS (mROS) generation and activation of RhoA/Rho kinase, redox-sensitive proteins. **Methods:** C57Bl/6J mice received control or high fat diet for 18 weeks. Vascular function was assessed in endothelium-denuded thoracic aortic rings in the presence or absence of PVAT. Concentration-effect curves for phenylephrine were performed. The groups were divided into: Control PVAT (-), Control PVAT (+), Obese PVAT (-) and Obese PVAT (+). ROS generation was measured by DHE and Amplex Red. The contribution of mitochondrial ROS to vascular changes promoted by PVAT was evaluated by using a mitochondrial uncoupler (CCCP 10^{-6} M) and an antioxidant (Peg-catalase 100 U/mL). Mitochondrial function was determined by the cellular respiration rate. RhoA inhibitor (Y27632 10^{-4} M) was used to assess the involvement of RhoA/Rho kinase in vascular reactivity. **Results:** PVAT decreased contractile responses in control aortas [(mN) Control PVAT (-): 5.6 ± 0.1 *vs*. Control PVAT (+): 4.2 ± 0.1, n=6]. Obesity promoted loss of PVAT anticontractile effect [(mN) Obese PVAT (-): 6.1 ± 0.1 *vs*. Obese PVAT (+): 6.1 ± 0.2, n=6] and increased PVAT ROS [DHE (arbitrary units (A.U.) – Control: 16.1 ± 1.9 *vs*. Obese: 27.1 ± 1.3, n=5; Amplex Red (A.U.) – Control: 16050 ± 1062, *vs*. Obese: 29173 ± 1896, n=8 and 6]. Peg-catalase increased contractile response in control aortas with PVAT [(mN) Control PVAT (+): 4.2 ± 0.1 *vs*. Control PVAT (+)-Pegcat: 5.3 ± 0.1, n=6]. However, Peg-catalase abolished increased contraction in aortas from obese mice [(pD_2) Obese PVAT (+): 7.3 ± 0.1 *vs*. Obese PVAT (+)-Pegcat: 6.4 ± 0.1, n=6 and 5]. Obesity also reduced basal PVAT mitochondrial respiration [(O_2 pmol.mg^{-1}.sec^{-1}) Control: 45.2 ± 1.9 *vs*. Obese: 23.2 ± 2.1, n=8 and 6]. Y27632 decreased contraction only in control aortas without PVAT [(mN) Control PVAT (-): 5.6 ± 0.1 *vs*. Control PVAT (-)-Y27632: 4.7 ± 0.4, n=6; Control PVAT (+): 4.1 ± 0.1 *vs*. Control PVAT (+)-Y27632: 4.0 ± 0.3, n=6]. Y27632 decreased contractions in aortas from obese mice with or without PVAT [(mN) Obese PVAT (-): 6.1 ± 0.3 *vs*. Obese PVAT (-)-Y27632: 5.1 ± 0.4, n=6; Obese PVAT (+): 6.2 ± 0.4 *vs*. Obese PVAT (+)-Y27632: 4.5 ± 0.3, n=6]. **Conclusion:** PVAT negatively modulates vascular contractions. PVAT anticontractile effects are abolished in obesity. Obesity induces PVAT mitochondrial dysfunction, culminating in increased ROS generation and augmented vascular contractility. RhoA/Rho kinase signaling in vascular smooth muscle contributes to PVAT-mediated increased vascular reactivity in obesity. **Financial support:** CAPES, CNPq, FAPESP. **Ethics committee:** Protocol nº 149/2014 FMRP.

06.003 NACHT, LRR and PYD domains-containing protein 3 (NLRP3) mediates endothelin-1- (ET-1)-induced contractile response sensitization in mice cavernous tissue.

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Introduction: Erectile function depends on the tone of the cavernous tissue (CT), and increased contraction and/or decreased relaxation responses contribute to erectile dysfunction. Endothelin-1 (ET-1) maintains the penis in the flaccid state and acts as an important mediator of chronic inflammation. NLRP3 is an innate immune system receptor responsible for the release of pro-inflammatory cytokines that induce inflammation and vascular dysfunction. **HYPOTHESIS:** ET-1-induced phenylephrine (PE) contractile responses sensitization in cavernous tissue is mediated by NLRP3 and enhanced activity of the RhoA/Rho-kinase pathway. **AIM:** To determine the effect of ET-1 incubation for 4 hours on the contraction induced by PE in CT. **Methods:** Contractile responses to PE were measured in CT of Wild-type (WT) and NLRP3 knockout mice ($NLRP3^{-/-}$) after incubation with ET-1 (0.1 uM) for 4 hours. MYPT1 activity was assessed by western blot. **Main Outcome Measures:** Potency (pD2) and maximal response (Emax) of PE cumulative concentration-response curves were compared between treatments. **Results:** ET-1 promotes increased contraction to PE and increased MYPT-1 activity in WT, but not in $NLRP3^{-/-}$ CT. **Conclusion:** NLRP3 contributes to ET-1-induced PE contractile responses sensitization in mice CT, possibly through the activation of the RhoA/Rho-kinase pathway. **Keywords:** Erectile dysfunction, Endothelin-1, NLRP3, Inflammation. **Financial Support**: CAPES, CNPQ, FAPESP, FAEPA. Research approval by the Animal Research Ethical Committee: CEUA 005/2015-1

06.004 Estrogen effects on cardiovascular and oxidative parameters of female rats under LPS endotoxemia: NO participation Castardo-de Paula JC[1], de Campos BH[1], de Jager L[1], Zalunqui NG[2], Pinge-Filho P[2], de Farias CC[3], Higachi L[3], Barbosa DS[3], Martins-Pinge MC[1]
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Introduction: It is recognized that gender differences exist in cardiovascular function and also in cardiovascular disease, and also, estrogen increases the bioavailability of nitric oxide (NO). However, no study has evaluated the interaction of both on cardiovascular and oxidative parameters during endotoxemia. **Methods**: Female wistar rats (220 to 270g) were subjected to ovariectomy or false surgery and divided into 3 groups: OVX (ovariectomized rats), OVX+E (ovariectomized plus estradiol treatment, 1 mg/Kg/day, p.o.) and SHAM (false surgery). After 8 weeks animals were submitted to artery and vein catheterization for blood pressure and heart rate recordings, while awake and freely moving, before and after LPS injection (5mg/Kg, IV) preceded by the injection of S-methylisothiourea sulphate (SMT; 3 mg/kg) or sterile saline (0,9%). Cardiovascular recordings underwent spectral analysis for evaluation of autonomic modulation. 2 hours after LPS injection, blood samples were collected for plasmatic measurements of the total radical-trapping antioxidant (TRAP), nitrite levels (NO_2), liperoxidation (LOOH) and Paraoxonase 1 (PON1) activity. Statistical analysis: RM-ANOVA or 1way-ANOVA, post-hoc Bonferroni's test. Mean ± SEM, significance level of 95%. **Results**: 2 hours after LPS, OVX+E treated with SMT presented a decrease of MAP, when compared to SHAM and to MAP at time zero (ΔMAP, mmHg: SHAM= 0,8 ± 3, OVX= -8,3 ± 3, OVX+E= -13,3 ± 4, n=8-9 per group). At this same time, all SMT+LPS groups presented, in percentage, an increase of sympathetic modulation and a decrease of parasympathetic modulation of HR, showed respectively by LFnu, LF/HF and HFnu parameters of HR variability. 2 hours after saline+LPS, OVX presented decreased TRAP when compared to SHAM (TRAP, uM trolox: SHAM= 379 ± 24, OVX= 153 ± 8, OVX+E= 312 ± 34). When treated with SMT+LPS, OVX did not presented altered TRAP after 2 hours, while estradiol presented reduced LOOH levels ($x10^3$ RLU: SHAM SMT+LPS= 4714 ± 563, OVX+E SMT+LPS= 1991 ± 310)**. Conclusion**: Since sympathetic activity is important to cardiac recovery in sepsis, our findings suggest that, in the cardiovascular system, treatment with estradiol could be protective in inflammatory challenges, since iNOS is inhibited. **Financial support**: CNPq. Institucional Animal Care Committe Protocol number: 276.2013.81.

06.005 A new nitric oxide generator induces a vasodilation in aorta and coronary and is able to reduce the blood pressure in normotensive and hypertensive rats. de Moraes TF[1], Rodrigues CNS[1], Oishi JC[1], Vatanabe IP[1], Rodrigues GJ[1 1]Universidade Federal de São Carlos – UFSCar – Ciências Fisiológicas

Introduction: Nitric oxide (NO) is a potent vasodilator and it can be generated by the ruthenium complex cis-[Ru(H-dcbpy$^-$)2(Cl)(NO$^-_2$)] (DCBPY). In aortas from hypertensive rats some NO donor presents lower potency in inducing relaxation. In addition, in previous study was verified that the relaxation to sodium nitroprusside is potentiated in the presence of endothelium. Thus, this study aimed to investigate the vasodilation effect of DCBPY in aorta and coronary artery (with and without endothelium) from normotensive and hypertensive rats and if this compound is able to induces a reduction on blood pressure in normotensive and hypertensive rats. **Methods:** Normotensive (2K) and hypertensive (2K-1C) wistar rats were used. To vascular reactivity study, aorta or coronary artery were isolated cleaned and mounted in a myograph. The relaxation was performed to DCBPY in pré-contracted vessels with phenylephrine to aorta and serotonin to coronary, in the endothelial presence (E+) or absence (E-). The potency (pD2) and maximum relaxant effect (ME) was measured. To direct mean arterial pressure (MAP) measurement, polyethylene canulate were implanted into the abdominal aorta (through the femoral artery, to record the blood pressure) and the iliac vein (to administer drugs), in anesthetized rats. The effect of DCBPY in different doses (0.1, 1.0 and 10.0 mg/kg) was measured. All experimental protocols were approved by the Ethical Committee of UFSCar (nº 4786180915). **Results:** The ruthenium complex DCBPY induced a concentration dependent relaxation in phenylephrine-contracted aortas from 2K (pD2 E-: 5.35 ± 0.13, n= 6; pD2 E+: 5.26 ± 0.07, n=6; ME E-: 112.00 ± 8.00%, n= 6; ME E+: 101.00 ± 3.53%, n=6) and 2K-1C rats (pD2 E-: 5.08 ± 0.13, n=6; pD2 E+: 4.96 0.08, n=8; ME E-: 118.30 ± 9.34%, n=6; ME E+: 104.60 ± 5.36%, n=8). The presence of the endothelium did not modify the effect of DCBPY in inducing the relaxation in aortas from 2K and 2K-1C rats. In coronary, the endothelium potentiated the vasodilator effect of DCBPY just in vessels from 2K (pD2 E+: 5.94 ± 0.26, n=6 > pD2 E-: 4.76 ± 0.27, n=6; $p<0.05$; ME E+: 43.36 ± 5.57, n=6 > ME E-: 22.88 ± 2.70, n=6, $p<0.05$) and not from 2K-1C (pD2 E+: 5.35 ± 0.23, n=6; pD2 E-: 5.55 ± 0.61, n=6; ME E+: 35.39 ± 6.67, n=6; ME E-: 27.76 ± 2.71%, n=5) rats. The efficacy in inducing relaxation to DCBPY is higher in aortic rings them in coronary rings, in the presence or absence of endothelium. The intravenous bolus injection of the DCBPY had an hypotensive effect in 2K (0.1 mg/Kg: 9.88 ± 1.25 mmHg, n= 8, 1.0 mg/Kg: 9.07 ± 0.82 mmHg, n= 8 and 10.0 mg/Kg: 13.69 ± 4.44 mmHg, n= 8) and 2K-1C rats (0.1 mg/Kg: 11.18 ± 2.95 mmHg, n= 8, 1.0 mg/Kg: 14.47 ± 1.93 mmHg, n= 8 < 10.0 mg/Kg: 23.70 ± 2.65 mmHg, n= 8, $p<0.05$). The hypotensive effect to 10.0mg/Kg of DCBPY was higher to 2K-1C than to 2K ($p<0.05$). **Conclusion:** Taken together our results indicate that the compound DCBPY induces a vasodilation in aortic and coronary rings from 2K and 2K-1C rats with no difference on sensibility between 2K and 2K-1C, as well as the intravenous bolus injection of the DCBPY had a hypotensive effect in 2K and 2K-1C rats with higher effect in 2K-1C rats. **Financial support:** FAPESP e CNPq.

06.006 Maternal exposure to fluoxetine reduced aortic contraction in female progeny by mechanism involving nitric oxide and prostacyclin Higashi CM[1], Sartoretto SM, Higachi L, Carvalho MHC, Pelosi GG, Barbosa DS, Gerardin DCC, Moreira EG, Akamine EH, Ceravolo GS[1 1]UEL – Fisiologia e Farmacologia

Introduction: Fluoxetine (FLX) is one of main drugs used for pharmacological treatment of depression during gestation and lactation. FLX is a relatively safe drug, but in humans and experimental animals, it may cause structural and functional alterations in exposed fetuses *in utero* (FORNARO et al., 2007). We have recently demonstrated in rat that maternal treatment with FLX, during gestation and lactation causes aortic hypo-contraction and increases nitric oxide (NO) levels in adult female offspring (HIGASHI et al., 2016). Thus, the aim of the present study was to investigate the mechanism related with this aortic hyporreactivity observed in female rats maternally exposed to FLX during pregnancy and lactation. **Methods**: Female Wistar rats were treated by gavage with: FLX (5mg/kg/day) or water (CTL) during pregnancy and lactation. The experiments were carried out in female offspring at 75 days old. The thoracic aorta was removed and cut in two rings, with (E+) and without (E-) endothelium. Cumulative concentration-effect curves to phenylephrine (Phe: 1nM-30µM) were constructed in the absence or presence of: nonspecific NO synthases (NOS) inhibitor (L-NAME 1µM), or inducible NOS inhibitor (L-NIL 1µM), or catalase (100U/mL), or cyclooxygenases (COX) inhibitor (indomethacin, INDO 10µM), or COX2 inhibitor (NS-398 1µM), or prostacyclin synthase inhibitor (tranylcypromine, tranyl 10µM). NO was measured in plasma by Griess method. Aortic expression of endothelial NOS (eNOS) and COX1 were measured by Western blot (WB) method. **Results** expressed as mean ± SEM of the maximal response (Rmax). (n) is the number of rats/group. Statistical analysis: one or two-way ANOVA and Tukey's test, differences with $p<0.05$. **Results**: Aortic rings E+ from FLX rats (1.52 ± 0.11 (10)) presented reduction in Rmax to Phe when compared to CTL (2.47 ± 0.19 (10)); p=0.0001. Endothelium removal increased Rmax to Phe, eliminating difference in aortic contraction between groups (CTL=3.12 ± 0.17 (10) vs FLX=3.30 ± 0.26 (10)). L-NAME increased (p=0.001) the Rmax to Phe in both groups when compared with their respective E+ rings without L-NAME, (CTL=3.20 ± 0.20 (7); FLX=2.97 ± 0.33 (7)). Neither L-NIL, catalase nor NS-398 interfered with Phe-response in E+ rings from both groups. INDO and tranyl did not interfere with Phe-response in E+ rings from CTL. However, INDO (CTL=2.14 ± 0.08 (7) vs FLX=2.27 ± 0.12 (7)) and tranylcypromine (CTL=2.22 ± 0.23 (7) vs FLX=2.23 ± 0.28 (7)) increased the Rmax to Phe in FLX rats (p=0.005) and equaled Rmax between CTL and FLX. NOx plasmatic levels were increased in FLX group (5.7 ± 0.40 (8)) compared to CTL (3.8 ± 0.22 (7); p=0.0001). There was no difference in eNOS and COX1 expression in aorta between groups. **Conclusion**: FLX exposure during intrauterine and neonatal development causes reduction in contractile aortic response and increased NO plasmatic levels in female adult offspring. The mechanism involved with aortic hyporreactivity depends of endothelium derived NO and prostanoids production probably by eNOS and COX1 increased activity. **Financial support**: CAPES/CNPQ Research approval by the Animal Research Ethical Committee: CEUA nº16166-2012.12

06.007 Participation of TRPM4/TRPM7 channels in the cardiac activities of carvacrol on animals with essential hypertension. Alves QL, Santos PV, Jesus RLC, Oliveira SCDS, Froes TQ, Castilho MS, Silva DF UFBA

Introduction: Carvacrol (CAR), a phenolic monoterpene, has been described to cause hypotension and bradychardia, our studies demonstrated a direct cardiac action of CAR in the contractility and force of isolated atria from spontaneously hypertensive (SHR) and normotensive controls rats (Wistar Kyoto -WKY). Therefore, the aim of this study was to investigate the involvement of TRPM4 and TRPM7 channels in cardiac activities of CAR. **Methods**: The role of TRPM4 and TRPM7 in cardiac activities of CAR was investigated using channels blockers and molecular modeling tools (morphological similarity). The values of $p < 0.05$ were considered significant. **Results**: In studies with isolated right and left atrium, CAR 100µM induced significant negative chronotropic (CE) and inotropic effect (IE), respectively, in both WKY and SHR rats. To investigate the role of TRPM channels in these responses, TRPM4 [lanthanum 100µM (LAN), flufenamic acid 10µM (FF) and 9-phenanthrol 10µM (9-PHE)] and TRPM7 [magnesium 1.5mM (MG)] blockers were used. LAN, FF, 9-PHE and MG significantly attenuated the negative CE of CAR 100µM in SHR, but did not in WKY rats. However, MG significantly reduced heart automaticity in SHR and WKY rats when compared to the control group. Interestingly, CAR in the presence of LAN, FF, 9-PHE and MG significantly induced an additional negative IE in the SHR. Furthermore, these compounds, in the absence of CAR, reduced the cardiac contractile force in SHR when compared to the control group. In WKY left atrium, the effect of CAR was changed only by 9-PHE, but the heart contraction force was significantly reduced in the presence of FF and 9-PHE in the absence of the monoterpene. Additionally, CAR shows 86.62% chemical similarity to 9-PHE, according to SURFLEX-SIM software, and 9-PHE and FF compounds have similar shape and electrostatic potential, as calculated by SYBYL®2.0, furthermore, there are data reporting that CAR inhibits TRPM7 channels in HEK cells (PARNAS et al.; 2009) and inhibits completely TRPM7 current input and output in human atrial myocytes (MACIANSKIENE et al, 2012). **Conclusions**: Taken together, these results suggest that TRPM7 channel seems to influence heart rate and force of contraction, whereas TRPM4 channels influence in the force of contraction, at least at the atrial level. Moreover, CAR, along with magnesium, might act in a signaling pathway modified by hypertension. The in silico results are in good agreement with in vitro data and point out that CAR has all the chemical requirements to bind to TRPM4. **References**: PARNAS, M.; et al. Cell Calcium, v.45, p.300, 2009**;** MACIANSKIENE, R.; et al. J Biomed Sci*,* v.19, p.75, 2012. **Financial Support**: FAPESB/CAPES. Research approval by Animal Research Ethical Committee: CEUA-ICS/UFBA (019/2011).

06.008 Mitochondrial DNA activates NLRP3 inflamassome and contributes to inflammatory response in the vasculature of type 1 diabetic mice. Pereira CA[1], Ferreira NS[1], Zanotto CZ[1], Carlos D[2], Tostes RC[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Bioquímica e Imunologia

Introduction: NLRP3 Inflammasome is a platform of the innate immune system that regulates inflammatory responses by caspase-1 activation and proteolytic processing of pro-IL-1β and pro-IL-18 to the mature cytokines. NLRP3 can be activated by several mechanisms including mitochondrial DNA (mitDNA). Circulating mitDNA is increased in diabetes, a condition associated with activation of the NLRP3 inflammasome. We tested the hypothesis that mitDNA release is increased in type 1 diabetes (T1D) leading to NLRP3 inflammasome activation and contributing to T1D-associated vascular inflammatory and oxidative processes. **Methods:** Wild type (WT) and NLRP3-deficient ($NLRP3^{-/-}$) mice were treated with: (i) vehicle (Veh, citrate buffer, 25 mM, pH 4.0) or (ii) streptozotocin (STZ, 40 mg/kg, freshly dissolved in citrate buffer, pH 4.0, *i.p.*) for 5 days. Vascular reactivity was determined in mesenteric resistance arteries, with a Mulvany & Halpern myograph. Cultured vascular smooth muscle cells (VSMC) were stimulated with mitDNA isolated from pancreatic islets of diabetic (dmDNA) and control (cmDNA) mice to evaluate NLRP3 inflammasome activation. Caspase-1 and IL-1β activation was evaluated in VSMC, mesenteric bed and pancreas by western blot analysis. Reactive oxygen species (ROS) generation was determined by fluorescence to dihydroethidium bromide. DNA was extracted, purified and amplified with mitochondrial primers by real-time (RT)-PCR. Data are presented as mean ± standard error of mean, Veh *vs.* T1D. **Results**: $NLRP3^{-/-}$ diabetic mice had attenuated hyperglycemia compared to WT diabetic mice [(mg/dL) 241.0 ± 27.7 *vs.* 337.6 ± 18.1, n=6-8, $p<0.05$]. Mesenteric arteries from diabetic mice exhibited decreased ACh-induced vasodilatation *vs.* the vehicle group [(E_{max}), 46.6 ± 4.0 *vs*. 91.5 ± 2.8, n=4-5, $p<0.05$], which was not observed in $NLRP3^{-/-}$ diabetic mice. Diabetes significantly increased vascular caspase-1 and IL-1β activation [arbitrary units (a.u.), 1.2 ± 0.1 *vs.* 0.8 ± 0.1; 4.8 ± 1.1 *vs.* 0.8 ± 0.5 *vs*. the vehicle group, respectively, $p <0.05$], but this activation was attenuated in diabetic $NLRP3^{-/-}$ mice. Increased inflammasome activation, mitDNA release in cytosolic fraction of pancreatic cells and circulating mitDNA were observed in diabetic mice. In addition, dmDNA, but not cmDNA, significantly increased NLRP3 inflammasome activation in VSMC (i.e. activated caspase-1 and increased IL-1β levels) [(a.u.), 4.2 ± 0.1 *vs.* 1.9 ± 0.1; 2.3 ± 0.1 *vs.* 0.7 ± 0.1, $p <0.05$]. NLRP3 activation was attenuated in VMSC from $NLRP3^{-/-}$ mice, but not in WT VSMC incubated with a TLR-9 antagonist. Increased ROS generation was observed in response to dmDNA, which was prevented by a mitochondrial uncoupler. **Conclusion**: T1D increases mitDNA release, which promotes vascular NLRP3 inflammasome activation via mitochondrial superoxide production, contributing to T1D-associated inflammatory response. **Financial Support:** FAPESP and CAPES. Approved by the Ethics Committee on Animal Experimentation of the Ribeirao Preto Medical School (026/2015).

06.009 Cardiovascular effects and vascular reactivity induced by linalool treatment of spontaneously hypertensive rats. Camargo SB[1], Simões LO[1], Medeiros CFA[1], Jesus AM[1], Evangelista A[2], Villareal CF[2], Fregoneze JB[3], Silva DF[1] [1]UFBA – Ciências da Saúde, [2]Fiocruz, [3]UFBA – Neurociências

Introduction: The essential oils extracted from plants are products of a wide variety of biological actions. Linalool (LIN) is a alcoholic monoterpene found at essential oil chemically known as 3,7-dimethyl-1,6-octadien-3-ol. **Methods**: Spontaneously hypertensive rats (SHR) aged 9-12 weeks were used. The (-)-linalool was solubilized in cremophor 2:1 and diluted with physiological saline. At acute studies to measure blood pressure, performed a surgical procedure of cannulation and intravenous injections of LIN were administered. At subchronic studies orogastric injections of LIN (100 mg/kg) daily given for 21 days and as control group captopril (30 mg/kg); vehicle (saline + cremophor). Each 5 days was performed measurement of arterial pressure by tail cuff method. At the end of treatment, reactivity concentration response curve was performed to phenylephrine (PHE) and sodium nitropusside (SNP) with the mesenteric artery. The heart removed was cleaned, weight and then placed in an oven for drying at 60° C for 24 hours. With the spleen was realized ELISA to dose IL-1b and IL-10 levels. **Results**: The intravenous acute administration of LIN (5, 10, 20 and 40 mg/kg) induced hypotension in SHR with MAP% = -19.64 ± 05.07; -41.28 ± 2.64; -46.52 ± 2.19; -56.64 ± 05.05 mmHg, n=5. This hypotensive effect was associated with bradycardia. The subchronic orogastric administration of LIN for 21 days in SHR induced maintenance of initial blood pressure levels and showed an antihypertensive significant effect from the 15th day reaching the end of treatment with: 158.44 ± 8.82, LIN, n=9; control group 201.09 ± 8.94, vehicle, n=7; and 126.11 ± 7.13, captopril, n=9 mmHg. Linalool was able to increase the production of anti-inflammatory cytokine IL-10 significantly; in addition, treatment with LIN was able to prevent the development of cardiac hypertrophy with significant decrease of heart mass index after 24 hours of dry 93.81 ± 4.41 mg/100g of body weight similar to that found in animals treated with captopril 98.50 ± 5.81 mg/100g and vehicle 221.73 ± 37.21 mg/100g. At vascular reactivity studies with mesenteric artery the curve of PHE (10^{-10}-10^{-6} M) at LIN group have significant decrease in pharmacological potency when compared to control. With SNP (10^{-13}-10^{-5}M) the LIN group showed a significant increase at pharmacological potency since the first concentration, suggesting that the LIN mechanism acting by a pathway linked to nitric oxide. **Conclusion**: The results suggest that LIN is able to induce hypotension and bradycardia acute intravenous via and after 21 days of orogastric subchronic treatment in SHR have a antihypertensive potential with decrease at MAP, being able to reduce cardiac mass of heart, anti-inflammatory potencial and decreased vascular reactivity to PHE contraction and increased pharmacological potency of vasorelaxation by SNP. **Financial Support**: FAPESB and CNPq. Ethical Aspects: Approved under Protocol CEUA - ICS/UFBA n° 085/2015. 1. NJOS, P. J. C. Zeitsc fur Naturfor., v.68, n. 5-6, p. 181, 2013 2. CAMARGO S. B. Journ. of Med. and Biol. Scie., v. 13, p. 381, 2014. 3. MENEZES I. A. Zeitsc. fur Naturfor., v. 65, n. 9-10, p. 562, 2010 4. SANTOS M.R.V. Braz. Journ. of Pharmac., v. 21, n, 4, p. 764, 2011.

06.010 Effects of artemether treatment on mice isolated cardiomyocytes contractility and calcium transient. Souza ACM[1], Mosqueira VCF[1], Richard S, Oliveira LT[1], Silveira APA[2], Rodrigues LA[2], Castro QJT[1], Guimarães HN[3], Grabe-Guimarães A[1] [1]UFOP – Ciências Farmacêuticas, [2]UFOP – Farmácia, [3]UFMG

Introduction: According to the World Health Organization (WHO, 2015), malaria is a public health problem and endemic in 97 countries. Malaria control requires an integrated approach including prevention, vector control and treatment with effective antimalarial drugs (WHO, 2015). It was already demonstrated the increasing resistance of *Plasmodium falciparum* to drugs such as chloroquine, amodiaquine, pyrimethamine and sulfadoxine (Lehane et al, 2012; Cheng et al, 2012). Artemisinin derivatives, such as artemether (ATM), are an alternative for malaria treatment, as well as the treatment protocols containing them (Mueller et al, 2006). ATM is a potent antimalarial drug, but there are few studies about its effect on cardiovascular system. This work aims to evaluate ATM effect on in vitro isolated mice cardiomyocytes. **Methods**: All procedures were approved by CEUA/UFOP (number 2014/14). Black C57/bl6 mice were used aged from 8 to 10 weeks. The animals were divided in two groups: a) treated with vehicle and b) treated with ATM 120 mg/kg/dose (n = 6, each), both given orally for 4 days, twice daily (12/12 hours). Two hours after the last dose administration, the animals were euthanized, the heart was quickly removed and the quiescent cardiomyocytes were obtained as described by Scamps et al. (1990), with modifications. Cardiomyocytes in Tyrode solution were labeled during 15 minutes with 2 mM of Indo-1. hen cells were taken to a plate at Ionoptix SoftEdge system (Milton, MA, USA) connected to a standard inverted fluorescence microscope and were stimulated at 1 Hz and 20 V during 20 seconds. It was registered and measured off line the transient Ca^{2+} and the contractility of a sarcomere selected portion of at least 9 isolated myocytes per heart. **Results:** It was observed in cardiomyocytes of mice treated with ATM a significant reduction contractility compared to vehicle treatment: 36 % reduction of sarcomere shortening and 20 % reduction of contraction velocity. There was 11 % reduction in diastolic transient Ca^{2+} and 36% reduction in peak h. **Conclusion**: ATM presents cardiotoxic effect, since it interferes on both Ca^{2+} transient and cardiomyocytes contractility, which are suggestive of heart failure. **Funding agencies:** Capes/COFECUB (**5119/14-6**), FAPEMIG (Rede NanobioMG and APQ 02346-11), CAPES and UFOP. **References:** CHENG Q; *et al*. Int J Parasitol Drugs Drug Resist 2:249, 2012. LEHANE A, *et al*. Int J Parasitol Drugs Drug Resist, 2:47, 2012. MUELLER E A, *et al*. Acta Trop 100:41-53, 2006. WORLD HELTH ORGANIZATION. Guidelines for the treatment of malaria, third edition, Geneva, 2015.

06.011 Aldosterone activates NLRP3/inflammasome in the vasculature of type 2 diabetic mice. Ferreira NS[1], Bruder-Nascimento T[1], Zanotto CZ[1], Pereira CA[1], Prado DS[1], Alves-Filho JC[1], Carlos D[2], Tostes RC[1] [1]FMRP-USP – Farmacologia, [2]FMRP-USP – Biochemistry and Immunology

Introduction: Diabetic patients and animal models of type 2 diabetes (DM2) display increased plasma aldosterone (aldo) levels. Aldo excess aggravates endothelial dysfunction in diabetes by promoting insulin resistance, fibrosis, oxidative stress and inflammation. Aldo activates proinflammatory transcription factors, stimulates the production of adhesion molecules and inflammatory cytokines. The NOD-like receptors comprise a group of pattern recognition receptors involved in a variety of host innate immune responses and leads to release of the pro-inflammatory cytokines IL-1β and IL-18. We hypothesized that aldosterone via mineralocorticoid receptors (MR) activates the inflammasome platform in the vasculature of DM2 mice. **Methods:** Mesenteric arteries and peritoneal lavage macrophages from control (db/+) and diabetic (db/db) mice treated with vehicle or spironolactone (spiro - MR antagonist, 50 mg/Kg/day), as well as cultured vascular smooth muscle cells (VSMC) and bone marrow-derived macrophages (BMDM) were used to determine aldosterone-induced inflammasome activation (expression of pro- and mature caspase-1 and IL-1β, caspase-1 activity). To determine whether aldo directly activates NLRP3/inflammasome in the vasculature and whether NLRP3 activation contributes to aldo-induced vascular injury, aldo was infused and bone marrow transplantation was performed. The following groups were constituted: WT□WT, WT□WT+aldo and WT□*NLRP3-/-*+aldo. **Results:** Mesenteric resistance arteries (MA) from db/db mice exhibited reduced acetylcholine (ACh) dilation, which was reversed by spiro treatment [maximum response (Emax) (% of relaxation): db/+: 78.5 ± 4.1; db/db: 40.5 ± 6.4*; db/+spiro: 77.04 ± 3.83; db/db+spiro: 62.83 ± 5.89 n=3-6, *p<0.05]. Spiro treatment reduced caspase-1 and mature IL-1β content in MA from db/db mice. Increased number of caspase-1-positive macrophages was found in the peritoneal lavage of db/db mice (vs. control mice), and Spiro treatment reduced macrophage caspase-1 activity [% of activity: db/+: 33.88 ± 2.53; db/db: 51.75 ± 7.43*; db/+spiro: 31.11 ± 1.98; db/db+spiro: 34.76 ± 3.80 n=4-7, *p<0.05]. Aldosterone *in vitro* increased mature IL-1β in VSMC (cont: 0.99 ± 0.01 n=4; LPS+Nigericine: 6.05 ± 2.07* n=3; Aldo 4h: 9.71 ± 2.58* n=5; LPS+Aldo 4h: 12.79 ± 1.85* n=5, p<0.05) and caspase-1 activity in BMDM (cont: 21.15 ± 1.80; LPS+Nigericine: 87.58 ± 3.74*; Aldo 4h: 32.71 ± 3.34*; LPS+Aldo 4h: 37.21 ± 2.18* n=3, p<0.05). *NLRP3 -/-* mice were protected against aldo-induced endothelial dysfunction [Emax: WT: 89,3 ± 2,9; WT+aldo: 39,8 ± 1,8*, *NLRP3-/-*+aldo: 87,7 ± 4,2*[#]. *P<0.05 *vs*. WT; [#]P<0.05 *vs*. WT+aldo]. Aldo induced endothelial dysfunction in WT□WT mice, but not in WT□*NLRP3-/-* mice [Emax: WT□WT: 95,1 ± 3,1; WT□WT+aldo: 57,1 ± 4,7*; WT□*NLRP3-/-*+aldo: 85,3 ± 3,1*[#]. *P<0.05 *vs*. WT□WT; [#]P<0.05 *vs*. WT□WT+aldo]. **Conclusion:** These results suggest that aldosterone via MR directly activates the inflammasome platform in the vasculature of DM2 mice and that NLRP3/inflammasome in the vasculature plays a crucial role on aldo/MR-induced vascular damage and on DM2-associated vascular dysfunction. **Financial support:** CAPES, CNPq, FAPESP. The project was approved by the local Ethics Committee (protocol: 012/2013-1).

06.012 Placental-fetal interface is affected positively by sodium nitrite and sildenafil and concomitantly shows reductions in hypertension-in-pregnancy Gonçalves-Rizzi VH[1], Possomato-Vieira JS[1], Nascimento RA[1], Silva KP[1], Caldeira-Dias M[1], Sandrim VC[1], Dias-Junior CA[1] [1]IBB-Unesp-Botucatu – Farmacologia

Introduction: Preeclampsia is a pregnancy-associated disorder characterized by hypertension with unclear pathogenesis. Reductions in nitric oxide (NO) bioavailability have been observed in preeclamptic women. Studies showed that oral administration of sodium nitrite may increase the bioavailability of NO. NO is a potent vasodilator and exerts its effects by increasing the levels of cGMP. The effects of cGMP are finalized by PDE5 (Dias-Jr, Thromb Res, 124, 349, 2009). Then, we hypothesized restoring NO bioavailability and improve NO-cGMP pathway with nitrite or sildenafil and their associations improve the parameters of hypertension in pregnancy. **Methods**: Female rats (220-250g) were mated with males for pregnancy. On pregnancy day 14, each animal was first placed into a single cage and randomized to one of the eight treatment groups (n=8 per group). Normal-pregnancy (NP), NP+nitrite, NP+sildenafil, NP+nitrite+sildenafil, Hypertensive pregnancy (HP), HP+nitrite, HP+Sildenafil, HP+nitrite+sildenafil groups. We developed hypertension in pregnancy using L-NAME (60mg/Kg/day, i.p). Sodium nitrite was used at dose of 15 mg/Kg/day by gavage, and Sildenafil citrate was used at dose of 10mg/Kg/day by gavage. We treated animals from 14th to 20th day of pregnancy. Systolic blood pressure was measured by tail cuff plethysmography at gestational days 14, 16, 18 and 20. Animals were killed on pregnancy day 21. **Results:** Systolic blood pressure was significantly increased in HP (145 ± 4mmHg/ 149 ± 3mmHg/ 140 ± 4mmHg on gestational days 16, 18 and 20, respectively). In HP+Nitrite (126 ± 3mmHg / 131 ± 3mmHg/ 127 ± 2mmHg), HP+Sildenafil (130 ± 3mmHg / 129 ± 2mmHg/ 130 ± 1mmHg in nitrite) and HP+Nitrite+Sildenafil (132 ± 2mmHg/ 130 ± 2mmHg/ 123 ± 4.7mmHg) were found reduced systolic blood pressure on days 16, 18 and 20 respectively. Gestational hypertensive disorders has the characteristics to intrauterine growth restriction, surprisingly the fetal weight was significantly higher in HP+Sildenafil (3.6 ± 0.03g) and HP+Nitrite+Sildenafil (3.7 ± 0.03g) when compared with HP group (3.7 ± 0.03g). The placental weight was significantly lower in HP group (0.53 ± 0.008g) *vs* NP group (0.63 ± 0.01g). However the treatment with Nitrite or Sildenafil and their association improved the placental weight (0.6 ± 0.15/ 0.61 ± 0.009/ 0.62 ± 0.1g respectively). Number of pups (litter size) was significantly lower in HP (9 ± 0.7) *vs* NP (13 ± 0.8). Nitrite (12 ± 0.5) and Sildenafil (12 ± 0.5) improved litter size. Also, lower numbers of viable fetuses were found only in HP group (7.7 ± 0.7). In HP+Nitrite (11 ± 0.6), HP+Sildenafil (11 ± 0.5) and HP+Nitrite+Sildenafil (11 ± 0.5) groups, vs. HP group presented higher number of viable fetuses. We found significant lower NO bioavailability in HP group (49 ± 7µmol/L) compared to NP group (72 ± 8 µmol/L). However, treatment with Nitrite (with or without) Sildenafil improves NO bioavailability (131 ± 11/ 88 ± 12 µmol/L respectively). **Conclusion:** Our data showed that sodium nitrite, Sildenafil citrate and their association has an antihypertensive effect and differences in fetal and placentas weights that may be related to an enhancement in blood flow promoted by NO-cGMP pathway. **Financial support**: Capes and Fapesp. **Ethics Committee** IBB/UNESP (Protocol 618/2014)

06.013 Correlation between cardiovascular disorder and early exposure to the ambient pollutant 1,2-naphthoquinone: role of transient receptor potential channel. Soares AG[1], Florenzano J[1], Rodrigues L[1], Cunha C[1], Teixeira SA[1], Brain SD[2], Muscará MN[1], Costa SKP[1]
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Introduction: Emerging mechanistic studies show that transient receptor potential channels (TRPs) acts as a molecular target of the multiple respiratory and cardiovascular disorders to air pollutants (AP; 1], whereas others suggest that AP-induced health effects varies among age (e.g. children) and their socio-economic status (SES), indicating SES as an additional mechanism by which AP affects health [2]. We showed that neonate mice exposure to 1,2-naphthoquinone (1,2-NQ), found in Diesel Exhaust Particle (DEP), increases susceptibility to lung allergic inflammation at adulthood [3]. Whether 1,2-NQ exposure affects cardiovascular function is unknown. We aimed to determine a critical link between cardiovascular disorder to early exposure to 1,2-NQ and the role of TRPV1 and TRPV4 channels. **Methods:** Neonate male C57BL/6 mice were exposed to 1,2-NQ (100 nM, 15 min) or vehicle on days 6, 8 and 10 of life [3]. After 33 days, mice were killed and both the positive (Norepinephrine, NE) and negative (Carbachol, CCh) chronotropic effect in the right atrium (RA) and the pulmonary artery (PA) segment (2 mm) responsiveness to Phenylephrine (Phe) and Acetylcholine (ACh) were recorded via a wire-myograph system. Data are presented as mean ± SEM for n=5 mice. Stats were performed by One-way ANOVA. **Results:** Maximum positive inotropic response to NE in RA from 1,2-NQ-pretreated mice (259 ± 27) did not significantly differ from vehicle-treated mice (218 ± 12). The exposure of 1,2-NQ shifted the NE concentration-response curve to the right and upward. The pD_2 of NE in the RA of 1,2-NQ treated mice was reduced (5.73 ± 0.1*; $P<0.05$) compared to VEH (6.63 ± 0.1). TRPV1 antagonist CZP (50 mg/kg; i.p. -30 min) reversed this effect as it produced left and upward shift in the positive inotropic concentration-response curve of NE. The pD_2 of NE in the RA of mice exposed to 1,2-NQ was 7.14 ± 0.1* in the presence of CPZ. The maximum negative chronotropic effect of CCh was not affected by 1,2-NQ, although 1,2-NQ produced ($P<0.05$) change in the pD_2 of CCh (6.12 ± 0.06*; $P<0.05$) compared to VEH (6.29 ± 0.04) or to mice treated with 1,2-NQ and CZP (5.83 ± 0.05*; $P<0.05$). Isolated PA from mice exposed to 1,2-NQ had a significantly higher constriction response to Phe (pD_2 7.57 ± 0.08*; $P<0.05$) than vehicle-treated mice (pD_2 6.69 ± 0.06). The blockade of TRPV1 (8.22 ± 0.11*; $P<0.05$) or TRPV4 (7.59 ± 0.08*; $P<0.05$) potentiated the pD_2 of Phe in 1,2-NQ treated mice. Emax response to ACh in PA of 1,2-NQ treated animals was higher (69 ± 1*; $P<0.05$) compared to VEH (46 ± 0.4). This effect was reversed by CPZ (14 ± 1*; $P<0.05$) or HC067047 (38 ± 3*; $P<0.05$). **Conclusion:** Neonatal exposure to 1,2-NQ may enhances susceptibility to the cardiovascular health effects of PED, which showed to be reversed by TRPV1 blockade. This is important as the public cardiovascular health impacts of AP could increase with neonatal exposure to 1,2-NQ. **Acknowledgments:** FAPESP, CNPq, CAPES Ethics Committee 48/2016 CEUA/ICB References: 1 Chem Res Toxicol. 26:750, 2013 2 Environ.-Health 211 (2008) 326–336 3 Arch Toxicol. 2014 Aug;88(8):1589-605

06.014 Estrone treatment improves endothelial dysfunction in ovariectomized Wistar rats. Oliveira TS[1], Campos HM[1], Bastos AM[1], Oliveira LP[1], Costa EA[1], Filgueira FP[2], Ghedini PC[1] [1]UFG – Farmacologia, [2]UFG – Ciências da Saúde

Introduction: The effect of hormone replacement therapy (HRT) on the prevention and outcome of cardiovascular disease has been the reason of a number of clinical trials. We have previously demonstrated that estrone (E1), one of the main components of the conjugated equine estrogen (CEE), have an endothelium-dependent vasorelaxant effect mediated by activation of estrogen receptors which in turn results in stimulation of NO/cGMP pathway by PI3K signaling in a Ca^{2+}/CaM complex -activation dependent manner. Therefore, the present study proceeded to examine the hypothesis that in vivo treatment with E1 corrects vascular dysfunction observed in ovariectomized rats (OVX). **Methods:** Experiments were performed in female Wistar rats, maintained on a 12 h light/dark cycle under controlled temperature and ad libitum access to food and water. Ovariectomy was performed in 12-weeks-old rats. After 30 days of ovariectomy, a group of OVX rats was treated during 30 days with vehicle (OVX) or E1 (825 µg/kg, sc) or 17β-estradiol (E2; positive control - 15µg/kg, sc). Female Wistar in physiological estrous were used as the control group (SHAM). Thoracic aortic rings (4 mm in length) were mounted in an isolated tissue chamber and submitted to vascular reactivity experiments. Endothelial integrity was assessed by testing the relaxant effect of acetylcholine (ACh, 10 µM) in vessels precontracted with 1 µM of phenylephrine (Phe). Concentration-response curves to the endothelium-dependent vasodilator acetylcholine (0.1 nM to 0.1mM) and to the nitric oxide donor sodium nitroprusside (SNP, 0.1 nM to 0.1 mM) were obtained. Relaxation induced by ACh and SNP was expressed as the percentage of the tonus obtained with Phe. Data are presented as mean ± SEM of 5-6 experiments and analyzed by Student's t-test or one-way ANOVA statistical tests, when appropriate. *P* values less than 0.05 were considered significant. **Results and Discussion:** OVX rats presented reduced uterus weight as compared to SHAM rats, and the treatment with E1 or E2 restored this parameter. The endothelium-dependent relaxation to ACh was reduced in aorta from OVX rats (Emax: SHAM 98.1 ± 0.6% vs. OVX 77.7 ± 2.9%; $P < 0.05$). There was no change in the relaxation response to the SNP. Treatment of OVX rats with either E1 or E2 for 30 days corrected the impaired ACh-induced relaxation (Emax: OVX + E1 96.1 ± 0.9% vs. OVX + E2 92.2 ± 2.8%), demonstrating beneficial effect of E1 on endothelial cells of these animals. Further studies will be performed to a better understanding of the E1 effects on vasculature during HRT. **Financial Support:** CAPES, FAPEG, CNPq Research approved by the Animal Research Ethical Committee from Federal University of Goiás (process number 20/2013)

06.015 Cardioprotective effect of ipriflavone in female spontaneously hypertensive rats submitted to the left coronary ligature. Castro QJT[1], Mosqueira VCF[1], Pereira SC[1], Souza ACM[1], Amancio GCS[1], Guimarães HN[2], Leite R[1], Grabe-Guimarães A[1] [1]UFOP – Farmácia, [2]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) is as a promising technology to improve the bioavailability of poorly water-soluble drugs like ipriflavone. **Objective:** The objective was to demonstrate the cardioprotective effect of ipriflavone in SEDDS administered in female spontaneously hypertensive rats (SHR) submitted to the left coronary ligature. **Methods**: All the procedures were approved by CEUA/UFOP (number 2013/02). The SHR were submitted to the heart coronary ligature. They were treated during 21 days with ipriflavone-SEDDS (30 mg/kg) or with blank SEDDS (0.5ml/100g), in a single daily dose. Sham animals treated with ipriflavone-SEEDS or blank SEDDS were used as control group. At the 22th day, under anesthesia (ketamine 100 mg/kg/xylazine 14 mg/kg), the lead II ECG signal was obtained. The cardioprotective activity of ipriflavone was determined for its ability to prevent electrocardiogram (ECG) intervals alterations induced by IV administration of norepinephrine (NE, 3 and 10 µg/kg). **Results:** For PR and QRS intervals of ECG NE did not induce significant alterations and there were no difference in animals treated with vehicle or ipriflavone. Twenty-one days after the coronary ligature, the NE challenge induced QT and QTc intervals increase and they were significantly reduced in ipriflavone treated animals compared to vehicle treatment. The reduction of the QT interval prolongation induced by 3 and 10 µg/kg of NE was respectively 78 % and 73 %, and for QTc it was observed 58 % and 53 % reduction after 3 and 10 µg/kg, respectively. **Conclusion:** Considering QT and QTc intervals prolongation are predictors of cardiac arrhythmias, the reduced alterations induced by NE in SHR treated female rats reveals the ipriflavone-SEDDS cardioprotective activity and its potential for further clinical investigation. **Funding agencies and Acknowledgments:** FAPEMIG (Rede NanobioMG; PPM00481-13; APQ 02346-11), CAPES, CNPq, PET Farmácia (PROGRAD/UFOP) and UFOP.

06.016 Ethnopharmacological investigation of the diuretic properties of native species of the southern pantanal. Tirloni CAS[1], Vasconcelos PCP[1], Silva AO[1], Lopes GB[2], Tomazetto TA[1], Gasparotto Júnior A[1] [1]UFGD – Ciências da Saúde, [2]UFGD – Ciências Biológicas

Introduction: In South America, several natural products are used as antihypertensive agents primarily due to their diuretic properties. Nevertheless, very few native species have been critically investigated despite the immense biodiversity available in these countries. In southern Pantanal *Acanthospermum hispidum* DC (Asteraceae), *Luehea divaricata* Mart. (Tiliaceae), and *Talisia esculenta* (A. St.-Hil.) Radlk. (Sapindaceae) are widely used as diuretic and antihypertensive drugs (Bieski, 2012). However, these species lack a thorough ethnopharmacological investigation due to their extensive popular use. **Aim:** Evaluate the acute diuretic activities of ethanol soluble fractions (ES) obtained from these species using normotensive male Wistar rats. **Methods:** The leaves of *Acanthospermum hispidum* (AH), *Luehea divaricata* (LD), and *Talisia esculenta* (TE) were collected from the local vegetation of the Federal University of Grande Dourados (UFGD) (Dourados, Brazil) (S22°11'43.7-W54°56'08.5). Dry leaves were extracted by infusion (1:10 w/v). The infusion was treated with 3 volumes of EtOH, which gave rise to a precipitate and an ethanol soluble fraction (ES). Male rats were separated in different groups (n = 5) and fasted overnight with free access to water. Before the treatments, all animals received physiological saline (0.9% NaCl) in an oral dose of 5 ml/100 g to impose a uniform water and salt load. Then, the first group received vehicle (deionized water) orally and it was used as control. Other groups of rats received, by oral route, ES-AH (30, 100 and 300 mg/kg), ES-LD (30, 100 and 300 mg/kg), ES-TE (30, 100 and 300 mg/kg), or HCTZ (hydrochlorothiazide, 25 mg/kg). The urine was collected in a graduated cylinder and its volume was recorded for 8 and 24 h. The urine excretion rate, pH, density, conductivity and content of Na^+, K^+, Cl^- and Ca^{+2} were measured in the urine of saline-loaded animals. Serum electrolytes, total protein, urea, and creatinine were also investigated. **Results:** Treatment with a single dose of ES-AH significantly increased diuresis after 24 h (ES-AH 30: 12.40 ± 1.45 ml/100g; Control: 7.98 ± 0.82 mL/100g; $p < 0.05$). In a similar way, ES-TE (30 mg/kg) also showed an increased urinary volume at ~ 32% after 24 hours of treatment. All other parameters evaluated were not affected by any treatment. **Conclusion:** The results presented here support, at least in part, the traditional use of ES obtained from *Acanthospermum hispidum* and *Talisia esculenta* leaves as a diuretic agent. In addition, it was shown for the first time that the pharmacological effects of ES obtained from *Luehea divaricata* do not support its popular use as a diuretic agent. **Financial support:** CNPq and FUNDECT **Animal Research Ethical Committee**: Process 16/2015- CEUA/UFGD **References:** Bieski, I.G.C *et al*. **Ethnopharmacology of Medicinal Plants of the Pantanal Region (Mato Grosso, Brazil)**, Evidence-Based Complementary and Alternative Medicine, 2012, 36p.

06.017 Influence of physical exercise in SHR rats on treatment with captopril. Castro QJT, Watai PY, Souza ACM, Paula DCC, Antunes LR, Locatelli J, Guimarães HN, Oliveira LKB, Silva SSC, Guimarães AG – UFOP

Introduction: Regular physical exercise is important to prevent and treat arterial hypertension (HA) besides helping weight loss (Diretrizes Brasileiras de Hipertensão VI, 2010, SBH). The effectiveness of pharmacological treatment of HA seems to be better when non-pharmacological treatment is present (RONDON and BRUM, 2003), although there are few evidence that can support dose reduction. In this context, we conducted an experimental study to evaluate the effectiveness of regular physical exercise on response to three doses of captopril in spontaneously rats (SHR). **Methods:** All the procedures were approved by CEUA/UFOP (2015/05). Physical exercise trained and sedentary male adults SHR received captopril 12.5, 25 or 50 mg/kg by oral route or vehicle (8 groups, n=6 each). Physical exercise was done on a treadmill velocity 18 m/min during 60 minutes per day, five days a week during eight weeks. After that it was recorded the systolic arterial pressure (SAP) using tail-cuff method. **Results:** SAP of SHR trained and treated with 25 and 50 mg/kg captopril had percentual reduction of 8.4% and 8.5%, respectively, related to sedentary groups treated with the same dose. SAP of SHR treated with vehicle and sedentary was 192 ± 7.8 mmHg, for SHR treated with vehicle and exercised it was 191 ± 4.9 mmHg, for SHR treated with captopril and sedentary it was 178 ± 9.5 mmHg, 168 ± 3.6 mmHg, 146 ± 4.9 mmHg and for SHR treated with captopril and exercised it was 179 ± 6.4 mmHg, 154 ± 3.8 mmHg and 134 ± 1.4 mmHg, respectively for 12.5, 25 and 50 mg/kg. **Conclusion:** Regular exercise can help arterial pressure reduction and can increase pharmacological treatment effectiveness as demonstrated in male adults SHR. Further studies are necessary to encourage dose reduction to patients that exercise regularly. **Funding agencies and Acknowledgments:** FAPEMIG, CAPES, CNPq, PET Farmácia (PROGRAD/UFOP) and UFOP.

06.018 Modulation of Intrarenal Gene Expression of Guanylate Ciclase-C, Guanylin and Uroguanylin and by Enalapril in 5/6 Nephrectomized rats. Alves NTQ[1], Costa PHS[1], Rodrigues FAP[1], Medeiros PHQS[1], Silveira JAM[1], Silva PLB[1], Viana DA[2], Nogueira Júnior FA[1], Ximenes RM[3], Havt A[1], Monteiro HSA[1] [1]UFC – Physiology and Pharmacology, [2]UECE, [3]UFPE – Antibiotics

Introduction: Chronic kidney disease (CKD) is characterized by loss usually slow, progressive and irreversible of renal function. It is suggested that, in this pathology, the natriuretic body's response to salt intake and to volume expansion can be reduced, as a result of damage of the nephrons. In this context, more studies are necessary for establishment of a link between DRC and regulation of natriuretic peptides, such as guanylin (Gn) and uroguanylin (UGn), and the effect of angiotensin II (Ang II) on regulation of these peptides. Thus, we sought to evaluate a possible modulation of the guanylin pathway by enalapril in the 5/6 nephrectomy model (nx5/6). **Methods**: We used male Wistar rats, weighing between 250-300g. The animals were divided into 4 groups (n=8): untreated control group treated or not with enalapril (10 mg/kg oral) (SHAM and SHAM+E) and group subjected to nx5/6 treated or not with enalapril (10 mg/kg oral) (Nx and Nx+E). At the end of the 10th week after surgery, we measured some markers of renal function, such as serum urea, glomerular filtration rate (GRF), proteinuria and the fractional excretion of sodium ($FENa^+$). Kidney samples were sent for histological analysis and evaluation of mRNA expression of Gn, UGn and membrane guanylate cyclase receptor type C (GC-C). All data were analyzed by one-way ANOVA, followed by Bonferroni posttest, with level of significance of *$p<0.05$. The experimental protocols were approved by the Federal University of Ceará Animal Research Ethical Committee (CEPA-UFC), license number of 72/14. **Results**: Nx compared to SHAM, increased levels of serum urea (Nx=108.0 ± 5.57 vs SHAM=96.83 ± 4.08mg/dL), proteinuria (Nx=129.10 ± 13.87 vs SHAM= 96.83 ± 4.07; mg/24hrs) and FENa+ (Nx=3.55 ± 0.56 vs SHAM=1.43 ± 0.16). Nevertheless, GFR was decreased in Nx group (Nx=0.44 ± 0.10 ± 0.04 vs SHAM= 0.97 ± 0.07mL/min). Nx+E, compared to Nx, showed reduced levels of proteinuria (Nx+E=129.10 ± 31.94 vs Nx=13.87 ± 6.46mg/24hrs) and FENa+ (Nx+E=2.02 ± 0.28 vs Nx=3.55 ± 0.56). However, Nx+E presented higher levels of GFR (Nx+E=0.70 ± 0.08 vs Nx=0.44 ± 0.10ml/min). Nx showed increased intrarenal gene expression of Gn (Nx=13.92 ± 5.13; SHAM=1.08 ± 0.20) and UGn (Nx=12.77 ± 7.00; SHAM=1.04 ± 0.13). On the other hand, Nx+E had reduced genes for UGn (Nx+E= 0.10 ± 0.03; Nx = 1.75 ± 0.96), when compared to Nx. **Conclusion**: There was an increase in the expression of guanylins by the kidney in response to Nx5/6. In addition, enalapril reduced UGn expression in kidney from nephrectomized rats. These data suggest a hyperactivation of guanylin pathway in CKD, and modulation of this peptide class by Ang II. **Financial support:** CNPQ, CAPES and FUNCAP. **Keywords**: Chronic kidney disease. Enalapril. Angiotensin II. Uroguanylin. Natriuresis.

06.019 Effect of physical training (Swimming) on sympathetic neurotransmission in SHR of different age groups. Garcia MP[1], Miranda-Ferreira R, Castro-Musial D[1], Souza BP[2], Jurkiewicz NH[1], Jurkiewicz A[1] [1]Unifesp – Farmacologia, [2]Unifesp

Introduction: It is well known that the physical training (PT) causes significant hemodynamic and autonomic changes influencing the cardiovascular system as for example, decreased activity of the sympathetic nervous system which leads to reduced peripheral vascular resistance and cardiac output, leading to decreased blood pressure, both in humans and in hypertensive animals. However, it is not known from when these changes start to appear. So the goal of our work is to study the effect of swimming on peripheral sympathetic neurotransmission; catecholamines released from sympathetic nerves, and the contraction of the smooth muscle, using as a model the vas deferens (VD) of SHR and their respective controls at different ages. **Methods:** The PT consisted of swimming sessions of 60 minutes, 5 days per week, for 4 weeks (to SHR-TRE 8 weeks) and 8 weeks (to SHR-TRE 12 and 20 weeks). Using animals NWR and SHR of 8, 12 e 20 weeks of life divided in: sedentary NWR (NWR-SED), sedentary SHR (SHR-SED) and trained SHR (SHR-TRE). After euthanasia, the vas deferens of SHR and NWR 8, 12 and 20 weeks were mounted in isolated organ bath with nutrient solution (LNV) and submitted 1g of tension for isometric contraction for the tests: **1)** Measurement of systolic blood pressure (SBP). To study the sympathetic neutransmissão were constructed: **2)** Concentration-effect curve with adrenergic agonist noradrenaline (NA 10^{-9}, $3x10^{-9}$, 10^{-8}, $3x10^{-8}$, 10^{-7}, $3x10^{-7}$, 10^{-6}, $3x10^{-6}$, 10^{-5}, $3x10^{-5}$, 10^{-4}, $3x10^{-4}$; **3)** Single stimulation with ATP 10^{-3}M; and **4)** KCl (70mM). Pharmacological parameters pD2 (apparent affinity) and Emax (maximum effect) were analyzed. **5)** Measure of secretion of catecholamines from sympathetic nerves of VD using Nicotine (10^{-6} M) and measured by means of electrochemical detector. The results were normalized by the weight in grams of VD. Statistical analysis was performed by ANOVA with post-test Tukey p<***0,05. **Results**: The SHR-TRE compared to SHR-SED showed: **1)** Reduction of SBP in 17% (8 weeks), 29% (12 weeks) and 24% (20 weeks) p <0.05 ***. 2) Decrease statistically significant of the maximum effect (Emax), showing a decrease in the contractile response of the VD in SHR-TRE; and pD2 in all trained groups. **3)** Decreased contractile purinergic response in SHR-TRE. **4)** Significant increase in the contraction of smooth muscle of VD in both phasic response as the tonic response in SHR-TRE of all groups. **5)** Decrease statistically significant of catecholamine secretion in all training groups. **Conclusion:** These results demonstrate that regular physical exercise can be used as a non-pharmacological treatment to control blood pressure. Since the effect of this begins before the onset of hypertension in SHR. Physical exercise (swimming) can reduce eventually eliminate the use of antihypertensive drugs, can also avoid the side effects of these drugs and decreasing cost of the treatments for these patients and public health. **Financial Support**: Capes, CNPq, FAPESP. All experimental procedures were approved by Ethical Committee of University Federal de São Paulo, Brazil (protocol N°: 78660203-15).

06.020 Novel sulfonylhydrazone compound (LASSBio-1773) ameliorates cardiovascular and renal dysfunction in streptozotocin-induced diabetic rats. Araújo JSC, da Silva JS, Trachez MM, Delgobbo MS, Silva TF, Lima LM, Barreiro EJ, Sudo RT, Zapata-Sudo G UFRJ – Farmacologia e Química Medicinal

Introduction: Diabetes mellitus (DM) is a metabolic disease associated to a hyperglycemic state if untreated could produce cardiac and renal complications. A new sulfonilhidrazone compound named as LASSBio-1773 ((E)-metil-4- (2-(3,4-dimetoxifenilsulfonilidrazono)metil)benzoato)) was previously described as a hypoglycemic agent. This work investigated the beneficial effects of LASSBio-1773 on the cardiac and renal dysfunction in diabetic rats. **Methods and results:** Male Wistar rats received a single injection of streptozotocin (STZ) (60 mg/kg) for diabetes induction. Glycose level (mg/dL) was increased from 102.6 ± 2.2 to 512.8 ± 22.0 (P< 0.05) mg/dL. Animals were treated with vehicle or LASSBio-1773 for 14 days after 8 weeks of onset of disease. LASSBio-1773 reduced glycemia to 454.8 ± 58.8 (P< 0.05) and 375.3 ± 51.1 (P< 0.05) after 3 and 14 days of treatment. Levels of plasmatic insulin (pmol/L) decreased in diabetic animals from 260.9 ± 37.9 to 69.4 ± 14.1 (P< 0.001) which was not altered during treatment. LASSBio-1773 also lowered cholesterol and triglycerides levels (mg/dL) from 53.5 ± 7.7 to 42.4 ± 4.5 and from 67.4 ± 14.8 to 48.1 ± 11.9, respectively. Diastolic pressure and mean pressure (mmHg) increased from 92.0 ± 1.7 to 112.4 ± 2.5 (P< 0.05) and from 101.7 ± 2.4 to 120.7 (P< 0,05) which were reversed to 97.0 ± 2.2 and 109.9 ± 2.2 after treatment with LASSBio-1773, without changes in heart rate. Endothelial dysfunction was evaluated using isometric tension recording of aorta from diabetic rats and LASSBio-1773 improved acetylcholine-induced relaxation. Renal dysfunction was investigated using metabolic cages and the following parameters were determined: 1. water intake (mL) increased from 33.4 ± 1.6 to 122.1 ± 10.4 (P< 0.05) and decreased to 58.2 ± 6.8 (P< 0.05) after treatment with derivative, 2. urea (mg/dL) increased from 17.9 ± to 35.7 (P< 0.05) and reduced to 26.9 ± 5.5 (P< 0.05) after treatment, 3. protein/creatinin ratio decreased from 1.1 ± 0.2 to 0.2 ± 0.1 (p< 0.05) which was not altered with treatment. CEUA/CCS/UFRJ 030/2016. **Conclusions:** Hypoglycemic activity was induced by LASSBio-1773 reflecting in an improvement of cardiovascular parameters, dyslipidemia, exercise intolerance and renal dysfunction. These findings support the development of new agent for the treatment of chronic complications of diabetes. **Financial support:** CNPq, FAPERJ, CAPES, INCT-INOFAR, PRONEX, PENSA RIO. **Keywords:** Diabetes mellitus, T1DM, sulfonylhydrazone, diabetic nephropathy, diabetic cardiomyopathy, drug development

06.021 Hydrogen Sulfide (H2S) donor reduces systolic blood pressure and stimulates nitric oxide production in rats with L-NAME-induced hypertension in pregnancy.
Possomato-Vieira JS, Gonçalves-Rizzi VH, Nascimento RA, Silva KP, Caldeira-Dias M, Sandrim VC, Dias-Junior CA IBB-Unesp-Botucatu – Farmacologia

Introduction: Preeclampsia (PE) is a pregnancy disorder characterized by elevated blood pressure (>140/90 mmHg) and often proteinuria. Studies indicate nitric oxide (NO) as a key factor in the maintenance of healthy pregnancy and alterations in NO pathway may be an underlying mechanism in PE. Several factors, such as the antiangiogenic factor soluble fms-tyrosine kinase-like (sFlt-1) and the endogenous gasotransmitter hydrogen sulfide (H_2S) may modulate NO bioavailability (Zhou, Q., Cell Biol. Int., 31, 61, 2011; Altaany, Z., J. Cell. Mol. Med., 17, 879, 2013). sFlt-1 is a well-recognized factor in PE and recent studies have shown H_2S important role in normal pregnancy and PE (Wang, K., Circulation, 127, 2514, 2013). Although H_2S crosstalk with NO has been described, this interaction in PE needs further investigation. **Methods:** Female Wistar rats (220-250g, n=32) were mated and after confirmation of pregnancy, rats were separated in individual cages and randomly assigned into four different groups. Normal pregnant (NP); pregnant+sodium hydrogen sulfide (Preg+NaHS, a donor of H_2S; 50 µmol/Kg, twice daily, intraperitoneally - i.p.); hypertension in pregnancy (HTN-Preg; N_ω-nitro-L-arginine methyl ester - L-NAME - 60mg/Kg, i.p.) and HTN-Preg+NaHS (L-NAME 60 mg/Kg, i.p. and NaHS 50µmol/Kg, twice daily, i.p.). Systolic blood pressure was measured by tail cuff plethysmography at gestational days 14, 16, 18 and 20. Animals were killed on pregnancy day 21. Fetal parameters were recorded and plasma was obtained for biochemical analysis and incubation of human umbilical vein endothelial cells (HUVECs). **Results:** Injection of L-NAME significantly elevated systolic blood pressure (SBP) on days 16, 18 and 20 in the HTN-Preg group (145 ± 4 mmHg, 149 ± 3 mmHg and 139 ± 4, respectively). NaHS reduced SBP on days 18 and 20 in the HTN-Preg+NaHS group (129 ± 3 mmHg and 126 ± 2 mmHg, respectively). HTN-Preg group showed a decrease in number of live fetuses (7.72 ± 0.68) and in litter size (9.27 ± 0.74) that was blunted by NaHS treatment (10.88 ± 0.99 and 12.63 ± 1.07 in HTN-Preg+NaHS group, respectively). Reductions in placentae weight occurred in both HTN-Preg and HTN-Preg+NaHS group compared with NP and Preg+NaHS group. Measurements of NO in plasma through Griess reaction, showed that treatment with NaHS caused an increase in NOx concentration in animals with L-NAME-induced hypertension (134.10 ± 14.52µmol/L in HTN-Preg+NaHS group) but not in normotense animals (63.50 ± 15.33µmol/L in Preg+NaHS group). In HUVECs treated with 10% of plasma from animals of the different groups, cells treated with plasma from HTN-Preg+NaHS group showed an approximately 2.5 fold increase in the production of nitrite compared to Norm-Preg and HTN-Preg group. Moreover, treatment with NaHS prevented the increases in sFlt-1 plasmatic levels in HTN-Preg group (174.30 ± 14.37 pg/mL versus 368.20 ± 54.17 pg/mL). **Conclusion:** Beneficial effects of NaHS on systolic blood pressure and fetal parameters may be related to an increase in NO bioavailability. Moreover, our results show that the negative co-relation between sFlt-1 and NO plasmatic levels may be underlined by NaHS. **Financial support**: CAPES and FAPESP Ethics Committee IBB/UNESP (Protocol# 619/2014)

06.022 TNF-α mediates oxidative stress and vascular inflammation induced by ethanol consumption in mouse aorta with and without perivascular adipose tissue. Simplicio JA[1], Cunha TM[1], Tirapelli CR[2] [1]FMRP-USP – Farmacologia, [2]EERP-USP – Farmacologia

Introduction: Chronic ethanol consumption increases blood pressure, reactive oxygen species (ROS) generation and induces vascular inflammation with increased production of TNF-α. However, the role of TNF on ethanol-induced vascular ROS generation and the involvement of PVAT in such response remains elusive. Objectives: evaluate the role of TNF-α in the induction of oxidative stress, vascular inflammation and the increase in blood pressure induced by chronic ethanol consumption and the involvement of PVAT in such responses. **Methods**: Wild type (wt) C57/BL6 and knockout mice for TNFR1 receptor ($TNFR1^{-/-}$) were treated with ethanol 20% (v/v) for 9 weeks. Systolic blood pressure (SBP) was measured weekly by tail cuff. The thoracic aorta with ($PVAT^+$) or without PVAT ($PVAT^-$) and plasma were used for determination of superoxide anion (O_2^-), thiobarbituric acid reactive species (TBARS), nitrate/nitrite (NOx), H_2O_2, reduced glutathione (GSH) and cytokines. Superoxide dismutase (SOD), and catalase (CAT) activities were also evaluated. *In situ* visualization of ROS and NO was performed by immunofluorescence using DHE (dihydroethidium) and DAF-2DA (4,5-Diaminofluorescein diacetate), respectively (Ethical committee: 12.1.1654.53.9). Groups were compared using two-way ANOVA / Bonferroni *post hoc* test ($p<0.05$ was considered significant). **Results**: Ethanol treatment increased SBP in wt animals (control: 115.7 ± 5.1, n=9/ ethanol: 130.7 ± 1.8, n=9) and this increase was less pronounced in $TNFR1^{-/-}$ mice. Ethanol increased vascular TNF-α levels (pg/mg protein) ($PVAT^-$, control:12.9 ± 2.6, n=8/ ethanol: 27.7 ± 4.8, n=8/$PVAT^+$,control: 32.1 ± 1.2, n=8 /ethanol: 41.8 ± 5.4, n=8) and IL-6 levels in wt ($PVAT^-$, control: 22.0 ± 1.4, n=6/ ethanol: 35.7 ± 1.5, n=8; $PVAT^+$, control: 40.7 ± 3.2, n=7/ ethanol: 48.7 ± 4.9, n=7). Ethanol increased O_2^- generation (RLU/mg protein) in wt aorta ($PVAT^-$, control: 149 ± 6.7, n=7/ ethanol: 250 ± 13.8, n=7; $PVAT^+$, control: 409 ± 48.3, n=6/ ethanol: 592 ± 51.5, n=7) but not in aortas from $TNFR1^{-/-}$ mice corroborating immunofluorescence experiments with DHE. The levels of TBARS (nmol/mg protein) increased in wt aorta ($PVAT^-$, control: 9.9 ± 1.3, n=9 / ethanol: 15 ± 1.9, n=10; $PVAT^+$, control: 8.5 ± 0.9, n=8/ethanol: 16 ± 2.3, n=8) and plasma (nmol/ml) after treatment with ethanol but these responses were not found in $TNFR1^{-/-}$ mice. Aortic levels of H_2O_2 (nmol/mg protein) decreased in wt mice after treatment with ethanol but not in $TNFR1^{-/-}$. The aortic levels of NOx (nmol/mg protein) were reduced after treatment with ethanol in wt mice ($PVAT^+$, control: 23 ± 4.7, n=7/ethanol: 9 ± 1.6, n=7; $PVAT^+$, control: 20 ± 4.6,n=5/ ethanol: 3 ± 0.5, n=6) and this decrease was not observed in $TNFR1^{-/-}$ mice, further corroborating immunofluorescence experiments with DAF-2DA. Treatment with ethanol increased the activity of SOD (inhibition %) in wt aorta ($PVAT^-$, control: 64 ± 4.3, n=7/ethanol: 83 ± 4.0, n=6; $PVAT^+$, control: 80 ± 2.6, n=7/ ethanol: 93 ± 4.2, n=7) and CAT (Units/mg) ($PVAT^-$, control:118 ± 12.5, n=6/ ethanol: 163 ± 14.8, n=7; $PVAT^+$, control: 87 ± 7.5, n=7/ ethanol:106 ± 14.4, n=7). Ethanol reduced plasma levels of GSH in wt animals. Such changes were not observed in $TNFR1^{-/-}$ animals. **Conclusions**: TNF-α is an important mediator of ethanol-induced vascular oxidative stress and hypertension. PVAT does not display a beneficial/protective action in reducing ethanol-induced oxidative stress. **Financial Support**: FAPESP/CAPES.

06.023 Northeastern Brazilian red wine is able to reduce oxidative stress and to improve vascular dysfunction in resistance arteries in hypertensive animals. Maciel MPM[1], Machado-Calzerra NT[1], Melo MP[1], Santos PF[1], Assis KS[1], Vieira RLP[2], Cavalcante AA[1], Albuquerque JGF[1], Meireles RLAM[3], Cordeiro AMTM[4], Ribeiro TP[1], Medeiros IA[1] [1]UFPB – Farmácia, [2]UFPB – Ciências da Saúde, [3]UFCG, [4]UFPB

Introduction: Hypertension is characterized by increased peripheral vascular resistance, caused mainly by vascular dysfunction, which is reflected by impaired endothelium-dependent dilation and enhancement of vasoconstrictor response to different agonists.There is increasing evidence that an elevation of oxidative stress is mediator of vascular injury in hypertension. The aim of the present study was to examine whether chronic treatmentof alcohol-free (Cabernet-Sauvignon)lyophilized northeastern Brazilian red wine – RIOSOL (LRW-RSCS)reduces oxidative stress and improves microvascular dysfunction in essential hypertension. **Methods**: All protocols were approved by CEUA-UFPB nº 010/2016. Phenolic compounds of the LRW-RSCS were quantifiedby high-performance liquid chromatography (HPLC) and DPPH method was used to evaluate the antioxidant capacity of LRW-RSCS. Moreover, spontaneously hypertensive rats (SHRs) were treated with LRW-RSCS(100 and 300 mg/kg) or vehicle and Wistar-Kyoto rats (WKY) received vehicle. All rats were treated by oral gavage once daily for 6 weeks. Vascular reactivity of resistance mesenteric artery (RMA) rings was assessed in tension myograph and fluorescence technique was used to determination of superoxide anion (O^{2-}) production by dihydroethidine (DHE) in RMA. **Results**: HPLC analysis identified nineteen different phenolic compounds in the LRW-RSCS. Furthermore, this lyophilized demonstrated potent DPPH free-radical-scavenging activity. At the end of the treatment, control SHR exhibited attenuated endothelium dependent relaxation, enhancement of vasoconstrictor response and increased O^{2-} production by DHEin comparison to WKY rats.The treatment with 100 and 300 mg/kg of LRW-RSCS induced a reduction in the vasoconstrictor response by Phe(pD_2:5.3 ± 0.06, n:5; pD_2:5.4 ± 0.1, n:5, respectively, $p<0.05$) compared to control SHR (pD_2:5.8 ± 0.1; n:5); increased endothelium-dependent vaso dilation induced by ACh (pD_2:8.78 ± 0.2, n:6; pD_2:8.65 ± 0.1, n:6, respectively, $p<0.05$) compared with control SHR (pD_2:7.8 ± 0.2,n:6). Protective effect of the LRW-RSCS treatment (100 and 300 mg/kg) on the endothelial function involves increased NO-mediated component of relaxation, assessed by ACh-induced vasodilation in the presence of indomethacin and TRAM 34 plus apamin (MR:88.6 ± 5.5%, n:6; MR:89.5 ± 1.3%, n:5, respectively, $p<0.05$), in comparison to control SHR (MR:58.2 ± 11.4%; n:5). Moreover, LRW-RSCS improvement of the EDHF-mediated component of relaxation, assessed byACh-induced dilation in the presence of indomethacin and L-NAME(MR:88.4 ± 5.4%; n:6; MR:89.2 ± 2.5%; n:6, respectively, $p<0.05$), compared with control SHR (MR:66.3 ± 8.7%; n:6). Treatment with LRW-RSCS (100 and 300 mg/kg) significantly reduced fluorescence intensity (% control WKY) of DHE probe (175.7 ± 9.4%, n:5; 168.3 ± 12.3%, n:5, respectively, $p<0.05$)when compared with control SHR (224.5 ± 5.7%, n:4). **Conclusion**: These results demonstrate that LRW-RSCS is rich in phenolic compounds with potent antioxidant activity. Moreover, treatment of the SHR with the LRW-RSCS reduces microvascular dysfunction by decreasing the tissue oxidative stress and increasing the bioavailability of NO and EDHFs. **Financial support**: CNPq and CAPES.

06.024 Increased cellular excitability and its cross-talk with activity of the sympathoadrenal axis and hypertension development by chronic ethanol consumption by normotensive and hypertensive rats. Bomfim GHS[1], Méndez-López I[2], Padín JF[2], Jurkiewicz A[1], García AG[2], Jurkiewicz NH[1] [1]Unifesp-EPM – Farmacologia, [2]Universidad Autónoma de Madrid – Farmacologia

Introduction: Chronic ethanol (EtOH) consumption and enhanced activity of the sympathoadrenal axis have been associated with development of hypertension. However, the sequence of alterations in the, electrophysiological properties, calcium handling and exocytosis from adrenal chromaffin cells (CCs) remain poorly understood. Therefore, this study aimed to establish the correlation between chronic EtOH consumption and alterations in the sympathoadrenal axis. **Methods:** Male Wistar Kyoto (WKY) and Spontaneously Hypertensive (SHR) rats were subjected to the intake of increasing EtOH concentrations (5-20%, for 30 days). The experiments were approved by the Animal Research Ethics Committee from UNIFESP with protocol number 84750303/15. **Results:** With respect to WKY/Control, in CCs of WKY/EtOH the following changes were found: **1)** mild depolarization and higher frequency of spontaneous action potentials (SAPs); **2)** decreased Ca^{2+} currents (I_{ca}) with slower inactivation; **3)** decrease of K^{+} currents both in voltage ($I_{K(v)}$) and Ca^{2+}-dependent component ($I_{K(Ca)}$); **4)** augmented cytosolic Ca^{2+} transients ($[Ca^{2+}]_c$) and **5)** pronounced increase in catecholamine release. In addition, we also found an increase in systolic blood pressure (SBP) and cardiac hypertrophy in both rats, as well as massive generation of H_2O_2 and enhanced activity of ALDH. **Conclusion:** The results suggest that the hypertension elicited by chronic EtOH consumption has pathogenic features common to SHR rats, with an augmented activity of the sympathoadrenal axis that gives rise to augmented $[Ca^{2+}]_{c,}$cellular excitability and exocytotic signals in CCs. This work was supported by the Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES (BEX 8477/13-2); the Fundação de Amparo a Pesquisa do Estado de São Paulo-FAPESP (2014/01569-0)

06.025 Effects of exercise on the cardiovascular response to repeated restraint stress in rats. Veríssimo LF[1], Volpini VL[1], Matsubara NK[1], Estrada VB[1], dos Santos DC[1], Marques LAC[1], Ceravolo GS[1], Gomes MV[2], Martins Pinge MC[1], Pelosi GG[1] [1]UEL – Ciências Fisiológicas, [2]UENP- Ciências da Saúde

Introduction: Stress activates various physiological systems in order to maintain homeostasis (Radley J, et al. *Neurosci Biobehav Rev*. (58), 79, 2015), integrating behavioral, endocrine, immune and cardiovascular responses (Herman JP. *Front Behav Neurosci*. (7), 61, 2013). During the restraint stress the major cardiovascular changes observed are the increase in mean arterial pressure (MAP) and heart rate (HR)(Tavares RF, et al. *Neuroscience*. (143), 231, 2006) . Physical training promotes acute effects as increased of sympathetic activity, HR and cardiac output (Thompson PD, et al. *Med Sci Sports Exerc*. (33), 438, 2001). Considering that, we aim to observe the effect of physical exercise practice on the cardiovascular responses to repeated restraint stress in rats. **Methods:** We used Wistar rats (50-90 days); one group performed physical training for 4 weeks according to Kashimoto et al (2005) protocol (Kashimoto RK, et al. *Behav Brain Res*. (296), 286, 2016) associated to 1h session of restraint stress for 5 consecutive days (repeated stress); other group was submitted only to repeated restraint stress protocol. After the fourth stress session, animals were anesthetized (tribromoethanol, 0,25g/Kg i.p.) and a catheter was introduced into the femoral aorta. The blood pressure recording was carried out during and after the last session of restraint repeated stress. **Results:** The physical training did not alter the pressor response during restraint stress (Time: MAP: $p<0,0001$, $F_{(15, 270)}=4,699$; Treatment: MAP: $p=0,3343$, $F_{(1, 18)}=0,9841$; Interaction: MAP: $p=0,2608$, $F_{(15, 270)}=1,214$; n= repeated stress (RS): 10, repeated stress trained (RST): 10), but reduced the tachycardic response (Time: heart rate (HR): $p<0.0001$, $F_{(15, 270)}=37,17$; Treatment: HR: $p=0,0095$, $F_{(1, 18)}=8,420$; Interaction: HR: $p<0,0001$, $F_{(15, 270)}=7,573$; n= RS: 10, RST: 10). Comparing the recovery of stress, physical exercise showed no change in MAP before and after the repeated restraint stress (before stress: RS: 94,8 ± 2,93 mmHg, n=10; RST: 100,0 ± 2,82 mmHg, n=10; after stress: RS: 97,5 ± 2,15 mmHg, n=10; RST: 95,19 ± 3,16 mmHg; n=10; $p=0,5397$; $F_{(3, 38)}=0,7314$). Regarding the HR, restraint stress caused an increase in that parameter in both groups, however, it was recovered in the trained group 30 minutes after the stress session (before stress: RS: 357,1 ± 9,21 bpm, n=10; RST: 344,3 ± 5,89 bpm, n=10; after stress: RS: 408,5 ± 11,98 bpm, n=10; RST: 365,6 ± 3,97 bpm, n=18; $p < 0,0001$; $F_{(3, 36)}=11,11$). **Conclusion:** The data suggested that physical training was able to reduce the tachycardic response to repeated restraint stress and induce a better recovery of heart rate in compared to control group, but the mechanisms involved on this response still need to be elucidated. **Financial Support:** Scholarship and funding of the project by CAPES and CNPq (proc. 478566/2013-1). **Animal Research Ethical Committee:** CEUA – UEL: 14441.2013.18

06.026 Treatment with enalapril prevents functional decline in hypertensive rats dos Santos DC[1], Veríssimo LF[1], Raquel HA[1], Volpini VL[1], Marques LAC[1], Gomes MV[2], Fernandes KBP[2], Michelini LC[3], Pelosi GG[1] [1]UEL – Ciências Fisiológicas, [2]UNOPAR, [3]USP

Introduction: Enalapril, an inhibitor of angiotensin converting enzyme (ACE), is an antihypertensive (AH) drug widely used[1]. Studies suggested that ACE inhibitors could have a beneficial effect on musculoskeletal system[2], however, if the use of ACE inhibitors is effective in sarcopenia[3] is still a question that remains unclear. Pharmacological strategies currently available to prevent or reverse sarcopenia are unsatisfactory or have conflicting results[4]. In this context, the aim of this study was to evaluate the acute and later effects of pharmacological treatment with enalapril in hypertensive animals subjected to maximal exercise test on a rodent treadmill. **Methods:** Adult spontaneously hypertensive rats were divided into 3 groups: Control Group (CG, n=11), it was given tap water; Enalapril group (ENA: ACE inhibitor group, 10 mg/kg/day[5], n=13); Losartan group (LOS: selective AT1 receptor antagonist group, 10 mg/kg/day[6], n=13). Animals were treated daily for 28 consecutive days and submitted to the maximal exercise tests[7] once a week. The maximum distance travelled at the end of each test was assessed. Seven days after the last treatment, a group of animals performed the maximal exercise test, in order to verify the late effect of the treatment. **Results:** After 14 days of treatment, we observed a decline on the distance travelled on CG, compared to the ENA, but there were no differences between GC and LOS, or LOS and ENA. However, after 28 days of treatment, a decline on the distance travelled on CG and LOS compared to ENA (Time: P=0.0448, $F_{(4,\ 136)}$=2.509; Treatment: P=0,0276, $F_{(2,\ 34)}$=3,996; Interaction: P=0,2758, $F_{(8,\ 136)}$=1,249; n=CG:11; ENA:13; LOS:13) was observed. Regarding the delayed effect treatment, we observed decline in the distance walked in CG, not observed in the other groups. One week after the end of the treatment, the ENA group still showed greater distance travelled during the maximal exercise test when compared to the CG (Basal: CG: 275,0 ± 21,5 m, n: 4; LOS: 121,8 ± 16,6 m, n: 6; ENA: 222,1 ± 27,9 m, n: 5; After 1 week: CG: 121,8 ± 16,6 m, n: 4; LOS: 222,1 ± 27,9 m, n: 6; ENA: 346,5 ± 76,3 m, n: 5; P=0,0020; $F_{(5,\ 24)}$=5,322). **Conclusion:** Enalapril prevented the decline of the distance travelled in hypertensive animals and this effect remains seven days later the end of the treatment. The mechanisms for this response still need to be elucidated. **References:** 1. Regulski M, et al. *Curr Pharm Des*. 2015;21(13):1764-75. 1. Buford TW, et al. *J Am Geriatr Soc*. 2012;60(7):1244-52. 3. Zhou LS, et al. *Drugs Aging*. 2015 Sep;32(9):727-35. 4. Sartiani L, et al. *Clinical Cases in Mineral and Bone Metabolism*. 2015;12(2):135-138. 5. Dalpiaz PLM, et al. *Braz J Med Biol* Res 2013, vol.46, n.2, pp. 171-177. 6. Abdulla MH et al. *Eur J Pharmacol*. 2009, 612(1-3):69-74. 7. Melo RM, et al. *Hypertension*. 2003; 42: 851-857. **Financial Support:** CAPES. **Animal Research Ethical Committee:** CEUA/UEL n. 30987.2014.73

06.027 β_1-adrenergic receptor activation induces vascular oxidative stress and hypertension in a model of chronic ethanol consumption. Vale GT[1], Tirapelli CR[2] [1]FMRP-USP – Farmacologia, [2]EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas

Chronic ethanol consumption induces hypertension and oxidative stress. Our study evaluated the participation of β_1-adrenergic receptor in the cardiovascular effects induced by chronic ethanol consumption. Male Wistar rats (200-250g) were divided into 4 groups: control (C), ethanol 20% (v/v) (E), control + nebivolol (10mg/kg/day p.o.) (CN) and ethanol 20% (v/v) + nebivolol (10mg/kg/day p.o.) (EN). (CEUA Protocol: 14.1.357.53.2). One-way ANOVA followed by Newman Keuls test ($p<0.05$) was used to analyze the results. Chronic ethanol consumption increased systolic blood pressure (SBP) (mmHg), and this response was prevented by nebivolol (C:118 ± 1.2,n=12; E:138 ± 1.9*,n=12; CN:117 ± 1.1,n=12; EN:119 ± 1.1,n=12). Ethanol caused no alteration on H_2O_2 but increased O_2^- aortic levels (RLU/mg protein) that was prevented by nebivolol (C:109.3 ± 8.9,n=6; E:204.1 ± 6.1*,n=4; CN:113.2 ± 10.5,n=6; EN:121.8 ± 14.5,n=6). Qualitative analyses using Dihydroethidium and 2,7-Dichlorofluorescin diacetate corroborates these results. Ethanol consumption increased plasma (nmol/ml) and aortic (nmol/mg protein) TBARS levels and nebivolol prevented these responses (Plasma: C:10.4 ± 1.3,n=9; E:20.6 ± 4.2*,n=8; CN:10.5 ± 1.3,n=12; CN:9.8 ± 1.3,n=10); (Aorta: C:31.5 ± 3.5,n=7; E:54.1 ± 5.1*,n=6; CN:28.5 ± 3.3,n=11; EN:32.3 ± 4.5,n=10). No differences were found in plasma 8-isoprostane. Nebivolol preventend ethanol-induced aortic expression of Nox1 (C:1.08 ± 0.13,n=4; E:1.26 ± 0.15*,n=5; CN:1.03 ± 0.08,n=5; EN:1.03 ± 0.11,n=5). Nebivolol (CN and EN) reduced Nox2 and Nox4 aortic expression (Nox2: C:0.49 ± 0.01,n=4; E:0.50 ± 0.03,n=6; CN:0.42 ± 0.01*,n=4; EN:0.43 ± 0.01*,n=4), (Nox4: C:0.70 ± 0.07,n=4; E:0.67 ± 0.05,n=5; CN:0.58 ± 0.01*,n=5; EN:0.57 ± 0.02*,n=6). Ethanol consumption decreased plasma (nmol/ml) and aortic (nmol/mg protein) Nitrate/Nitrite levels that were prevented by nebivolol treatment (Plasma: C:38.2 ± 2.4,n=6; E:22.9 ± 3.1*,n=10; CN:31.1 ± 3.9,n=10; EN:32.6 ± 2.9,n=7); (Aorta: C:18.5 ± 2.1,n=8; E:5.6 ± 0.8,n=5*; CN:13.7 ± 2.9,n=7; EN:15.5 ± 2.9,n=7. Qualitative analyses using 4,5-Diaminofluorescein corroborates these results. Treatment with nebivolol (CN and EN) increased eNOS expression in the aorta (C:0.43 ± 0.03,n=4; E:0.44 ± 0.03,n=5; CN:0.69 ± 0.08*,n=4; EN:0.65 ± 0.05*,n=5. No difference was found in plasma superoxide dismutase (SOD) activity and treatment with nebivolol (CN and EN) reduced aortic SOD activity (% inhibition rate) (C:94.2 ± 12.5,n=6; E:96.7 ± 5.9,n=6; CN:48.8 ± 6.2*,n=6; EN:44.4 ± 5.7*,n=7) and SOD2 expression (C:1.21 ± 0.04,n=5; E:1.17 ± 0.11,n=5; CN:1.02 ± 0.13*,n=5; EN:0.99 ± 0.10*,n=5). No difference was found in SOD1 expression, catalase activity (plasma and tissue) or on aortic GSH levels. Treatment with nebivolol and ethanol+nebivolol increased and reduced GSH levels (µg/ml), respectively (C:3.8 ± 0.6,n=9; E:4.3 ± 0.8,n=8; CN:7.2 ± 0.8*,n=10; EN:1.3 ± 0.2*,n=10). Ethanol consumption increased plasma renin levels (pg/ml) and Angiotensin Converting Enzyme activity (ACE) (RFU) and both responses were prevented by nebivolol (Renin: C:7.63 ± 1.01,n=8, E:12.59 ± 1.82*,n=9; CN:9.12 ± 1.77,n=9; EN:9.45 ± 1.92,n=7), (ACE: C:1153.5 ± 137,n=7, E:1607.6 ± 106*,n=7; CN:1342.3 ± 37,n=9; EN:1383.2 ± 114,n=7). We conclude that both the increase in blood pressure and vascular oxidative stress induced by ethanol are mediated by β_1-adrenergic receptor activation. **Financial support**: CNPq.

06.028 AT_1 receptor activation induces vascular oxidative stress and hypertension in a model of ethanol withdrawal. Gonzaga NA[1], Tirapelli CR[2] [1]FMRP-USP – Farmacologia, [2]EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas

Introduction: Ethanol withdrawal (EW) induces hypertension and oxidative stress[1]. Our study evaluated the participation of angiotensin II on the cardiovascular effects induced by EW. **Methods**: male *Wistar* rats (250g) were divided into 6 groups: Control: animals received water *ad libitum* for 21 days and daily gavage (DG) of vehicle (CV) or losartan (LST-10mg/kg/day - CL); Ethanol: animals were treated with an ethanol 9% (v/v) for 21 days and DG of vehicle (EV) or LST (EL); Ethanol Withdrawal: animals were treated in the same way that ethanol group for 21 days and after that ethanol was removed and the animals received water *ad libitum* until the 23rd during EW period (48h) rats received DG of vehicle (EWV) or LST (EWL). Systolic blood pressure (SBP) was measured by plethysmography. The levels of thiobarbituric acid reactive substances (TBARS), Nitrate/Nitrite (NOx), glutathione (GSH), and the activity of superoxide dismutase (SOD) were measured by colorimetric assay. The levels of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) were detected by chemiluminescence of lucigenin and fluorimetry, respectively. Two-way ANOVA followed by Bonferroni was used to analyze the results. The protocols were approved by the Ethics Committee (CEUA:11.1.1432.53.5). **Results**: EW increased SBP (mmHg; n=10) that was prevented by LST (CV=121 ± 1; CL=122 ± 0.8; EV=121 ± 1 EL=122 ± 0.8; EWV=154 ± 1*; EWL=122 ± 1). EW increased O_2^- generation (RLU/mg protein; n=8) (CV=248 ± 19; CL=214 ± 18; EV=228 ± 18; EL=214 ± 21; EWV=655 ± 51*; EWL=199 ± 24), reduced H_2O_2 aortic levels (µmol/L/mg protein; n=8) (CV=0.50 ± 0.05; CL=0.49 ± 0.06; EV=0.46 ± 0.05; EL=0.49 ± 0.08; EWV=0.21 ± 0.02*; EWL=0.53 ± 0.05), and these responses were prevented by LST. Qualitative analyses *in situ* using Dihydroethidium and 2,7-Dichlorofluorescin diacetate corroborates these results. EW increased plasma (nmol/ml; n=9) and aortic (nmol/mg protein; n=13) TBARS levels and LST prevent these responses (Plasma: CV=10 ± 1; CL=12 ± 1; EV=13 ± 1; EL=14 ± 1; EWV=20 ± 2*; EWL=9 ± 1; Aorta: (CV=5.8 ± 0.8; CL=4.5 ± 0.6; EV=6.3 ± 1; EL=4.7 ± 0.7; EWV=10 ± 1*; EWL=6.7 ± 0.8). EW decrease plasma (nmol/ml; n=12) and aortic (nmol/ml; n=10) NOx levels and LST prevented these responses (Plasma: CV=13 ± 0.9; CL=13 ± 1; EV=11 ± 0.7; EL=12 ± 1; EWV=7 ± 1*; EWL=11 ± 0.9; Aorta: CV=26 ± 3; CL=24 ± 1; EV=24 ± 3; EL=25 ± 2; EWV=16 ± 1*; EWL=22 ± 3). Qualitative analyses *in situ* using 4,5-Diaminofluorescein corroborates these results. No difference was found in plasma or aortic SOD activity. In plasma but not in aorta, EW increased GSH levels (µg/ml; n=8) and LST did not prevent this response (CV=2.1 ± 0.1; CL=1.8 ± 0.2; EV=2.4 ± 0.2; EL=2.7 ± 0.3; EWV=16.2 ± 2*; EWL=18 ± 2*). **Conclusion**: EW increases SBP, systemic and vascular oxidative stress and these responses are mediated by AT_1 receptor. **Financial support**: CNPq and FAPESP [1]Gonzaga NA, Mecawi AS, Antunes-Rodrigues J, De Martinis BS, Padovan CM, Tirapelli CR. Ethanol withdrawal increases oxidative stress and reduces nitric oxide bioavailability in the vasculature of rats. Alcohol. 2015 Feb; 49(1): 47-56.

06.029 Phosphodiesterase-5 inhibitors and novel N-acylhydrazone derivative agonist of adenosine A2A receptor ameliorate pulmonary hypertension-induced impairment of skeletal muscle function in rats. Silva AMS, Carvalho FIS, Alencar AKN, Fraga CAM, Barreiro EJ, Zapata-Sudo G, Sudo RT UFRJ

Introduction: Exercise intolerance is a cardinal symptom of patients with pulmonary hypertension (PH), a cardiovascular disease with high morbidity and reduced quality of life. This work studied the potential of the phosphodiesterase-5 (PDE5) inhibition and adenosine A2A receptor (A2AR) activation in rats with PH related exercise intolerance and skeletal muscle contraction. **Methods:** Male Wistar rats received a single intraperitoneal injection of monocrotaline (60 mg/kg) for PH induction. Studied groups were: control + vehicle (DMSO), MCT + vehicle, MCT + sildenafil (PDE5 inhibitor; 170 µmol/kg/day p.o.), MCT + lodenafil (PDE5 inhibitor; 170 µmol/kg/day p.o.) and MCT + LASSBio-1359 (A2AR selective agonist; 170 µmol/kg/day p.o.). Animals were treated with vehicle or drug for 14 days after the onset of PH (n = 6 per group). Treadmill test was performed to access the exercise performance. Right ventricular systolic pressure (RVSP) and transthoracic echocardiography analysis confirmed success of PH and showed a pulmonary hypertension-induced left ventricular (LV) dysfunction. Afterwards, functional and structure studies were performed in isolated *extensor digitorum longus* (EDL) and *soleus* (SOL) of rats in the presence and absence of treatment with substances. **Results:** Time to exhaustion (s) was reduced from 1188 ± 43.4 (control) to 188.8 ± 26.1 (MCT + vehicle) and increased to 695.7 ± 23.1 (MCT + sildenafil), 853.2 ± 64.5 (MCT + lodenafil) and 778.5 ± 56.3 (MCT + LASSBio-1359; $P < 0.05$ *vs* MCT + vehicle). RVSP (mmHg) was increased from 23.83 ± 0.9 (control) to 82.5 ± 4.1 (MCT + vehicle; $P < 0.05$), confirming PH onset, and it was reduced to 40.8 ± 1.2 (MCT + sildenafil), 31 ± 1 (MCT + lodenafil) and 36.3 ± 0.9 (MCT + LASSBio-1359; $P < 0.05$ *vs.* MCT + vehicle). LV ejection fraction (%) was reduced from 66.1 ± 1.6 (control) to 31.1 ± 1 (MCT + vehicle) and increased to 50.1 ± 1.1 (MCT + sildenafil), 57.7 ± 2.4 (MCT + lodenafil) and 76.4 ± 0.9 (MCT + LASSBio-1359; $P < 0.05$ *vs* MCT + vehicle). The skeletal muscles cross-sectional areas (CSA; cm^2) were reduced from 0.14 ± 0.01 (control) to 0.08 ± 0.01 in EDL and from 0.15 ± 0.01 (control) to 0.09 ± 0.01 in SOL (MCT + vehicle; $P < 0.05$). Sildenafil, lodenafil and LASSBio-1359 reduced the skeletal muscle atrophy, as depicted by the increased CSA in both EDL (0.15 ± 0.01, 0.15 ± 0.01 and 0.12 ± 0.01, respectively) and SOL (0.13 ± 0.01, 0.14 ± 0.01 and 0.12 ± 0.01, respectively; $P < 0.05$ *vs* MCT + vehicle). *In vitro* protocol of muscle fatigue showed a reduction of the force development (mN/cm2) in EDL from PH rats [80.1 ± 20.5 compared to control (187.1 ± 15.2); $P < 0.05$]. The same occurred in SOL from PH rats [46.9 ± 13.3 compared to control (110.9 ± 10.7); $P < 0.05$]. Sildenafil, lodenafil and LASSBio-1359 increased skeletal muscle force development in both EDL (156 ± 17, 184.9 ± 7.2 and 126.5 ± 6.3, respectively) and SOL (124.1 ± 4.6, 130.2 ± 5.2 and 100.5 ± 5.1, respectively; $P < 0.05$ *vs* MCT + vehicle). **Conclusion:** Beside sildenafil, the novel A2A receptor agonist LASSBio-1359 and the PDE5 inhibitor, lodenafil, represent new candidates for treatment of PH. **Financial support:** CNPq, FAPERJ, CAPES, INCT-INOFAR, CRISTÁLIA **Research approval:** DFBCICB 020

06.030 Antiplatelet effects of MK571 (MRP4 inhibitor) and bay 60-2770 (soluble guanylyl cyclase activator) in human platelets: A new perspective in cardiovascular therapeutics.
Silvério-Mendes CM[1], Sollon CS[2], Anhê GF[2], De Nucci G[2], Mónica FZ[2], Antunes E[2] [1]Unicamp – Farmacologia, [2]Unicamp

Introduction: Multidrug resistance proteins (MRP) are transmembrane proteins capable of pumping cyclic nucleotides out of cells, thereby affecting signaling events mediated by cGMP and cAMP[1]. MRP4 is highly expressed in human platelets[1]. Nitric oxide-independent soluble guanylyl cyclase (sGC) activators are reported to reactivate haem-oxidized sGC in vascular diseases[2]. We tested the hypothesis that MRP4 inhibition potentiates the anti-platelet activities of sGC activation. Therefore, this study was undertaken to investigate the interactions of MK571 (MRP4 inhibitor) and BAY 60-2770 (haem-independent sGC activator) in human washed platelets. **Methods:**The experimental protocols, CAAE n° 46832315.8.0000.5404 were approved by the Human Ethics Committee of the State University of Campinas (UNICAMP). Human washed platelet aggregation assays and measurements of cAMP and cGMP levels, as well as monitorization of intracellular calcium levels $[Ca^{2+}]i$ in platelets loaded with a fluorogenic Ca^{2+}-binding dye (FluoForte) were performed. **Results and Discussion:** BAY 60-2770 (0.001–10 µM) produced significant inhibition of collagen (2 µg/mL) and thrombin (0.1 U/mL)-induced platelet aggregation, which was markedly potentiated by prior incubation with MK571 (10 µM). BAY 60-2770 significantly increased the cGMP levels that paralleled a marked $[Ca^{2+}]i$ reduction, both of which were potentiated by MK571. The cAMP levels remained unchanged by BAY 60-2770. MK571 alone had no significant effect in any studied assay. **Conclusion:** MRP4 inhibition by MK571 potentiates the cGMP signaling pathway leading to a marked anti-platelet activity. A combination of MRP4 inhibition and sGC activation in platelets could be of therapeutic interest in cardiovascular diseases associated with thrombotic complications. **References:** [1]Jedlitschky G. et al, Blood 119: 3394, 2012, [2]Evgenov, O.V., Nat Rev Drug Discov, 5, 755, 2006. **Acknowledgments:** CNPq and FAPESP. **Financial support:** CNPq and FAPESP.

06.031 Protective effect of rimonabant against the increased reactivity to vasopressin after induction of sepsis by the cecal ligation and puncture (CLP) model. Leite MCG[1], Souza P[2], da Silva-Santos JE[2], Zampronio AR[1] [1]UFPR- Farmacologia, [2]UFSC – Farmacologia

Introduction: During severe sepsis, cardiovascular hyporeactivity to catecholamines and other vasoconstrictors is one of the most pronounced effects of human septic shock and contributes to the high mortality rate associated with septic shock (Dellinger et al., Crit Care Med, 32:858, 2004). Previous results from our group showed that oral and icv administration of rimonabant increases the survival rate and increased circulating arginin-vasopressin levels 12 h after CLP. The aim of this study was to investigate whether treatment with rimonabant has influence on vascular reactivity to phenylephrine and vasopressin. **Methods**: sepsis was induced in male Wistar rats (180-200 g) by cecal ligation and puncture (CLP) and 4 hours after induction animals were orally treated with rimonabant (10 mg/kg) or vehicle (10% carboxymethylcellulose). Six and 12 h after CLP, the descending thoracic aorta was removed and the vascular reactivity of 3-4 mm long rings to vasoactive agents was evaluated. All procedures were approved by the Institutional Ethics Committee of UFPR (protocol # 828). **Results**: Six hours after induction of sepsis, aortic rings obtained from the CLP group showed a decreased vascular reactivity to phenylephrine, compared with vessels from the sham animal (CLP group:1,22 g Sham group:1,93 g). Treatment with rimonabant did not alter this response. Six and twenty four hours after sepsis induction, there were no differences between groups in the vascular reactivity to AVP and phenylephrine, respectively. At 24 h after the CLP surgery, the vessels from the CLP/vehicle group showed a hyperresponsiveness to vasopressin, compared to false-operated animals (CLP group:1,34 g Sham group:0,79 g), the treatment with rimonabant prevented this hyperresponsiveness (Rim group: 0,60 g). **Conclusion**: The data described in this study demonstrate that the treatment with the selective blocker of the cannabinoid CB_1 receptor rimonabant prevented sepsis-associated impairment in the responsiveness to vasopressin induced by the CLP model of severe sepsis. **Financial support**: CNPq and Capes. Animal Ethics Committees: 828/2014

06.032 Function of AT1 and AT2 Receptors in atrial contractions from hypertensive or diabectis induced-STZ RATS. Musial DC[1], Miranda-Ferreira R[1], Pena MGG[1], Bomfim GHS[1], Arranz JA[2], Padín JF[2], Jurkiewicz A[1], García AG[2], Jurkiewicz NH[1] [1]Unifesp-EPM – Farmacologia, [2]Universidad Autónoma de Madrid – Farmacologia

Introduction: Diabetes mellitus and hypertension are diseases that have a strong connection and a high cardiovascular risk. Due this, we decided study the function of angiotensin II receptors in atria isolated from diabetics or hypertensive rats. **Methods:** We distributed the animals in three groups: Control - WKY (Wistar Kyoto), Diabetic and SHR (Spontaneous hypertensive rats). Diabetes was induced in Wistar Kyoto rats by I.P of streptozotocin at dose of 60 $mg.kg^{-1}$. The diabetic rats were sacrificed after 30 days of injection. All animals 4 month old in moment of the experiment. We evaluated the glucose and blood pressure by tail coff; atrial expression and contractions from AT1 and AT2 receptors in right (RA) and left atria (LA). The RA and LA was removed and mounted in isolated organ bath to study the contractions. The LA the contractions was induce by electrical field stimulation (2Hz; 5ms, 20-40V) in the presence of AT1 and AT2 agonist or antagonist. We also analyzed the expression of AT1 and AT2 receptor by Western Blot. The experiments conducted were approved by the Ethics Committee in Research from Universidade Federal de São Paulo. **Results:** The glucose in SHR compared with WKY was no diference, but the diabetic rats were 3.7 fold compared with WKY. In relation the blood pressure diabetic rats were increase (163 ± 2 mmHg) compared with WKY (131 ± 2 mmHg), but the SHR (211 ± 4 mmHg) was higher than diabetic rats. The expression of AT1 in LA from SHR was higher than diabetics, no difference in SHR or diabetic when compared with control, and no diference in AT2 as well. To study AT2 receptor we blocked the AT1 receptor with losartan (10^{-6} $M.l^{-1}$) and the atria was stimulated by AngII (10^{-12} - 10^{-6} $M.l^{-1}$), no diference in RA was observe, but in the LA the diabetic and SHR had a decrease in the response compared to WKY, we also used novokinin (10^{-12} - 10^{-6} $M.l^{-1}$ AT2 agonist) and the response was decrease in LA and RA from SHR compared with WKY, no change in pD_2 was observe. To study AT1 receptor we blocked AT2 with PD123177 (10^{-6} $M.l^{-1}$) and the atria was stimulated by AngII (10^{-12} - 10^{-6} $M.l^{-1}$), we observed in LA and RA an increase in contractile force in diabetic and SHR compared with WKY. We also did a time-effect curve with caffeine 10^{-2}M, thapsigargin 10^{-6}M e ryanodine 10^{-7}M to evaluate the calcium intracellular stock concentration, and we observed that in atria from diabetic and SHR have calcium levels higher than WKY. **Conclusion**: Our data show some alterations in atrial functions in AT1 (increase of response) and AT2 (decrease of response) receptors, but no alterations in expression in this receptors was observed, probably these alterations was due intracellular change, like an increase in calcium intracellular stocks. The important factor that we need highlight is that in diabetic or SHR the angiotensin II receptor have the function alterated and this could be contribute to any cardiovascular changes in these models. **Financial support**: CNPq, CAPES e FAPESP. Research approval by the Animal Research Ethical Committee from UNIFESP: 6610210214

06.033 THE Cav1-BKCa interaction involved in the negative feedback control of the contraction of mesenteric arteries is lost in hypertensive humans. Garcia DCG[1], Costa ED[2], Rezende BA[3], Wainstein AJA[3], Lemos VS[2], Côrtes SF[1] [1]ICB-UFMG – Farmacologia, [2]ICB-UFMG – Biofísica e Fisiologia, [3]Faculdade de Ciências Médicas – BH – Ciências da Saúde

Introduction: The control of the myogenic tone of resistance arteries is essential for the regulation of blood pressure. This adjustment involves a fine tune of positive and negative feedback mechanisms regulated by the communication between the plasma membrane (PM) and the sarcoplasmic reticulum (SR), known as PM-SR junction. In vascular smooth muscle cells (VSMC) the interaction between Ca_v1, BK_{Ca} and ryanodine receptors (RyRs) for the activation of the negative feedback during contraction is not well described in human resistance arteries. Moreover, in hypertension, the feedback control of the contraction of VSMC might be altered. The present work investigated the mechanisms in the PM-SR junction involved in the negative feedback of the contraction induced by caffeine (a RyRs agonist) in mesenteric arteries from normotensive and hypertensive humans. **Methods**: The procure and the experimental protocols with human mesenteric arteries were approved by the ethics committee (CAAE: 03885312.0.0000.5149). All protocols were performed in endothelium-denuded vessels. The results were expressed as mean ± SEM of the peak and the area under the curve (AUC) of the responses to caffeine of at least five experiments. The data were statistically analyzed by unpaired Student's *t*-test and considered significantly different when $P < 0.05$. **Results:** Caffeine (10 mM) induced a transient contraction in arteries from normotensive humans (peak = 2.9 ± 0.2 mN/mm; AUC = 60.3 ± 6.9 mN/mm.s^{-1}) and hypertensive humans (peak = 3.1 ± 0.3 mN/mm; AUC = 54.0 ± 5.3 mN/mm.s^{-1}). In Ca^{2+}-free solution, the contraction induced by caffeine was significantly reduced ($P < 0.001$) in normotensive (peak = 0.7 ± 0.3 mN/mm; AUC = 17.4 ± 4.8 mN/mm.s^{-1}) and hypertensive (peak = 0.4 ± 0.2 mN/mm; AUC = 8.7 ± 1.3 mN/mm.s^{-1}) arteries. Nifedipine (10 µM), a Ca_v1 calcium channel blocker, increased the contraction induced by caffeine ($P < 0.05$) in arteries from normotensive humans (peak = 3.8 ± 0.3 mN/mm; AUC = 85.4 ± 5.9 mN/mm.s^{-1}), but did not have any significant effect in arteries from hypertensive humans (peak = 2.9 ± 0.3 mN/mm; AUC = 57.7 ± 6.0 mN/mm.s^{-1}). Paxilline, a BK_{Ca} channel blocker, also increased the contraction in arteries from normotensive humans (peak = 5.2 ± 0.5 mN/mm; AUC = 92.9 ± 14.1 mN/mm.s^{-1}), but did not induce any significant effect in arteries from hypertensive humans (peak = 3.2 ± 0.5 mN/mm; AUC = 56.9 ± 7.9 mN/mm.s^{-1}). **Conclusion:** The mechanism of negative feedback stimulated by the release of Ca^{2+} in the PM-SR junction involved the activation of Ca_v1 and BK_{Ca} in mesenteric arteries from normotensive humans is lost in arteries from hypertensive humans. **Financial support:** CNPQ, FAPEMIG

06.034 The NO-sGC-cGMP pathway is impaired in mesenteric arteries from rats with periodontitis. Jesus FN, Teixeira SA, Napolitano M, Costa SKP, Muscará MN USP – Farmacologia

Introduction: During the last years it has become evident that periodontal disease has systemic consequences. We have previously described that NO is a key regulator of some of the effects of periodontal disease on remote organs such as heart and kidney, and that endothelial dysfunction and decreased contractile response to norepinephrine *in vitro* also occur in aorta. Based in these results, we decided to evaluate the influence of periodontitis on rat mesenteric artery vasomotricity *in vitro*. **Methods**: Male Wistar rats (10-14 wk-old) were anesthetized with ketamine plus xylazine and periodontitis was induced by placing (subgingivally) a cotton ligature around both the left and right lower first molars (group P); sham rats (S) had the ligature immediately removed. Seven days later, the rats were killed by anesthesia overdosing and the mesenteric bed was dissected for isolation of the third-branch artery. The vessels were mounted on a wire myograph (containing Krebs solution bubbled with O_2/CO_2 at 37ºC) in order to evaluate the *in vitro* response to acetylcholine (ACh), sodium nitroprusside (SNP), sildenafil (Sil), the H_2S donor GYY4137, the sGC activator BAY60-2770, as well as in the presence of the K_{ATP} channel blocker glibenclamide (Gli) or the non selective K+ channel blocker tetraethylammonium (TEA). From the concentration-response curves, potency (pD_2) and maximal response (Emax) values were calculated. The vessels were also analysed for COX and NOS isoenzyme mRNA and Western blotting for sGC, catalase (CAT), superoxide dismutase (SOD) and nitrotyrosine-containing proteins (NT). Activities of the antioxidant enzymes SOD, CAT, glutathione (G) peroxidase (GPx), reductase (GR) and transferase GT) were also measured. Gingiva samples and ligatures were analysed for the presence of bacterial DNA by PCR. Differences between the groups were analysed by unpaired Student t-test. **Results**: ACh, SNP and Sil showed significantly lower relaxant potencies in arteries from group P in comparison with those from the S animals, with respective pD2 values 7.9 ± 0.2 vs. 7.3 ± 0.1 ($P<0.05$), 7.1 ± 0.1 vs. 6.4 ± 0.2 ($P<0.01$) and 10.3 ± 0.2 vs. 8.6 ± 0.2 ($P<0.001$), with unaltered Emax values. In the presence of TEA or Gli, the differential responses to ACh and SNP were abolished. No differences were observed between the groups in terms of artery diameter, or the relaxant responses to GYY4137 or BAY60-2770. Arteries from group P showed significantly increased iNOS mRNA (1.0 ± 0.1 vs 20.9 ± 5.9; $P<0.01$), NT (77.5 ± 11.8 vs 122.6 ± 15.1; $P<0.05$) and CAT activity (2.3 ± 0.2 vs 3.2 ± 0.3; $P<0.05$) parallel to lower SOD activity (0.7 ± 0.1 vs 0.4 ± 0.1; $P<0.01$) in comparison with group S. Significantly increased bacterial DNA was found in gingiva and ligatures obtained from group P in comparison with group S (24.0 ± 4.4 vs 47.4 ± 8.2; $P<0.05$). **Conclusions**: We conclude that during the early phase of ligature-induced periodontitis in rats, functional changes related to the NO-sGC-cGMP pathway occur in the mesenteric artery, which may be secondary to impaired hyperpolarization and/or nitration of selected protein tyrosine residues. **Financial Support**: FAPESP, CNPq and CAPES. Ethics Committee for Animal Experimentation: protocol CEUA-ICB 170, book 2/113, 2011.

06.035 Bradykinin increases blood pressure in endotoxemic rats Anton EL, Corrêa T, Fernandes D, Assreuy J, da Silva-Santos JE UFSC – Farmacologia

Introduction: It is well known that BK is a potent endothelium-dependent vasodilator in several vessels. Notably, systemic administration of BK in healthy subjects results in hypotension. In spite of its putative role in blood pressure regulation, the participation of BK in the cardiovascular effects of sepsis remains poorly investigated. The main hypothesis of this study was that blood pressure responses to BK are impaired in experimental models of sepsis. **Methods**: Female Wistar rats (200-280 g) received intraperitoneal (i.p.) injections of sterile saline (1 ml/kg), or lipopolysaccharide (LPS; 1 mg/kg), and were anesthetized at 6, 24, 48, or 72 h after for direct measurement of the mean arterial pressure (MAP). The hypotensive effect of intravenous (i.v.) BK (6, 20 and 60 nmol/kg), and acetylcholine (ACh; 60 nmol/kg) were assessed before and after the treatment with prazosin (0,5 mg/kg i.v.), HOE 140 (20 nmol/kg s.c.), losartan (15 mg/kg, i.v.), enalapril (3 mg/kg, i.v.), Y-27632 (0,1 mg/kg, i.v.), or indomethacin (10 mg/kg i.v.). In additional experiments, the effects of BK and ACh were evaluated in perfused mesenteric vascular bed obtained from the control and LPS 24 h groups. **Results**: Both the basal MAP and the hypotensive effects induced by BK and ACh remained unaltered in the LPS groups, which presented hematological evidence of systemic inflammation (i.e. leukocytosis), compared with the control group. However, in the LPS 24 h and 48 h groups, BK-induced hypotension was followed by a sustained and enhanced pressor effect, which was fully reproducible in perfused mesenteric bed preparations. For instance, in the LPS 24 h group, the MAP increased by 9 ± 2, 18 ± 5 and 24 ± 3 mm Hg in the control group, and by 29 ± 4, 42 ± 3 and 56 ± 4 mm Hg, after administration of 6, 20, and 60 nmol/kg BK, respectively. The hypertensive response to BK was not reduced by the selective antagonist of the alpha-1 adrenergic receptor prazosin, but was fully abolished by either HOE-140, a selective B2 receptor antagonist, or losartan, an angiotensin II (AII) AT1 receptor antagonist. We also found that the hypertensive effect of AII (60 nmol/kg) was increased from 52 ± 5 mm Hg in control animals to 75 ± 3 mm Hg in the LPS 24 h group. Notably, administration of HOE-140 was able to prevent the enhanced effect of AII in the LPS 24 h group. Moreover, the angiotensin-converting enzyme inhibitor enalapril, which also prevents BK breakdown, enhanced the hypertensive effect elicited by BK in the LPS 24 h group. Although the cyclooxygenase inhibitor indomethacin has not had any influence on the effect of BK in the MAP, the Rho-associated kinase (ROCK) inhibitor Y-27632 avoided the enhanced hypertensive effect of BK in LPS-treated animals. **Conclusion:** These results disclose that in spite of the typical hypotension produced by BK in healthy animals, in endotoxemic rats, an experimental model used to simulate systemic inflammation seen in sepsis, administration of BK produces biphasic blood pressure responses, characterized by hypotension followed by sustained pressor effect. The hypertensive response induced by BK in endotoxemic rats appears to be mediated by both bradykinin B2 and angiotensin II AT1 receptors, and is fully dependent on activation of ROCK. **Financial support:** CNPq (448738/2014-7). This study was approved by CEUA/UFSC (PP00566).

06.036 Effects of adjuvant induced arthritis on THE ANG II responses in rat aorta Tozzato GPZ[1], Chies AB[2] [1]IBB-Unesp-Botucatu, [2]FAMEMA – Farmacologia

Background: Life expectancy of patients with rheumatoid arthritis (RA) is lower due to increased mortality in consequence of cardiovascular disease. Indeed, this occurs due to endothelial dysfunction resulting from severe inflammatory activity related to RA. Moreover, evidences demonstrate the involvement of renin angiotensin system (RAS) in cardiovascular injury associated to RA and arthritis experimental models. It is suggested that RAS might impair the endothelial function due the angiotensin II type 1 receptor (AT_1) activation by angiotensin II (Ang II). Furthermore, evidences observed that testosterone modulates the Ang II in cardiovascular system. **Objectives:** To investigate the effects of adjuvant induced arthritis (AIA) on the responses of rat aorta to Ang II and acetylcholine (ACh). Moreover, to determine if eventual changes of aorta responses induced by AIA may be influenced by a reduction of plasma testosterone. **Methods:** Bilateral orchidectomy (ORQ) was performed in twelve weeks old male Wistar rats. The SHAM-orchidectomized group was also subjected to this surgical procedure, but the testis were preserved. Twenty days after ORQ, AIA was induced by a single intradermal injection of 0,05g of heat-killed *Mycobacterium tuberculosis* in 0,1mL mineral oil in the palmar surface of the right hind paw. Inflammation signs appeared into 15-20 days after AIA immunization. SHAM-immunized animals only received 0,1mL mineral oil. Twenty-one days after the onset of the disease these animals were sacrificed and rings (3 mm) of thoracic aorta were obtained to be set up in organ bath containing Krebs-Henseleit solution at 37^oC, pH 7,4, gassed with 95% O_2 and 5% CO_2, under basal tension of 1,5g. Some preparations had their endothelium mechanically removed. Preparations with or without endothelium were challenged with cumulative concentrations of Ang II or ACh in absence or presence of 1µmol losartan, 10µmol indomethacin or 100µmol L-NAME. Vascular tone modifications were recorded by isometric force transducers and expressed as concentration-response curves. Log of EC_{50} and maximal response R_{max} (n=6-9) were compared by two-way ANOVA/Bonferroni (significance when P<0,05). **Results:** No changes of Ang II responses were observed in consequence of AIA and or ORQ, in aorta with or without endothelium. The presence of L-NAME did not reveal any change of response in consequence of AIA and or ORQ. However, in the presence of indomethacin, the AIA reduced the AngII pEC_{50} either in SHAM-ORQ (from 8,24□0,09 to 7.70□0,16; P<0,05) or in ORQ animals (from 7.94□0,06 to 7.15□0,2; P<0,01). There also was an evident but not statistically significant reduction in the R_{max} (from 41,84□6,23 to 21,31□4,78) and pEC_{50} (from 6,02□0,13 to 5,69□0,17) to ACh in AIA group, in comparison to the SHAM-immunized group. **Conclusions:** The data suggest that the presence of prostanoids assure that the responses to Ang II are not influenced by AIA. However, in absence of prostanoids, AIA reduces the Ang II responses inasmuch the ORQ does not modify this phenomenon. Moreover, it is possible that AIA has induced endothelial dysfunction in the studied aorta. However, this conclusion depends on further experiments. **Financial Support**: CAPES. This research was approved by CEUA-FAMEMA protocol n° 1026/14.

06.037 Differential modulation of iNOS-derived nitric oxide on alpha-1 adrenergic agonists-induced vascular contraction in sepsis Bernardelli AK[1], da Silva-Santos JE[1]
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Introduction: Hypotension, a hallmark of sepsis, is putatively associated with a generalized hyporesponsiveness of the vascular system to vasoactive agents. Nevertheless, the impaired vascular reactivity of mesenteric arteries from rats subjected to the cecal ligation and puncture (CLP) model of sepsis showed a remarkable reduction in their responses to phenylephrine, but not to norepinephrine (BERNARDELLI et al., 2016, in press), in spite of the well known agonistic effects of these ligands on alpha-1 adrenergic receptors. In this study, we hypothesized that the activation of the same receptor by distinct ligands in vessels from septic animals may result in a differential modulation of intracellular mediators, such as nitric oxide, affecting the ability of vessels to increase their tone. **Methods:** Male Wistar rats (3-4 months old) were used in this study. Sepsis was induced by the CLP model, and the animals were euthanized 6 h (CLP 6 h group) or 18 h (CLP 18 h group) later for removal of the superior mesenteric artery, which was mounted in an organ bath apparatus. Only endothelium-intact preparations were used in this study. The effects of cumulative concentrations (1 nM–300 □M) of phenylephrine (PE) and norepinephrine (NE) were evaluated in ring-like preparations of the mesenteric artery, with or without the previous incubation with the nitric oxide synthase inhibitors L-NAME (100 μM), or 1400W (10 μM). The results obtained in vessels from CLP 6 h and 18 h groups were compared with those obtained from control animals (not subjected to CLP). **Results:** The maximal contractile responses (E_{max}) to PE were reduced from 0.8 ± 0.04 g in mesenteric arteries from control animals to 0.53 ± 0.02 and 0.28 ± 0.01 g in preparations from the CLP 6 h and CLP 18 h groups, respectively. However, the responses to NE were not significantly reduced in arteries from these same animals. The non-selective inhibitor of the nitric oxide synthase L-NAME was able to shift to the left the concentration response curves and increased the E_{max} to both PE and NE in all experimental groups, independently of the degree of refractoriness induced by the septic insult. On the other hand, the selective inhibitor of the inducible isoform of nitric oxide synthase (iNOS) 1400W, which had no effect on PE- and NE-induced contraction in mesenteric arteries obtained from the control group, also did not improve the contractile responses for these vasoconstrictors in preparations from the CLP 6 h groups, in spite of the reduced reactivity to PE found in these vessels. Interestingly, 1400W was able to increase the E_{max} to PE from 0.20 ± 0.01 g to 0.58 ± 0.02 in arteries from the CLP 18 h group. However, incubation with 1400W had no effect on the responses to NE in mesenteric arteries from the CLP 18 h group (E_{max} = 0.42 ± 0.2 and 0.44 ± 0.01, without and with 1400W, respectively). **Discussion:** Although high amounts of nitric oxide have been putatively associated with generalized vascular refractoriness in sepsis, our results suggests that the influence of nitric oxide, mainly derived from the inducible isoform of NOS, on the vascular contractility induced by activation of alpha-1 adrenergic receptors, may vary accordingly with the vasoactive agent used. **Financial support:** CNPq (448738/2014-7). This study was approved by CEUA/UFSC (PP00566).

06.038 Simvastatin induces cardiac repairment through Notch 1 activation in chronic Chagas Cardiomyopathy Guzmán-Rivera D, González-Herrera F, Lapier M, Pesce B, Maya JD University of Chile – Molecular and Clinical Pharmacology Program, Biomedical Sciences Institute (ICBM), Faculty of Medicine.

Introduction: Trypanosoma cruzi is the causal agent of Chagas Disease and it is endemic in Latin American and worldwide as a consequence of population migration. This disease has two phases: the acute phase is often asymptomatic and without the proper treatment it progresses to the chronic phase, affecting the heart or gastrointestinal tract. Chronic Chagas cardiomyopathy (CCC), the most severe clinical manifestation of the chronic phase, involves progressive myocarditis and inflammatory infiltrate affecting the ventricular wall causing cardiovascular complications such as heart failure, arrhythmias, thromboembolism and sudden death[1,2]. Although investigation in trypanocidal therapy, no one drug can stop or reverse the progressive heart tissue damage. Simvastatin is a drug that decreases blood cholesterol, also has anti-inflammatory effects and inhibits platelet aggregation. Our group described that simvastatin improves endothelial function and reduces myocardial inflammation caused by T. cruzi through 15-epi-lipoxin A4 production, an anti-inflammatory molecule[3]. A report suggested that simvastatin activates Notch pathway after a stroke in endothelial cells enhancing blood flow[4]. Also, after a myocardial infarction, Notch 1 increases cardiomyocytes proliferation, promotes angiogenesis and regeneration and decreases fibrosis and cardiac remodeling[5]. CCC progress with myocardial inflammation, endothelial damage with microfocal ischemia and fibrosis. Therefore, an interesting therapeutic strategy is investigating drugs that can modulate Notch pathway, to revert the heart damage induced by T. cruzi and could be rapidly incorporated in Chagas disease treatment. We propose that simvastatin reverts cardiac damage in chronic infection by T. cruzi though Notch 1 pathway activation. **Methods**: HUVEC cells were infected by T. cruzi Dm28 strain during 16 hours, treated with 5 µM simvastatin and 100nM 15-epi- lipoxin and assayed by flow cytometry and qPCR. Sv/129 mouse were chronically infected with T. cruzi Dm28 strain and treated with simvastatin 5mg/Kg/day during 20 days. At 80 day post infection animals were sacrificed and the tissue assayed by inmunohistochemistry. **Results**: Notch signaling pathway is activated as evidenced by increased Notch 1 expression on the cell surface and migration to the nucleus of the Notch intracellular domain in HUVEC cells. *In vivo*, Notch 1 expression increases correlating with an increase in cardiomyocyte proliferation and angiogenesis, evidenced by Ki67 and isolectin. **Conclusion**: Thus, we concluded that simvastatin enhances cardiac conditions in spite of T. cruzi infection through Notch 1 activation by increasing blood flow and cardiomyocytes proliferation. Acknowledgements: FONDECYT Project No.1130189 (JDM). Ethical Committee: Animals handling and experimental procedures were authorized by Bioethics Committee of the Faculty of Medicine of the University of Chile {protocol #FMUCH 0528}. **References**: [1]Rassi, A.J.R. Lancet Vol 375, 1388 (2010) [2]Ribeiro, A.L. Cardiology Vol 9, 576 (2012) [3]Campos-Estrada, C. PLoS Negl Trop Dis Vol 9, 1 (2015) [4]Zacharek, A. Stroke Vol 40, 254 (2009) [5]Li, Y. Trends Cardiovas Med 20, 228 (2010)

06.039 Sodium nitrate decreases xanthine oxidoreductase nitrite reductase activity and the antihypertensive effect of sodium nitrite. Angelis CD[1], Pinheiro LC[2], Tanus-Santos JE[2]
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Introduction: Antihypertensive effect was shown for sodium nitrite(1), and this effect results of tissue nitrite reduction to nitric oxide (NO) within smooth muscle cells (SMC)(2). The enzyme xanthine oxidoreductase (XOR) catalizes nitrite reduction to NO in vascular tissue(3). This enzyme also reduces nitrate to nitrite(4), and to NO, however, at a much lower rate than it reduces nitrite to NO(5). Thereore, it is possible that the presence of nitrate decreases the formation of NO from nitrite by XOR, and decreases the antihypertensive effect of nitrite. **Methods:** The effect of nitrate on XOR activity was measured by fluorescence with a Amplex® Red Xanthine/Xanthine Oxidase Assay Kit following the manufacturer´s instructions. In brief, a working solution containing Amplex red reagent, horseradish peroxidase (HRP) and xanthine was added to increased concentration of XOR. Another series was made in the presence of nitrate 300µM. Nitrite reductase activity was measured by injecting XOR 100 mU/ml into a purge vessel containing nitrite 1 mM, NADPH 1 mM and 1 mM of NADH in 1 mM phosphate buffer pH 7.4, purging with nitrogen in line with a gas-phase chemiluminescence NO analyser (Sievers Model 280 NO analyzer; Boulder, CO, USA). The effect of nitrate on nitrite reductase activity was evaluated by adding it at 30 mM before adding XOR. Rats received Nω-nitro-L- arginine methyl ester (L-NAME) 100 mg/Kg orally to increase MAP and forty min later received nitrate (0.4 microMol/kg) or vehicle. Then increasing doses of sodium nitrite were infused into the femoral vein. Invasive MAP was assessed via a cannula in the femoral artery and recorded with a data acquisition system (MP150CE; Biopac Systems Inc., CA, USA). **Results:** XOR activity measured by Amplex red showed that the incubation of the XOR (0, 0.039, 0.078, 0.156, 0.3125, 0.625 mU/ml) with the working solution resulted in mean fluorescence (n=2/concentration) of 622.8, 825.4, 1034.7, 1588.6, 2310.7 and 3748.4 respectively. When nitrate 300 µM was added, the mean fluorescence values (n=2/concentratin) decreased to 567.7, 685.2, 929.1, 1214.3, 1806.9, 2906.7 respectively. The results of nitrite reductase activity assay by chemiluminescence showed that the rate of NO formation by XOR in the presence of nitrite 1 mM (mean ± SE: 256.4 ± 22.5) decreased when nitrate 30 mM was added (104.9 ± 8.4), (p<0.05, n=3). Nitrite dose-dependently decreased MAP by -1.9 ± 1.2, -4.9 ± 2.2, -13.2 ± 2.5, -25.3 ± 3.8, and -43.4 ± 4.9 mmHg in response to nitrite 1, 3, 10, 30 and 100 microMol/kg, respectively (n=5-6/dose). This effect was attenuated (p<0.05) by nitrate (0.8 ± 2.2, 0.8 ± 2.4, -5.7 ± 2.7, -17.9 ± 3.5, and -32.0 ± 3.2 mmHg, respectively). **Conclusion** Our results show that sodium nitrate decreases XOR nitrite reductase activity resulting in NO formation, and this effect may explain how nitrate attenuates the antihypertensive effects of nitrite. **References:** 1.Classen, H-G. J Am Coll Nutr. 9:500; 1990. 2. Alzawahra W.F. Am J Physiol Hear Circ Physiol. 295:H499; 2008 3. Li H. J. Biol. Chem. 283: 17855; 2008 4. Jansson E. A. Nat. Chem. Biol. 4:411 5. Millar T. M. FEBS Let.. 427:225; 1998 **Financial Support**: CAPES Approval ethical committee: 179/2014

06.040 The impact of protein (de)nitrosylation in septic shock Benedet PO[1], Menegatti ACO[2], Horewicz VV[1], Gonçalves MC[1], Terenzi H[2], Assreuy J[1] [1]UFSC – Farmacologia, [2]UFSC – Bioquímica

Introduction Nitric oxide (NO) overproduction during sepsis has been reported as one of the main contributors to the sepsis cardiovascular dysfunction. One of the mechanisms whereby NO exerts some of its effects is the reaction with thiol groups of cysteine residues (sulphydryls) in a process called S-nitrosylation, producing S-nitrosothiols. It has been reported that the increased levels of S-nitrosylated proteins contribute to pathogenesis of septic shock. The aim of the present study is to show that the denitrosylation of proteins could contribute for the improvement in hemodynamic parameters and to the higher survival of septic animals. To better understand the role of protein S-nitrosylation/denitrosylation in sepsis, we used DTNB [5,5'-dithio-bis-(2-nitrobenzoic acid)], an oxidizing agent of sulfhydryl groups. **Methods** Wistar female rats were anesthetized and submitted to cecal ligation and puncture (CLP) procedure. Twelve hours after surgery, animals received DTNB or vehicle. Twenty-four hours after CLP, biochemical, hematological and hemodynamic parameters were evaluated as well as the reactivity of aorta rings towards phenylephrine. In addition, we studied the S-nitrosylated proteins in the aorta and its co-localization to calcium-dependent potassium channels. Finally, the survival of septic animals was also evaluated. All procedures have been approved by our institutional Animal Ethics Committee (protocol number PP 00790/CEUA/UFSC). **Results** Septic rats presented increases in plasma concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), indicating liver dysfunction. Sepsis induced cardiac injury as demonstrated by increased plasma levels of creatine kinase-MB (CK-MB). Plasma levels lactate and blood glucose also were increased in septic animals besides inducing a pronounced hypotension, hyporesponsiveness to phenylephrine and increased levels of S-nitrosylated proteins in the aorta. Our results showed that calcium-dependent potassium channels were more S- nitrosylated during sepsis. DTNB treatment performed 12 h after sepsis induction (i) reduced plasma AST, ALT and decreased CM-MB levels; (ii) decreased tissue hypoxia, as indicated by lower lactate and glucose levels; (iii) attenuated leukocytopenia, leucopenia and thrombocytopenia; (iv) significantly reduced the hematocrit and hemoglobin, indicating a decreased vascular leakage; (v) attenuated the hypotension and normalized the reactivity to phenylephrine; (vi) caused a substantial reduction of nitrosylated proteins in aorta, notably calcium-dependent potassium channels and (vii) reduced the mortality of septic rats by 40%. **Conclusions** Our results show that S-nitrosylation of protein contributes to sepsis-induced vascular dysfunction and organ injury. The key finding however is that DTNB-induced protein denitrosylation restored hemodynamic functions, decreased organ damage and increased survival of septic rats even if administered after sepsis onset. **Financial support**: CNPq, CAPES, FAPESC and FINEP.

06.041 NOS-1 long-lasting inhibition caused by a nanoemulsion of 7-nitroindazole Barp CG[1], Mendes C[2], Lemos-Senna E[2], Assreuy A[1] [1]UFSC – Farmacologia, [2]UFSC – Farmácia

Introduction: Evidence suggests that nitric oxide (NO) produced by the neuronal isoform of nitric oxide synthase (NOS-1) is an important factor several aspects of the physiology and of the pathology, including a prominent role in the septic cardiovascular dysfunction. In this latter situation, the likely mechanism is that NOS-1-derived NO works as a signal to induce smooth muscle cells to express the inducible NOS-2 isoform. For instance, inhibition of NOS-1 using the selective inhibitor 7-nitroindazole (7-NI) was found to improve mortality and vascular reactivity during sepsis. However, 7-NI has a very short half-life in plasma (~2 h), thus limiting its use as a tool to unravel NOS-1 involvement. Therefore, we developed and characterized a 7-NI oil-in-water nanoemulsion formulation in order to increase its biological effects and maintain prolonged inhibitory levels. **Methods:** 7-NI nanoemulsion (NE 7-NI) was formulated using spontaneous emulsification method. We evaluated the release profile of 7-NI in nanoemulsion NE comparatively with free compound in vitro at 37^{o}, 75 rpm. We also used cells from a rat aorta smooth muscle cell line (A7r5) for incubate for 4 h with 7-NI nanoemulsion or free 7-NI 24, 8 and 4 hours before cell stimulation. Cells were then stimulated with lipopolysaccharide (10 µg/ml) and interferon-gamma (200 U/ml) that activates NOS-1. NO production by this isoform was evaluated by relative fluorescence of DAF FM-DA probe. **Results:** 7-NI nanoemulsion showed an encapsulation efficiency of 71.1 ± 0.6% and its average diameter was of 236.6 ± 21.3 nm. NE 7-NI showed a slower release profile compared with 7-NI. The rapid (4 h) exposure of cells to 7-NI nanoemulsion, but not to the free compound, reduced NO production by NOS-1 when incubated 8 and 4 h before cell activation. **Conclusion:** Our results show that 7-NI encapsulated in nanoemulsion has a long-lasting effect, if compared to the free compound. This new preparation can be useful when prolonged inhibition of NOS-1 and NO production may be of interest, such as during cardiovascular dysfunction of sepsis. **Financial support**: CNPq, CAPES, FINEP and FAPESC.

06.042 Intrauterine and lactation exposure to fluoxetine affects endothelial response in aorta of rats subjected to acute restrain stress. Marques BVD[1], Novi DRBS[1], Zanluqui NG[2], Higashi CM[1], Picinin R[1], Pinge-Filho P[2], Gomes GGP[1], Ceravolo GS[1] [1]UEL – Ciências Fisiológicas, [2]UEL – Ciências Patólogicas

Introduction: Fluoxetine (FLX) is commonly used in the treatment of gestational and postpartum depression. However, this drug crosses the placental barrier and is secreted in breast milk. Studies in rats have been shown that intrauterine and postnatal exposure to FLX reduced the activation of the amygdala neurons in acute restraint stress, decreasing limbic system response to the stressor stimulus. Thus, the aim of the present study was to evaluate whether exposure to FLX during pregnancy and lactation alter vascular adaptations promoted by acute restraint stress. **Methodology**: Female Wistar rats were treated by gavage with: FLX (5mg/kg/day) or water (CTL) during pregnancy and lactation. The experiments were carried out in male offspring at 75 days old, treated or not with reserpine (4mg/kg, intraperitoneal, 28 hours before the experiment). FLX and CTL rats were submitted to a single stress session of immobilization, during 1 hour, in a 5×27 cm metal tube (FLX-ST and CTL-ST). The thoracic aorta was removed and cut into rings (5mm) with (E+) and without (E-) endothelium. Cumulative concentration-effect curves were constructed to the vasoconstrictor phenylephrine (PHE: 1nM-30 µM) in the absence or presence of L-NAME (1µM) and also to vasodilators acetylcholine (Ach: 1nM-30 µM) and sodium nitroprusside (SNP: 0.1nm-3 µM). Nitric oxide (NO) was measured in aorta by Griess method. **Results** expressed as mean ± SEM of the maximal response (MaxR) to PHE and % relaxation for vasodilators, (n) represents the number of rats/group. Statistical analysis: one-way ANOVA followed by Tukey's test, differences with $p<0.05$. **Results**: The MaxR for PHE in E+ rings was similar between CTL and FLX rats. The acute stress reduced MaxR to PHE in E+ rings of CTL-ST rats compared to the CTL not stressed [CTL: 2.67 ± 0.11 (20) vs CTL-ST: 1.93 ± 0.11 (16)], and L-NAME incubation equaled those MaxR. In FLX rats stress did not change the MaxR to PHE in E+ aortic rings. Reserpine treatment restored the vasoconstriction in E+ aortic ring from CTL-ST [2.59 ± 0.14 (7)] and CTL-ST [2.45 ± 0.20 (9)], but did not interfere with MaxR to PHE in aortic rings from FLX [FLX: 2.33 ± 0.30 (5) vs FLX-ST: 2.19 ± 0.20 (8)]. The contraction induced by PHE in E- rings, as well as relaxation promoted by ACh in E+ rings were similar between the groups CTL, CTL-ST, FLX and FLX-ST. The % of relaxation to SNP was reduced in aortic rings from CTL-ST [94.00 ± 1.84 (7)] when compared to CTL [99.03 ± 0.49 (9)], and similar between FLX and FLX-ST. The NO concentration was higher in aorta from CTL-ST [CTL-ST: 4.49 ± 0.47 (6)] compared to CTL rats [2.28 ± 0.29 (5)], and was no similar between FLX and FLX-ST groups. **Conclusion**: In CTL rats, acute stress-induced sympathetic activity seems to increase the NO production in aorta, which modulates the vasoconstrictor response to PHE. Moreover, the endothelial and NO modulation were lost in FLX-maternally exposed rats, probably because these animals have less sympathetic activation during acute restrain stress. **Financial support**: CNPq Ethical Committee: CEUA 16166-2012.12

06.043 Evaluation of metabolic parameters in rat exposed to fluoxetine during early development. Moura KF, Marques BVD, Higashi CM, Costa GB, Barrionuevo DR, Ceravolo GS
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Introduction: Depression is a multifactorial disease that affects different age groups (Gaynes et al., 2005). Fluoxetine (FLX) is an antidepressant worldwide prescribed throughout life stages, including pregnancy and breastfeeding. FLX is classified as category C by the FDA because it crosses placental barrier and is secreted in breast milk (Heikkinen et al., 2002). It is has been reported that early developmental fluoxetine exposure affects central nervous system areas related to neuroendrocrine regulation (Pawluski et al., 2012). Therefore, the objective of the present study was evaluate whether intrauterine and lactational exposure to fluoxetine causes metabolic changes related with diabetes and obesity in adult male and female offspring. **Methods:** Male Wistar rats 75 days of age, whose mother was treated with fluoxetine (FLX) (5mg/kg) or water (C) during pregnancy and lactation were used. Lee's obesity index was calculated as follows: body weight1/3(g)/nasal-anal length (cm)×100. Visceral adipose tissue (periepididymal and retroperitoneal) were excised and weighted and the values expressed as fat pad weight/100g of body. After 4 hour of food deprivation the basal glycemia and insulin tolerance test (kITT) were evaluated. For statistical analysis, T-student test was used and differences considered with $P<0.05$. Values expressed as mean ± S.E.M, (n) is the number of rats/group. **Results:** The body weight (C: 288.00 ± 18.61 (7) vs FLX: 310.10 ± 14.59 (8)), Lee's index (C: 29.85 ± 0.31 (7) vs FLX : 30.81 ± 0.35 (8)) and the periepididymal fat (C: 0.95 ± 0.04 (6) vs FLX : 0.91 ± 0.04 (7)) and retroperitoneal fat (C: 0.79 ± 0.08 (6) FLX vs 0.80 ± 0.05 (7)) were similar between FLX and C rats. Also, the baseline glycemia (C: 123.10 ± 4.98 (7) vs FLX: 124.30 ± 6.25 (6)) and kITT (C: 2.57 ± 0.11 (7) vs FLX: 2.42 ± 0.27 (6)) did not differ between the groups. **Conclusion**: The results reveal that maternal exposure to FLX (5mg/Kg) during pregnancy and lactation did not induced obesity or insulin resistance in adult male offspring.

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06.044 Vasorelaxant Effect of Asenapine Involves Endothelium-Dependent and -Independent Mechanisms Bastos AM[1], Campos HM[1], Oliveira TS[1], Brito RB[1], Costa EA[1], Filgueira FP[2], Ghedini PC[1] [1]UFG – Farmacologia, [2]UFG – Ciências da Saúde

Introduction: Second-generation antipsychotics (SGA) are associated with increased risk of weight gain, cardiovascular diseases and diabetes mellitus. Asenapine (ASE) is a SGA that presents a lower relation with metabolic alterations when compared to others SGAs. In addition, it was reported the ASE protects endothelial function by modulation of NO release and decreases the blood arterial pressure in rats. Considering the vascular effects promoted by ASE, this study aims to investigate the action mechanisms involved in the vasorelaxant effect of this drug. **Methods:** The vasorelaxant effect of ASE was evaluated in preparations of thoracic aorta rings of male Wistar rats 10-12 weeks of age, using organ chambers. Concentration-response curves to ASE (1 nM – 30 µM) were constructed in phenylephrine pre-contracted aortic rings with either intact or denuded endothelium. Relaxation responses to ASE in intact endothelium rings were performed in the absence or presence of the L-NAME (100µM), a nitric oxide synthase inhibitor, or the ODQ (10µM), a guanylate cyclase inhibitor. Data are presented as mean ± SEM of 5-7 experiments and analysed by Student's t-test or one-way ANOVA statistical tests when appropriate. P values less than 0.05 were considered significant. **Results:** ASE induced concentration-dependent relaxation in endothelium-intact aortic rings (E_{max} = 99.06 ± 0.55%; pEC_{50} 7.20 ± 0.07) and endothelium-denuded vessels (E_{max} = 98.34 ± 0.64%; pEC_{50} 6.19 ± 0.04). The incubation of endothelium-intact aorta rings with L-NAME or ODQ, increase significantly ASE pEC_{50} value (6.33 ± 0.04 and 6.15 ± 0.03 respectively). **Conclusion:** These preliminary results suggest ASE induces endothelium-dependent and -independent relaxation in the rat thoracic aorta and the participation of the NO/cGMP pathway in this effect. **Financial Support:** CAPES, FAPEG, CNPq Research approval by the Animal Research Ethical Committee of UFG (process number 20/2013)

06.045 Modulation of cardiac ATPases involved with calcium homeostasis in rats fed with cholesterol rich diet obtained by eggs and butter supplementation. Silva RM[1], Marques EB[1], Scaramello CBV[1] [1]UFF – Fisiologia e Farmacologia

Introduction: Previous data of our group (Silva *et al*., SBFTE2012, panel 06.035) suggest that rats submitted to cholesterol rich diet obtained by supplementation of standard chow with eggs and butter (CRDEB) presented an inferior energy expenditure, a superior body mass index, a higher abdominal/thoracic circumferences ratio and a dyslipidemic profile 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 CRDEB 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 in animals fed with CRDEB (Silva *et al*., SBFTE2012, panel 06.035) being the degree of left ventricle dilation related to the severity of left ventricle filling impairment (Mottram and Marwick, Heart, 91(5):681, 2005). The aim of this work was to investigate if CRDEB modulates cardiac ATPases involved with calcium homeostasis. **Methods:** After weaning male Wistar rats were randomly divided into two groups: G1- fed with commercial chow (Nuvilab®); G2-fed with CRDEB. After 30 or 60 days of diet, rats were euthanized and cardiac homogenates were obtained (Bambrick *et al*., J Pharmacol Meth, 20:313, 1988) to the evaluation of different proteins expression - calcium ATPases from plasma membrane (PMCA) and sarcoendoplasmic reticulum (SERCA), Na^+/K^+ ATPase (NKA) and dephosphorylated phospholambam (PLB) (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 $P<0.05$(*). **Results**: It was observed a significant decrease of cardiac NKA expression after 30 days of CRDEB (G1=3.127 ± 0.277 x G2=1.055 ± 0.344**). SERCA expression was decreased just after 60 days of CRDEB (G1=1.432 ± 0.189 x G2=0.643 ± 0.147*). No differences were observed about PLB and PMCA expression. **Conclusions**: PLB is an important regulatory protein of cardiac function, increasing SERCA activity just when phosphorylated (MacLennan and Kranias, Nat Rev Mol Cell Biol, 4(7):566, 2003). Even if our data suggest that SERCA activity is not reduced in CRDEB fed animals, the decrease of SERCA and NKA expression are factors that contribute to abnormal intracellular calcium homeostasis in failing hearts (Sulaiman *et al*., Am J Physiol Heart Circ Physiol, 298(3):H833, 2010; Swift *et al*., Cardiovasc Res, 78:71, 2008). **Financial Support**: FAPERJ, CAPES, CNPq, PROPPI/UFF. The use of animals was according to local Ethics Committee (CEUA/UFF00099/2011).

06.046 Evaluation of cardiotoxic activity of free ATM and into a nanocarrier. Souza ACM[1], Mosqueira VCF[1], Richard S, Vidal-Diniz AT[1], Silveira APA[2], Rodrigues LA[2], Castro QJT[1], Guimarães HN[3], Guimarães AG[1 1]UFOP – Ciências Farmacêuticas, [2]UFOP, [3]UFMG

Introduction: Artemether (ATM) is a semisynthetic artemisinin derivate. It is one drug of choice to treat severe malaria caused by *Plasmodium falciparum* (Brossi et al, 1988; Hortelano et al, 2013). The artemisinin derivatives are used to uncomplicated malaria resistant or multiresistant to classical antimalarial drugs, besides being used also to treat cancer (Ooko et al, .2015). However, artemisinin derivatives like ATM, induce serious adverse effects such as neurotoxicity and cardiotoxicity (Brewer et al, 1994; CLASSEN et al, 1999). In order to circumvent the parasite resistance to antimalarial drugs and reduce the adverse effects it was proposed a formulation of ATM in nanocarrier. The nanocarriers have shown an important role as they allow: (i) delivery of high amounts to action sites in order to prevent the development of resistant parasites strains (White 1997; MOVELLAN et al, 2014) (ii) improve the efficiency, (iv) innovation of antimalarial therapy since they allow evaluation of drugs not tested before due to, for example, the high toxicity and non-specificity, and (v) increase immune response in vaccine formulations (MOVELLAN et al, 2014). Thus, this study aims to evaluate the cardiotoxic activity of ATM in a nanocarrier formulation administered orally. **Methods:** All procedures were approved by CEUA/UFOP (2014/14). Black C57/bl6 mice were used aged between 8 to 10 weeks. The animals were divided in four different groups (n = 6, each) that were treated orally during 4 days, two administration daily (12/12 hours) with: a) vehicle, b) empty nanocarrier, c) free ATM (40, 80 or 120 mg/kg) and d) nanocarrier containing ATM (40, 80 or 120 mg/kg). The mice were anesthetized (ketamine 100 mg/kg and xylazine 14 mg/kg) and the lead II ECG signal was obtained before treatment (baseline), 2; 6 and 24 hours after the last dose. The ECG parameters, PR, QRS, QT and QTc intervals, were obtained off line. **Results:** It was observed significant prolongation of QT (12, 3; 23, 8 and 18, 6 %, respectively to doses and related to baseline) and QTc (14,6, 16,9 and 12,03 %, respectively to doses and related to baseline) intervals in mice treated with free ATM compared to the group receiving vehicle in time 6 hours. The maximum prolongation of QT and QTc intervals occurred 6 hours after the last oral administration at doses of 80 and 120 mg/kg. However, the maximum prolongation of these intervals observed when mice were treated with nanocarrier containing ATM were 4,9 %, 7.4 % and 1.6 % for QT and 1,8 %; 5.1 % and -1.6 % for QTc, for 40; 80 and 120 mg/kg, respectively. **Conclusion:** The ATM in nanocarrier was able to reduce significantly, *in vivo*, the cardiotoxicity caused by this drug, since it was observed reduction of QT and QTc intervals prolongation, which are risk factors for sudden death. **Funding agencies and acknowledgments:** FAPEMIG (Rede Nanobiomg, PPM 03/2013, APQ 02346-11), CAPES, CAPES/COFECUB (1737/2013) and UFOP. BREWER TG, *et al*. *Am J Trop Med Hyg*, 51:251–59, 1994. BROSSI A, *et al. J Med Chem*, 645-650, 1988. CLASSEN W, *et al. Exp Toxicol Pathol,* 51: 507–16, 1999. HORTELANO M, *et al*. *Anal Ped (Barcelona)*, 782(124):e.1e.8, 2013. MOVELLAN J, *et al*. *Biomaterials*, 35:7940-7950, 2014. OOKO E, *et al*. *Phytomedicine,* 22(11):1045-54, 2015. WHITE NJ. *Antimicrob Agents Chemother*, 41:1413-22, 1997.

06.047 Acute and repeated restraint stress cause similar cardiovascular response in rats.
Marques LAC[1], Volpini VL[1], Veríssimo LF[1], Santos DC[1], Matsubara NK[1], Estrada VB[1], Ceravolo GS[1], Gomes MV[2], Pelosi GG[1 1]UEL, [2]UNOPAR

Introduction: The effects of stress are related to the most disorders seen in the modern life including cardiovascular diseases, altering the health condition and well-being as whole (CASTRO, 2004). Stress exposure increases the levels of circulating catecholamine, heart rate and mean arterial pressure (WITTSTEIN, 2012). Habituation involves a series of body's changes, such cardiovascular changes, in order to reduce the impact of stress in the body. An orchestrated central circuit processes inputs and outputs pathways in order to modulate the physiological response to a stressful event. (HERMAN, 2013). The objective of this work was to compare the effects of acute and repeated stress on cardiovascular parameters. **Methods:** Wistar male rats (70-90 days) were divided in two groups: acute restraint stress group (AS), animals submitted to restraint stress protocol for only one session during 1 hour, and repeated restraint stress group (RS), animals submitted to stress protocol for 1 hour/day for 5 consecutive days. The stress protocol was based in (KASHIMOTO et al., 2016). Cardiovascular parameters (blood pressure and heart rate) were recorded by catheterized femoral artery during the last session of stress. **Results:** There was no statistical difference in the temporal analysis of variation of mean arterial pressure (Time: DMAP: $p < 0,0001$, $F_{(15, 255)} = 9.972$; Treatment: DMAP: $p = 0.2966$, $F_{(1, 17)} = 1.160$; Interaction: DMAP: $p = 0.7672$, $F_{(15, 255)} = 0.7164$; n = AS: 9, RS: 10) and heart rate (Time: DHR: $p < 0.0001$, $F_{(15, 255)} = 22.52$; Treatment: DHR: $p = 0.5768$, $F_{(1, 17)} = 0,3237$; Interaction: DHR: $p = 0.0306$, $F_{(15, 255)} = 1,834$; n = AS: 9, RS: 10) caused by AS or RS. Groups showed no difference in mean arterial pressure before and after the restraint stress (before stress: AS: 100,9 ± 2,96 mmHg, n = 20; RS: 94,8 ± 2,93 mmHg, n = 10; after stress: acute stress: 105,4 ± 2,58 mmHg, n = 18; repeated stress: 97,5 ± 2,15 mmHg, n = 12; $p = 0,0770$; $F_{(3, 56)} = 2,405$). However, an increase of heart rate was observed after stress, but the values did not differ between the groups (before stress: AS: 374,3 ± 9,32 bpm, n = 20; RS: 357,1 ± 9,21 bpm, n = 10; after stress: AS: 433,1 ± 12,43 bpm, n = 18; RS: 408,5 ± 11,98 bpm, n = 10; $p < 0,0001$; $F_{(3, 54)} = 9,069$). **Conclusion:** This work suggest no habituation of cardiovascular response to repeated restraint stress with a protocol of 5 days, indicating that acute and a short time of repeated stress had a similar cardiovascular behavior during the restraint stress session. **References:** CASTRO, A.P., Revista. L. E., 12, p859, 2004 HERMAM, J., Frontiers B.N., 7, p1, 2013 KASHIOMOTO, R.K., Behavior B.R, 296,p286, 2016; WITTSTEIN, I. L., Cellular M. B.,32,p847, 2012; **Financial Support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq proc. 478566/2013-1). The Ethics Committee of Londrina State University approved all animal experimentation (CEUA Number 14441.2013.18/ UEL)

06.048 Endothelium-dependent vasorelaxant effect of the kuromanin compound in rat thoracic aorta. Campos HM[1], Bastos AM[1], Oliveira TS[1], Costa EA[1], Gil ES[2], Filgueira FP[3], Ghedini PC[1] [1]UFG – Farmacologia, [2]UFG – Farmácia, [3]UFG – Ciências da Saúde

Introduction: Kuromanin is a phenolic compound present in fermented beverages of jabuticaba (*Myrciaria jaboticaba*) fruits. Previous study showed the vasorelaxant effect of jabuticaba beverages and suggested the participation of kuromanin compound in this effect (de Sá, J Funct Foods 8:169, 2014). Considering there are no studies showing the vascular effect of kuromanin, this study investigated the effect produced by kuromanin in isolated rat aortic rings and the possible mechanism involved in this event. **Methods:** Concentration-response curves to kuromanin (10^{-9} – 10^{-3} M) were evaluated in rat aortic rings with *endothelium-intact* or *endothelium-denuded* pre-contracted with phenylephrine (Phe; 10^{-6}M), using an isolated organ-chamber technique. To evaluate the involvement of nitric oxide (NO) in the relaxation produced by kuromanin, endothelium-intact aorta rings were incubated with L-NAME (100 µM). After the incubation period, aorta rings were stimulated with Phe and cumulative concentration-effect curves for kuromanin were constructed. The results are presented as the mean ± SEM of 6–8 experiments. Statistical significance was determined using Student's t-test or one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test when appropriate. A P value of <0.05 was considered statistically significant. **Results:** Kuromanin induced concentration-dependent relaxation in endothelium-intact aortic rings (E_{max}= 44.42 ± 3.79 %), whereas in endothelium-denuded vessels the effect was not significantly (E_{max}= 0.49 ± 0.10 %). The incubation of endothelium intact aorta rings with L-NAME significantly reduced the kuromanin-induced relaxation (E_{max}= 1.75 ± 0.69 %). **Conclusion:** The results showed the vasorelaxant effect of kuromanin is endothelium-dependent and involves the participation of the NO mediator. **Financial Support:** FAPEG, CAPES and CNPq Research approved by the Animal Research Ethical Committee from Federal University of Goiás (process number 20/2013)

06.049 Spontaneous and isoprenaline-evoked response of isolated heart preparations from rats submitted to early weaning. Alvim-Silva T[1], Barros RBM[1], Marques EB[1], Oliveira DF[2], Nascimento JHM[2], Scaramello CBV[1] [1]UFF – Fisiologia e Farmacologia, [2]UFRJ – Biofísica

Introduction: Some mothers are not able to nurse their babies and maternal employment can be a barrier to breastfeeding (Witters-Green, Families, Systems, & Health, 21(4): 415, 2003). Metabolic imprinting describes the relation between nutritional experiences of early life and later diseases (Waterland and Garza, Am J Clin Nutr, 69:179, 1999). Early weaning is associated to body mass index and fasting glycemia raise along with impairment of lipid profile, increasing cardiometabolic risk, diastolic dysfunction and lower tolerance to exercise (Barros, RBM. Doctoral thesis. Pos Graduation Program in Cardiovascular Sciences, 2016). The aim of the present work was to study spontaneous and isoprenaline-evoked cardiac activity of isolated heart preparations from rats at 150 and 365 days of age submitted to early weaning. **Methods:** Male Wistar rats were randomly assigned into Control (C) -breastfed during 21 days- and Early Weaning (EW) -breastfed during just 18 days- groups. After weaning, pups received water and food *ad libitum*. Rats were heparinized before euthanasia; the hearts (n=3-6 each group) were excised and submitted to appropriated experimental conditions to monitor cardiac activity (Marques *et al*., Int J Cardiol, 195:48, 2015). LV developed pressure (LVDP; mmHg) and cardiac performance indexes ($mmHg.s^{-1}$) such as LV contractility rate (+dp/dt) and LV relaxation rate (−dp/dt) were determined before and after addition of isoprenaline (0.3-300nM). Data were presented as mean and standard error of the mean, analyzed by Two-Way ANOVA test and Student *t* test and considered statistically different if $p<0.05$(*). **Results**: Spontaneous LVDP of heart preparations from C diminished with aging (101.33 ± 6.94 x 74.64 ± 5.16*) while hearts from EW at 150 days of age presented higher spontaneous +dp/dt (2898.12 ± 132.17 x 3444,95 ± 188,02*). Isoprenaline increased LVDP and cardiac performance indexes in a concentration-dependent manner. Preparations from EW at 150 days of age showed a greater increase of LVDP (126.40 ± 11.85% x 173.10 ± 13.80*%), but a lower raise of –dp/dt (193.09 ± 16.23% x 142.47 ± 10.88*%) with 300nM isoprenaline comparing to C. On the other hand, at 365 days of age the same concentration of the β-agonist promoted a minor effect over LVDP (317.68% ± 53.35% x 163,49% ± 11,55%*), (+)dp/dt (399.22 ± 65.96% x 196.89 ± 30.35%*) and (-)dp/dt (416.38 ± 70.96% x 159.24 ± 11.97%*) of heart preparations from EW. **Conclusions**: Systolic function at rest appears to be unaffected by aging but left ventricular diastolic function seems to deteriorate (Spurgeon *et al*., Am J Physiol, 277:H2083, 1999). Literature reports compensatory mechanisms in obesity-induced cardiac dysfunction aiming intracellular Ca^{2+} homeostasis restoration in cardiac myocytes (Relling *et al*., J Hypertens, 24:549, 2006.). However, increased activity/expression of SERCA may not be effective at all stages of cardiac dysfunction (Dorn and Molkentin. Circulation, 109(2):150, 2004). In addition, cardiac β-adrenergic dysfunction is generally related to heart failure (Lymperopoulos et al., Circ Res 113 (6); 739, 2013). **Financial Support**: FAPERJ, CNPq, CAPES, PROPPI/UFF. The use of animals was approved by Ethics Committee (CEUA/ UFF389-13).

06.050 Are involved H3 and H4 receptors in the regulation atrial in Wistar-EPM1 rats?

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Introduction: Histamine is a biogenic amine which is able to modulate the sympathetic neurotransmission in the heart. However, the histamine effects in the inotropism and chronotropism atrial are contradictory and also need of additional investigations. **Aims:** in the previous presentation, we show the participation of H1 and H2 receptors in atrial contraction and now intend to enter further work, also showing the involvement of receptors H3 and H4. **Methods:** We used 4-month-old male Wistar rats weighing approximately 300 g. The left (LA) and right atria (RA) were isolated and mounted in isolated organ chambers bathed with Krebs-Henseleit solution (95% O_2, 5% CO_2 - 36.5°C). The LA was electrically stimulated (2 Hz; 5 ms, 20-40 V) and after 40 minutes of stabilization, cumulative concentration-response curves for histamine (10^{-5} to 10^{-2} M) in the absence or presence of H3-antagonist (Tioperamide 10^{-7}M) or H4-antagonist (JNJ 7777120 10^{-4} M) receptors were performed, after 20 minutes of antagonist application. All experimental procedures were approved by Ethical Committee of Universidade Federal de São Paulo - Brazil (protocol: 9098250214). **Results:** The histamine produce a biphasic response characterized by an initial negative inotropic effect (0.5-1 min of duration) followed by a positive inotropic effect which reaches the plateau after 4-5 minutes, in the LA and RA. In the maximal concentration of histamine, the negative inotropic effect was intensified by about 42% and 70% in LA and RA, respectively, compared to basal. Further, the positive inotropic effect induced by histamine was increased about 69% in LA and 63% in RA in relation to basal. We also found a reduced chronotropism of RA by about 30 % in the maximal concentration of histamine. When the H3 antagonist was used in the control group, there was a significant increase of 63.7% of the negative inotropic effect in the left atrium and a blockade of the positive inotropic effect of 96.7% and 56.7% in the left and right atrium, respectively, in the last dose of histamine, and a blocked 45.4% of the positive chronotropic effect. In the presence of H4-antagonist, in order to accomplish the analysis of receptors, the experiment showed blocking up to 37,8% of the negative inotropic effect in LA and 61% in RA, showing that among all studied receptors, it is present in the atrial muscles and is responsible for decrease contractility. **Conclusions:** Our data suggest that the H3 and H4 receptors are present in the atrial muscle and are responsible for modulation of inotropic and chronotropic effects, given that the H4 receptor is solely responsible for the regulation of negative inotropic effect . **Financial Support:** Fapesp and CAPES.

06.051 NLRP3 inhibition protects against aldosterone-induced endothelial dysfunction

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Introduction: Aldosterone (Aldo) is a mineralocorticoid hormone produced by the adrenal gland, which can trigger endothelial dysfunction. NLRP3 is a NOD-like receptor and integrates the family of receptors of the innate immunity. The role of NLRP3 in the immune system is well defined, but its function in the vasculature keeps to be elucidated. We tested the hypothesis that aldosterone instigates the endothelial dysfunction dependent of NLRP3. **Methods**: Rings from thoracic aorta were separated from male C57BL6/J mice with 10 weeks of age and incubated with aldosterone (Aldo) (10-7M) for 1 hour, in presence or absence of MCC950 [NLRP3 inhibitor, (10-6M)], and then concentration effect-curves (CEC) to acetylcholine (ACh) were obtained. In some experiments L-NAME [10-4M, nitric oxide synthase inhibitor (NOS)] and indomethacin [10-5M, cyclooxygenase (COX) inhibitor] were pre-incubated. **Results**: Aldo incubation impaired the ACh-induced vascular relaxation [Maximal effect (Emax): Vehicle (Veh): 86,6 ± 3,9 vs Aldo: 67,9 ± 4,1*, (% of relaxation), *P<0.05], and the MCC950 pre-incubation prevented the Aldo-induced impaired vascular relaxation [Emax: 88,2 ± 6,5 (% of relaxation) Indomethacin pre-incubation restored, similar to the vehicle, the impaired relaxation triggered by the Aldo incubation, but not for the pre-incubation of MCC950. On the other hand, L-NAME completely abolished the relaxation for incubations at the same level. **Conclusion**: Overall, our findings suggest that NLRP3 modulates the aldo-induced endothelial dysfunction involving the NO-dependent mechanisms. However, further studies are necessary to understand the role of NLRP3 in the vasculature. **Financial support**: FAPESP 2015-24796-4 Research approval by the Animal Research Ethical Committee number: protocol n° 012/2013-1

06.052 Chronic ethanol consumption causes renal oxidative stress and increases susceptibility to sepsis Ricci ST, Ceron CS, Vale GT, Tirapelli CR [1]EERP-USP – Farmacologia

Chronic ethanol consumption increases the production of reactive oxygen species (ROS), causing renal damage. Sepsis is an intense inflammatory and systemic response that occurs in response to infection, which can cause kidney damage leading to acute renal failure by several mechanisms, including increased oxidative stress. The aim of this study was to investigate whether oxidative stress induced by chronic ethanol consumption may increase susceptibility to sepsis. Male C57/Bl6 mice were divided into 4 groups: control, ethanol [9 weeks, 20% (v/v)], control+sespsis and ethanol+sepsis. Seven days after mild sepsis induction, a survival curve was performed to evaluate the mortality of the animals. 24 hours after induction of sepsis animals were sacrificed and the renal cortex was used for determination of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), thiobarbituric acid reactive species (TBARS) and nitrate/nitrite (NOx) levels, enzymatic and non-enzymatic antioxidant activity, Western immunoblotting for Nox1 and Nox4 and qualitative analyses using the dyes Dihydroethidium and 2,7-Dichlorofluorescin diacetate. Statistical analysis: One-way ANOVA followed by Newman Keuls test ($p<0.05$) was used to analyze the results. Chronic ethanol consumption decreased the survival rate (%) of animals subjected to sepsis (CS:100%; ES:60%). Ethanol consumption increased O_2^- generation (RLU/mg protein) and H_2O_2 (nmol H_2O_2/mg protein) levels that was not observed in ethanol+sepsis group (O_2^-: C:160.5 ± 33.9, n=7; E:325.8 ± 54.5, n=7; CS:64.5 ± 6.4, n=9; ES:136.8 ± 10.4, n=6); (H_2O_2: C:1.66 ± 0.23, n=7; E:3.36 ± 0.3, n=6; CS:2.25 ± 0.2, n=7; ES:1.55 ± 0.1, n=5). Qualitative analyses using Dihydroethidium and 2,7-Dichlorofluorescin diacetate corroborated these results. Ethanol treatment causes an increase in TBARS (nmol/mg protein) in control animals and subjected to sepsis (C:4.8 ± 0.5, n=13; E:8.4 ± 1, n=11; CS:6.9 ± 0.8, n=11; ES:8.3 ± 1.1, n=11). Ethanol consumption increased catalase activity (U/min/mg protein) that was not observed in ethanol+sepsis group (C:231.5 ± 22.2, n=7; E:350.24 ± 44.1, n=8; CS:182.14 ± 22.3, n=10; ES:246.37 ± 20.9, n=9). Ethanol treatment increased renal GSH levels (µg/mg protein) in control animals and in those subjected to sepsis (C:10.3 ± 0.3, n=10; E:12.8 ± 0.9, n=8; CS:10.7 ± 0.9, n=10; ES:14.7 ± 0.7, n=10). There was no difference in superoxide dismutase activity (% inhibition) as well as protein expression of Nox1 and Nox4 in the renal cortex. In summary, chronic ethanol consumption increases renal ROS generation , which is associated with increased susceptibility to sepsis. **Financial support**: CNPq.

06.053 Pharmacological evaluation of agonist of estrogen receptor (GPR30) on skeletal muscle fatigue in male Zucker Diabetic Fat rats. Costa GC, Silva AMS, da Silva JS, Gabriel D, Sudo RT, Zapata-Sudo G UFRJ

Introduction: G1 is a selective agonist of GPR30, a receptor which was recently described to play actions in skeletal muscle such as increase of glucose uptake. Zucker diabetic rats (ZDF) were used to mimic the type 2 diabetes in which exercise intolerance and fatigue can be observed in addition to glucose intolerance due to insulin resistance. The present work investigated the pharmacological effects of G1 on cardiac and skeletal muscle function in ZDF. **Methods**: Male Zucker Lean (ZL) and ZDF rats (24-32 weeks old) were randomly divided in the following experimental groups: ZL + vehicle (peanut oil), ZL + G1, ZDF + vehicle and ZDF + G1. Animals were treated s.c. with vehicle (100 µg/kg/day) and G1 (100 µg/kg/day) for four weeks (n = 6 per group). Treadmill test was performed to access the exercise performance and the skeletal muscle function was evaluated through the isometric tension recording of EDL and soleus muscle. **Results**: Time to exhaustion (s) was reduced from 1237 ± 155 (ZL + vehicle) to 791.5 ± 164.6 (ZDF + vehicle) which was not altered by the treatment of G1. In vitro protocol of muscle fatigue showed a reduction of the force development (mN/cm^2) in EDL muscles from 209.7 ± 26.3 (ZL + vehicle) to 72.5 ± 21.8 (ZDF + vehicle) which was improved after treatment with G1 to 353.2 ± 5.5 (ZL + G1) and 84.6 ± 3.4 (ZDF + G1). In soleus muscles, there was a decrease from 203.2 ± 5.9 (ZL + vehicle) to 115.3 ± 28.7 (ZDF + vehicle) and an improvement after treatment to 207.9 ± 6.2 (ZL + G1) and 141.5 ± 3.5 (ZDF + G1). Oral glucose tolerance test was performed in all groups and G1 did not alter the intolerance in ZDF rats. Insulin level (µg/L) was increased from 0.23 ± 0.05 (ZL + vehicle) to 3.60 ± 0.09 in diabetic rats (ZDF + vehicle) but decreased to 2.10 ± 0.08 (ZDF + G1) after treatment with G1. Left ventricular diastolic pressure (mmHg) increased from 9.8 ± 0.6 (ZL + vehicle) to 15.4 ± 2.4 (ZDF + vehicle), confirming the ventricular dysfunction and was partially recovered after treatment, 11.2 ± 2.4 (ZDF + G1). **Conclusions**: G1, an agonist of GPR30, increased skeletal muscle contractility and improved the ventricular dysfunction in diabetic rats. **Financial support**s CNPq, CAPES, FAPERJ, INCT/INOFAR Research approval: DFCBICB 042/15

06.054 Aldosterone-induced NLRP3 inflammasome activation Ramalho FN, Ferreira N, Zanotto CZ, Alves-Filho JC, Tostes RC, Bruder-Nascimento T FMRP-USP – Farmacologia

Introduction: Aldosterone is a hormone with mineralocorticoid activity produced, mainly, by the adrenal glomerulosa and is closely associated with cardiovascular inflammation [1]. It is well established that the innate immunity modulates the genesis of cardiovascular disease. Among innate immunity receptors, NOD-like receptors (NLRs) are a family of receptors that can be activated by damage-associated molecular patterns (DAMPs), such as ROS and induce the production of a multiprotein complex: the NLRP3 inflammasome [2]. Here, we hypothesized that aldosterone activates NLRP3 inflammasome in macrophages. **Methods**: To access the activation of the inflammasome, bone-marrow derived macrophages (BMDM) were isolated from C57BL/6 (WT) and NLRP3 knockout (*NLRP3-/-*) mice and stimulated with Lipopolysaccharide (LPS, 1ug/ml, for 4 hours) and Nigericin (Nig, 20uM, for 45 minutes), as positive controls for the activation of inflammasome, and aldosterone (10^{-6} M, for 8 hours).**Results**: Aldosterone increased the caspase-1 activity, measured by western blotting and flow cytometry [vehicle: 100 ± 3.5 vs aldosterone: 137.6 ± 8.2* (% of the vehicle), *P<0.05], in BMDM from WT mice, and the NLRP3 deletion abrogated this event. Also, aldosterone increased the mature form of IL-1β in BMDM from WT mice, as well as increased IL-1β secretion [vehicle: 11.58 ± 2.7; aldosterone: 28.99 ± 4.4*, (% of the vehicle) *P<0.05], analysed by western blotting and ELISA, respectively; BMDM from *NLPR3-/-* mice did not present increase of mature form of IL-1β and its secretion. To evaluate whether aldosterone is able to perform the prime and second signal, we combined the follows stimulus: aldosterone (8h) + Nig (45min) and LPS (4h) + aldosterone (4h), both combinations increased the caspase-1 activity. Using the NF-kB luciferase stable RAW264.7 cell line, aldosterone activated the NF-kB after 4h of incubation [vehicle: 100 ± 2.0 vs aldosterone: 160.8 ± 10* (% of the vehicle); *P<0.05]. **Conclusion**: Taking all together, aldosterone, by itself, has potential to activate the inflammasome. Efforts should be focused on elucidating the signalling pathways on aldosterone-induced inflammasome activation as well as its implication in drug discovery. References: [1] Schiffrin EL. Effects of aldosterone on the vasculature. Hypertension. 47:312–318. Review, 2006 [2] Kumar H. Pathogen recognition by the innate immune system. Int Rev Immunol. 30:16-34. Review, 2011. **Financial support**: Coordination for the Improvement of Higher Education Personnel (CAPES, 88887.092495/2015-00), from the São Paulo Research Foundation (FAPESP, 2013/08216-2 (Center for Research in Inflammatory Disease), and from the University of São Paulo NAP-DIN (11.1.21625.01.0). Research approval by the Animal Research Ethical Committee number: (protocol n° 012/2013-1).

06.055 Effects of tramadol hydrochloride in oxidative stress in ischemia and reperfusion injury on kidney of rats Monteiro AM[1], Gonçalves BH[1], Rocha CRO, Barros EMN, Brandão FMV, da Silva HC, Junior JBLN, da Silva LL, Pinto LCS, de Oliveira RCS, Couteiro RP, Junior RFGR UEPA –Cirurgia Experimental

Introduction: The Syndrome of Ischemia and Reperfusion (SIR) is responsible for deleterious effects on various organs, present in clinical situations such as acute myocardial infarction. In the kidney, the SIR is the main cause of Acute Renal Failure. To minimize injuries, techniques were created, such as remote per-conditioning (PercR) consisting of ischemia and reperfusion cycles applied by the tourniquet on the hindlimb of the animals. Recent studies have demonstrated that tramadol, an opioid anesthetic can reduce Oxidative stress (OS), being used in the experimental myocardial ischemia. Therefore, this study sought to evaluate the effects of Tramadol and PercR in renal SIR in rats. Method: The research received financial support from Fadesp(Foundation to Support and developmet of research) with a scientific iniciation scholarship, as well as the experimental surgery laboratory. The study was conduced with the approval of the ethics committee on animal testing, protocol number 08/2015, using 25 Wistar rats (Rattus norvegicus), aged approximately 90 days and weighing between 250-300g. The animals were divided into 5 groups: Sham (S), ischemia/reperfusion (I/R), Per-conditioning (Per), Tramadol (T) and Per-conditioning + Tramadol (Per+T). After right nephrectomy of all animals, the left kidney of rats in groups I/R, Per, T and Per+T was subjected to ischemia for 30 minutes. The OS in blood and kidney tissue was assessed by measurement of thiobarbituric acid reactive substances (TBARS) 24 hours after the reperfusion period. **Results**: TBARs blood showed difference in groups T(0,31mg/dl) and Per+T(0,19mg/dl) to the I/R group (0,29mg/dl) ($p<0.01$), as well as showed significant difference between the groups Per(0,15mg/dl) and T(0,31mg/dl) ($p<0.05$). In renal tissue, TBARs showed important difference between the groups I/R(0,29mg/ml) and Per (0,22mg/ml) ($p<0.01$). **Conclusion**: the study concluded that the remote per- conditioning associated with the application of tramadol hydrochloride was able to reduce the tissue damage caused by ischemia, because they reduce lipid peroxidation degree. This can be observed by analysing the blood TBARs values, which are higher when using only Tramadol(0,31mg/dl) and reduced when using this drug associated to the remote per-conditionnig technique(0,19mg/dl).

06.056 Characterization of the vasodilatior effects of organic nitrates GTN, NTHF, NCOE and BIS-NTHF in human umbilical veins. Silva TAF[1], Alustau Fernandes MC[2], Melo MP[1], Maciel PMP[3], Machado NT[3], Gomes SM[4,5], Mendes-Junior LG[3], Mendes-Neto JM[6], Furtado FF[7], Queiroz TM[8], Brandão MCR[9], Athayde-Filho PF[9], Medeiros IA[10] [1]UFPB – Acadêmico, [2]UFCG-CFP/ESTC, [3]UFPB – PPgPNSB, [4]FAMENE, [5]Médico-Residente, [6]UFS – PROCFIS, [7]UFPB – ETS, [8]UEPB, [9]UFPB – CCEN, [10]UFPB – CCS/DCF

Introduction: Organic nitrates are the most commonly nitric oxide (NO) donors used in the treatment of cardiovascular diseases. The effect of glyceryl trinitrate (GTN) has been characterized in several animal and human blood vessels. The tetrahydrofurfuryl nitrate (NTHF) and 13-cis-9-octadecanoate acetate nitrate (NCOE) are NO donors, whose effect has been characterized in animal vessels. 1,2-bis (tetrahydrofuran-2-yl) ethane-1,2-diildinitrato (BIS-NTHF) is a novel compound (two molecules of NTHF). The aim of this study was to characterize the effect of these four organic nitrates both in isolated human umbilical veins. **Methods**: Human umbilical cords were obtained from healthy pregnant women hospitalized at HULW/UFPB and Candida Vargas Maternity. The HUV connective tissue was removed and cut into 5 mm long rings segments. The rings were suspended by metallic bar for isometric tension recordings in a Kreb's Henseleit solution at 37 ºC, gassed with 95% O_2 and 5% CO_2. **Results**: The study of nitrates showed that these compounds have relaxed the basal tone of HUV (40.8 ± 5.2%-GTN; 53.9 ± 3.0%-NTHF; 57.4 ± 1.7%-NCOE; 37.8 ± 4.6%-BIS-NTHF). All nitrate-induced vasorelaxations in HUV pre-contracted with serotonin (5-HT) (98.3 ± 3.3%-GTN; 92.0 ± 3.3%-NTHF; 96.7 ± 1.8%-NCOE; 101.1 ± 2.9%-BIS-NTHF). In this situation, GTN (pD_2 = 5.30 ± 0.08M) was the most potent nitrate in causing vasodilation, while NCOE (pD_2 = 4.08 ± 0.06M) was the less potent agent in relaxing HUVs. When HUV rings were pre-contracted with KCl 60 mM, vasodilation induced by GTN (pD_2 = 4.32 ± 0.09M) or NTHF (74.1 ± 22% and pD_2 = 4.18 ± 0.08 M), were attenuated. The pre-incubation of GTN or BIS-NTHF inhibited contraction stimulated by 5-HT in HUV (88.4 ± 4.2% and 92.7 ± 3.5%, respectively), whereas NTHF or NCOE (31.4 ± 5,1% and 13.7 ± 1.2%, respectively) led to lower inhibition when compared with the other nitrates. GTN, NTHF and BIS-NTHF inhibited the phasic (95.0 ± 1.8%-GTN; 67.3 ± 6.3%NTHF; 93.3 ± 3.5%-BIS-NTHF) and tonic (97.3 ± 0.9%-GTN; 75.8 ± 6.2%-NTHF; 97.8 ± 0.7%-BIS-NTHF) components of the contraction induced by 5-HT in the absence of extracellular Ca^{2+}. NCOE was more effective to inhibit the tonic (84.7 ± 4.5%) contractions than phasic (31.7 ± 5.6%). Pre-incubation with ODQ 10 µM significantly attenuated the vasodilator response to nitrates ((74.9 ± 3.0% and 3.94 ± 0.15 M-GTN; 70.0 ± 2.1% and 4.02 ± 0.11 M-NTHF, except NCOE (95.7 ± 5.2%). Pre-incubation of 10 mM TEA did not alter the effects induced by nitrates in HUV (97.5 ± 1.8%-GTN; 100.2 ± 1.8%-NTHF; 100.6 ± 3,2%-NCOE). **Conclusion**: The results obtained in this study demonstrates that nitrates studied are able to induce relaxation of HUV rings, both in basal tone as against contractions induced by 5-HT or KCl. The mechanism of nitrates action involves, at least, the activation of sCG, and the inhibition of intracellular calcium mobilization. **Financial Support**: CNPq CEP/HUWL approved all protocols of this study (nº 016/10 and 1.200.327)

06.057 Effects of low dose of hydrocortisone in rats with hemorrhagic shock Khayat YF[1], Tavares MLC[2], Monteiro AM[2], Mainardi CR[2], Feijó DH[2], Dias DV[2], Junior RFGR[2], Brito MVH[2], Bohne MR [1]CESUPA, [2]UEPA

Introduction: The shock is a complex state that affects the different sistems of the animal organism, caused by the hypotension, and as a consequence, low concentration of tissue oxygen. The crystalloid must be used especially in non-emergency cases due to its low cost and less interference on the coagulation activities and kidney function. The use of corticosteroid in the treatment of hypovolemic shock remains controversial. There are questions about the correct moment to introduce that therapy, which corticosteroid would be ideal and the effective dose to the treatment. The aim of this study is evaluate the effects of low dose of hydrocortisone in rats with hemorrhagic shock. **Methods**: It was used 20 rats (Rattus norvegicus) Wistar inbreed, male, randomized distributed into 04 groups with 05 animals each: Sham Group (GS) to establish the normal stadards of the sample; Shock Group (GC), hemorrhagic shock of 30% of their volemia, without treatment; Saline Solution Group 7,5% (GSS7,5%), hemorrhagic shock with subsequent volume reposition of 7,5% saline solution; Hydrocortisone Group (GH)**,** hemorrhagic shock with subsequent dose of hydrocortisone. Were analysed samples of Arterial Blood Gas (ABG) before the induction of shock in the animals and after their treatments. The variations of the mean arterial pressure (MAP) was also analysed during the experiment. At the end, small intestine samples were obtained to analyse histological alterations. **Results**: The MAP was elevated to levels statistically significant after the shock induction in the groups GSS7,5% and GH, being the most effective improvemente in the first compared to GC. About the ABG, hydrocortisone was able to raise the PH of the arterial blood compared to GC (p=0,0372, Test t), maintain higher levels of bicarbonate ions (p=0,0395, Test t), lower levels of lactate (p=0,002, Test t) and higher levels of excess bases (p=0,007, Test t). In relation to histological changes, the GH presented fewer injuries than GC, but the value was no statistically significant and the GSSH7,5% had injuries statistically similar to GSham and different from GC. In conclusion, the hydrocortisone was able to improve the metabolic acidosis, the levels of MAP and reduce the intestinal mucosa lesion of rats submitted to hemorrhagic shock. **Financial support** and acknowlesgements: financial support from LCE/UEPA. The Ethics Committee in the Use of Animals approved he research, protocol 04/2015. REFERENCES: BRITO, M.V.H. et al. Viabilidade celular da mucosa do intestino delgado de ratos, após correção de choque hipovolêmico com solução de NaCl 7,5%. Acta. Cir. Bras., v.18, n.6, p. 326-331, 2003. CASSERLY, I.P. et al. Quantitative assessment of the conformational change in the femoropopliteal artery with leg movement. Catheter. Cardiovasc. Interv., v.74, n.5, p. 787–798, 2009. FRIEDMAN, G.; SORIANO, F.G.; RIOS, E.C. Sepsis volume reposition with hypertonic saline solution. Rev. Bras. Ter. Intensiva., v.20, n.3, p. 267-77, 2008.KEEFE, J. Shock and initial stabilization. In: NORKUS, C.L. Veterinary technician's manual for small animal emergency and critical care. Ames: Wiley-Blackwell, cap. 2, p. 25-43, 2012. DELLINGER. R.P. et al. Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: 2008. Intensive. Care. Med. v.34, n.1, p. 17-60, jan. 2008.

06.058 Long-term treatment with carvacrol produces antihypertensive effects and improvement of endotelial function in spontaneously hypertensive rats. Dantas BPV, Almeida AJPO, Santos PF, Lima FO, Castro MVEA, Carvalho CA, Ribeiro TP, Medeiros IA
UFPB – Pharmaceutical Sciences

Introduction: Carvacrol has been described as an agonist/antagonist of different transiente receptor potential (TRP) channels and voltage-dependent calcium channels (Cavs). In previous studies have been shown to be hypotensive effect and vasodilatation in aorta and mesenteric arteries [1-3]. **Aim:** This study examined whether chronic intake of carvacrol (100mg/kg/day) prevents hypertension and endothelial dysfunction in spontaneously hypertensive rats (SHR). **Methods:** Male spontaneously hypertensive rats (SHR) and wistar kyoto (WKY) at 12 weeks of age were randomly divided into normotensive control (WKY), WKY treated with carvacrol (100 mg/kg/day), hypertensive control (SHR) and SHR treated with carvacrol (100 mg/kg/day) groups. Systolic blood pressure (SBP) was determined by tail cuff sphingomanometry and vascular reactivity using aorta rings (2–3 mm), suspended in organ chambers with physiological Kreb's solution, at 37 °C, with a gas mixture of 95% O_2 and 5% CO_2 (pH 7.4). Changes in isometric tension were recorded using a force transducer (Miobath-4,WPI, Sara-sota, FL, USA) coupled to an amplifier-recorder (Miobath-4) to computer. Protocols to evaluate adjacent pathways were performed in the presence of mixes: Tram-34 (1µM) + apamin (300nM) + indomethacin (10µM) or Tram-34 (1µM) + apamin (300nM) + L-NAME (100µM) or L-NAME (100µM) + Indomethacin (10µM). **Results:** Carvacrol decreases high SBP in SHR (values were WKY: 115.50 ± 3.7 mmHg; WKY + carvacrol: 117.50 ± 6.5 mmHg; SHR: 167.50 ± 5.5 mmHg and SHR + carvacrol: 132.33 ± 4.8 mmHg), that effect was associated with blunted acetylcholine-induced relaxations of aorta rings (WKY: 79.10 ± 2.28%, WKY + carvacrol: 77.86 ± 2.58%, SHR: 78.17 ± 3,60% and SHR + carvacrol: 90.40 ± 3.00%) affecting the endothelium-dependent the NO (WKY: 72.84 ± 2.15%, WKY + carvacrol: 60.31 ± 3.32%, SHR: 79.82 ± 0.57% and SHR + carvacrol: 89.83 ± 2.33%). In addition, the vascular contracture response induced by phenylephrine was increased in aorta rings of SHR treated (WKY: 0.83 ± 0.06g, WKY + carvacrol: 0.86 ± 0.04g, SHR: 0.97 ± 0.05g and SHR + carvacrol: 0.78 ± 0.03g). The chronic intake of carvacrol (100mg/kg/day) improves vascular and endothelial function, especially the endothelial-derived mechanisms. **Conclusion:** The present findings indicate that chronic intake of carvacrol reduced the hypertension and prevented endothelial dysfunction in SHR rats, most likely by improvement of vascular function. **Financial support:** CAPES and CNPq. Research approval by the Animal Research Ethical Committee protocol **080/2016.** **Referencias:** 1. Dantas BP et. al. Vascular Pharmacology 67:48-58, 2015. 2. Scott Earley, Joseph E. Brayden Physiol Rev. 95(2): 645–690, 2015 3. Scott Earley, Albert L. Gonzales, Zarine I. Garcia Mol Pharmacol. 77(4): 612–620, 2010

06.059 Doxycycline-attenuation of ER-stress components: GRP78-eIF2 α is an additional mechanism to metalloproteinase inhibition in kidney protection after ischemia-reperfusion. Leal AC, Gonsalez SR, Melo PA, Lara LS UFRJ – Farmacologia

Introduction: Ischemia is a major cause of acute kidney injury (AKI). It has been demonstrated that renal ischemia-reperfusion (I/R) leads to an augmented activity and expression of matrix metalloproteinases (MMPs), as well as the activation of endoplasmic reticulum stress (ER). Doxycycline (Dc) is a tetracycline that have shown various pharmacological effects in subclinical doses, such as decreased inflammation, modulation of reactive oxygen species and MMPs. Preliminary results of our group showed that treatment with 3 mg/kg of Dc prevents the decrease in renal function promoted by I/R. However, the molecular mechanism of Dc was not fully understood. **Objective:** To investigate the mechanisms by which Dc prevents the decrease of kidney function promoted by I/R. **Methods:** Male Wistar rats were divided into 3 groups: (a) control (ctr, sham-operated); (b) I/R: ischemia was induced by applying a non-traumatic vascular clamp the renal pedicles for 30 min, followed by reperfusion for 24 hours and (c) I/R+Dc: two hours before ischemia, 3 mg/kg Dc was administered intraperitoneally. **Results:** We observed that renal I/R increases by two times MMP-9 protein content and MMP-2 activity, as expected; Dc treatment prevent this increase. Furthermore, I/R there is an increased activation of the stress of the ER due to increased expression of GRP78 (142%) and phosphorylation of the transcription factor eukaryotic 2 (p-eIF2α, 168%), which operates in stressful insults attenuating protein synthesis. The Dc treatment normalizes the GRP78-eIF2α decreasing direct or indirect the expression of apoptotic protein caspase-12. **Conclusion:** These results indicate that part of the molecular mechanism of Dc in protecting renal function after I/R involves the attenuation of ER stress components: GRP78-eIF2α and caspase-12. Furthermore, MMP modulation by Dc could be an additional epithelial integrity. **Finacial support**: CNPq, FAPERJ and INBEB. **CEUA-CCS**: 083/15

06.060 The anti-apoptotic arm of Endoplasmic Reticulum (ER) stress: GRP78/eIF2α/CHOP is involved in the survival of mesangial cells submitted to hypoxia-reoxygenation. Silva RC, Mendes LVP, Tortelote GG, Dias WB, Lara LS UFRJ

Introduction: Renal injury caused by ischemia-reperfusion (I/R) process is a major cause of acute renal failure reducing glomerular filtration rate (GFR), a marker of failure in kidney function. Mesangial cells are modified smooth muscle cells and, due to their contractile ability and central location in the glomerulus, can modulate GFR and renal blood flow. We postulate that alterations in intracellular O-GlcNAc levels and endoplasmic reticulum stress (ER stress) in mesangial cells elicited by I/R may contribute to impaired glomerular hemodynamics. **Objective:** To determine the role of intracellular O-GlcNAc levels and ER stress in mesangial cell subjected to hypoxia-reoxygenation (H/R) process. **Methods:** Immortalized mouse renal mesangial cells culture was incubated for 30, 45 and 60 min, or not (control group) with antimycin A (inhibitor of mitochondrial electron transport chain complex III), leading to hypoxia. Reoxygenation was followed by 24 h with cell culture media DMEM. Trypan blue staining was used to count dead cells. The ratio of apoptosis and necrosis was analyzed by flow cytometry. Cells resistant to this process (H/R group) and control group were homogenized and underwent to qRT-PCR and Western bolt (WB). Visualization and distribution of ER stress proteins were examined by immunofluorescence. **Results:** We choose the 45 min incubation time with antimycin A as the protocol for H/R, since under this condition we obtained about 30% of dead cells and 70% of resistant cells. The flow cytometry at this protocol indicated 23% of apoptosis and 44% of necrosis. The ER stress and O-GlcNAc levels were evaluated in resistant mesangial cells comparing to the control group. We observed an increase in about two times of GRP78 protein content and a decrease of 70% in gene expression. Analysis of downstream ER stress proteins by WB and immunofluorescence showed activation of eIF2α (194%), a decrease of 34% CHOP and 64% caspase 12 protein content. HIF1α protein content was decreased by 25%. Furthermore, the O-GlcNAcase/O-linked β-N-acetylglucosamine transferase (OGA/OGT) ratio gene expression was 13.4 arbitrary units indicating an altered intracellular glycosylation level. **Conclusion:** Our data indicate that H/R induces in the resistant mesangial cells activation of the anti-apoptotic arm of ER stress: GRP78/ eIF2α leading to a diminished CHOP/caspase 12 protein content. These events may also provoke a reduction in GRP78 and OGA/OGT ratio gene expression, and a reduction in HIF1α protein content as a negative feedback. **Financial support:** CNPq, FAPERJ, INBEB

06.061 Influence of maternal exposure to Metformin on metabolic and cardiovascular parameters of male and female rat offspring. Novi DRBS[1], Forcato S[1], Vidigal CB[1], Loiola GH[1], Gerardin DC[1], Ceravolo GS[1] [1]UEL – Ciências Fisiológicas

Introduction: Maternal exposure to drugs during the intrauterine development may later influence the health of offspring in adulthood. Metformin is a biguanide commonly used for the treatment of patients with type 2 diabetes mellitus, polycystic ovary syndrome and gestational diabetes. Although the metformin crosses the placenta and has been detected in the umbilical cord at concentrations equal to the maternal blood, it is considered a safe drug throughout gestation. Treatment of obese and diabetics subjects with metformin causes significant weight loss and reduction of adipose mass. On the other hand, exposure to this drug during gestation can increase offspring's body weight and mesenteric fat after high fat diet. Therefore more studies are required to characterize the functional changes caused by maternal exposure to metformin during initial phases of pups. **Aims:** To evaluate the metabolic and cardiovascular parameters in adult rats female and male exposed to metformin during pregnancy and lactation. **Methods:** Wistar female rats were treated with metformin 293mg/kg/day, by gavage from gestational day (GD) 0 to the GD 21 (METG) or GD 0 until lactation day 21 (METGL). Controls dams received water by gavage at the same periods (CTRG and CTRGL). It was evaluated in male and female offspring with 75 days of age: Lee index, the weight of the retroperitoneal fat pads (g/100 of body weight), body weight (g), mean arterial blood pressure (MAP, mmHg) and heart rate (HR, bpm) by an indirect method. The data are expressed as mean □ SEM, (n) is the number of rats per group. **Results** were considered statistically significant if $p < 0.05$ and compared by Student *t*-test (CEUA/UEL: 6996.2015.02). **Results:** Lee index and body weight of males or females metformin rats of different treatments were similar to their respective controls of the same sex. In female offspring, the weight of the perigonadal fat pad was reduced in METG [0.44 ± 0.02 (10)] when compared to CTRG [0.68 ± 0.11 (8)] (p= 0,03), but was similar between groups METGL [0.60 ± 0.06 (8)] and CTRGL [0.49 ± 0.07 (9)]. In male offspring, metformin exposure reduced retroperitoneal fat pad in both groups [CTRG 1.45 ± 0.12 (6)) vs METG 1.06 ± 0.13 (6) (p= 0,04) and CTRGL 1.51 ± 0.12 (7) vs METGL 1.06 ± 0.13 (7)] (p= 0,03). The fasting glycemia, as well as MAP and HR were similar between metformin rats and their respective control of the same sex. **Conclusion:** Metformin exposure during gestational period reduced the retroperitoneal fat pad weight in male and perigonadal fat pad weight in female adult offspring. The exposure during gestation and lactation reduced retroperitoneal fat pad in male, but not in female. The Lee index, body weight, fasting glycemia, as well as MAP and HR were similar between metformin rats and their respective control in the both periods of treatment. With our results it is possible to conclude that metformin exposure during development did not cause metabolic alterations related with obesity or diabetes, but is reduced the visceral fat pad weight. More studies are required to investigate the mechanism related to this alteration. **Financial Support:** CAPES

06.062 The Venous Endothelium: A comparative study between Vena Cava and Portal Veins of Rats. Trindade MR, Assunção HCR, Torres TC, Landgraf RG, Fernandes L Unifesp-Diadema – Ciências Biológicas

Introduction: Venous reactivity plays a fundamental role in the circulatory functioning since the venous compartment contains about 70% of the total volume of blood at rest, which can be rapidly displaced in certain situations, altering cardiac debit and the whole circulation. Nevertheless, the vascular modulation exerted by endothelial cells in the venous circuit is poorly investigated. The endothelium modulates the vasculature by releasing a number of factors, including vasoconstrictor and vasodilator prostanoids (PGH_2 and PGI_2, respectively), generated from the enzymatic action of cyclooxygenase (COX) on aracdonic acid. The aim of this study was to establish and characterize primary cultures of endothelial cells from Vena Cava (VC) and Portal Vein (VP) of rats, targeting a comparative analysis of these territories, particularly with regard to the generation of prostanoids. **Methods:** Wistar male adult rats were anesthetized and subjected to laparotomy. Inferior VC and VP segments were washed in PBS, dissected, sectioned in the longitudinal direction and plated with the endothelial face turned down. Tissues were covered with DMEM (20% fetal bovine serum; 10 ml/L penicillin/streptomycin), pH 7.4, and kept in 5% CO_2 incubator at 37°C. After cell migration, explants were removed and cells were sub-cultured using trypsin (0.125%). Cultures (n= 8-10) were studied between the 4^{th} and 5^{th} passages. The characterization of the cultured endothelium was performed by detection of von Willebrand Factor (vWF), endothelial nitric oxide synthase (eNOS) and endothelin ET-B receptor, using immunofluorescence technique. The basal release of PGH_2 and PGI_2 was determined in samples of the supernatant of cultures, by enzyme immunoassay. Expression of COX-1 (constitutive) and COX-2 (inducible) was determined by Western blot, using 60 □g of total protein and □-actin as endogenous control. **Results:** After 5 days, cell migration was consistently detected and tissues were removed. The time course from cell migration to confluence of the venous endothelial cultures was 7 to 10 days. Immunofluorescence staining was positive for vWF, eNOS and ET-B receptor in all cells analyzed. The basal production (pg/mL) of PGH_2 was significantly higher by cells from VC (11.5 ± 1.5) in comparison to VP (4.5 ± 0.7*), while PGI_2 levels were similar between cultures (20.0 ± 4.6 for VC *vs* 15.1 ± 1.2 for VP) * P <.05. Western Blot assays demonstrated a consistent expression of COX-1 and COX-2 in both cultures, with no statistical differences between groups. Values found for COX-1 were: 1.41 ± 0.20 for VC *vs* 1.30 ± 0.11 for VP. Values for COX-2 were 0.84 ± 0.13 for VC *vs* 0.99 ± 0.21 for VP (arbitrary units). **Conclusion:** Under basal situation, the venous endothelium produces prostanoids that may be responsible for modulation of venodilation (PGI_2) and venoconstriction (PGH_2). In comparison to VP, the VC endothelium exhibits higher ability to produce PGH_2, with no change in expression of COX. These preliminary results indicate major functional differences between the endothelial cells of the tested beds. The methodology established in this study enables further investigation of the endothelium physiology in different venous territories. Supported by CAPES, FAPESP (14/18760-4; 15/23584-3). CEUA UNIFESP 4208150216.

06.063 Matrix Metalloproteinase (MMP)-2 contributes to early hypertrophic and eutrophic remodeling in hypertension by different regulation of Calponin-1. Parente JM, Maciel EA, Castro MM FMRP-USP – Farmacologia

Introduction: Increased activity of MMP-2 degrades many extra- and intracellular proteins that contributes to hypertension-induced maladaptative vascular remodeling and dysfunction. MMP-2 may also contribute to the phenotype switch of vascular smooth muscle cells (VSMCs) from the contractile to a synthetic form. This effect confers to VSMC the ability of migration and proliferation, thus contributing to hypertension-induced arterial thickening. Calponin-1 is an important differentiation marker of VSMC and it is also an intracellular proteolytic target of MMP-2 in the aorta of endotoxemic rats, an effect that lead to vascular dysfunction [1]. The aim of this study is to investigate whether MMP-2 contributes to hypertension-induced vascular remodeling or dysfunction of conductance and resistance arteries by regulating calponin-1. **Methods:** Male wistar rats were submitted to the two kidney-one clip (2K-1C) model of hypertension and were treated with doxycycline (inhibitor of MMPs activity at 30 mg/kg/day) or water for one week. Control groups were submitted to the same surgical procedure and treatment, except for the clip placement. Systolic blood pressure (SBP) was daily accessed by tail cuff plethysmography. After one week, mesenteric arteries and aortas were removed to analyze the activity of MMP-2 by in situ zymography, and calponin-1 by immunofluorescence. Morphological analysis was also done with hematoxylin and eosin staining. **Results:** SBP increased in 2K-1C rats at the first week (146.2 ± 1.5 vs. 118.5 ± 4.1, $p<0,05$) and treatment with doxycycline did not reduce it ($p>0,05$). The morphological analysis showed that at one week, the 2K-1C aortas started to increase the media per lumen ratio (M/L) and the cross-sectional area (CSA) in relation to Sham groups. Mesenteric arteries have already presented eutrophic vascular remodeling with increased M/L (9.1 ± 1.2 vs. 7.0 ± 0.75) and unchanged CSA (206999 ± 25.7 vs. 175543 ± 28.8), and treatment with doxycycline may revert it. Gelatinolytic activity was increased in both mesenteric arteries and aortas of 2K-1C rats ($p<0,05$ vs. Sham groups) and doxycycline reduced it (mesenteric arteries: 26.1 ± 5.2 to 15.7 ± 2.6; aortas: 34.9 ± 5.2 to 27 ± 5.1). Calponin-1 levels were lower in the 2K-1C aortas (22 ± 1.5 vs. 25.4 ± 1.3) and treatment ameliorated it (22 ± 1.5 vs. 29.1 ± 1.4). On the other hand, calponin-1 levels were increased in the mesenteric arteries of 2K-1C (17.6 ± 2.5 vs. 10.7 ± 0.8) and treatment may revert this alteration (17.6 ± 2.5 vs. 13.1 ± 2.3). **Conclusion:** The results showed that MMP-2 diverges in regulate calponin-1 by MMP-2 in the vasculature in hypertension. In conductance arteries, the reduction of calponin-1 by MMP-2 may contribute to the phenotype switch of VSMC, which leads to hypertrophic remodeling in the late phase of hypertension. In resistance arteries, MMP-2 may contribute to eutrophic remodeling and vasoconstriction by increasing calponin-1levels. This effect may be particularly related to the extracellular mechanisms of MMP-2. **References:** [1] Castro, MM. Arterioscler Thromb Vasc Biol. 32(3);662;2012. **Acknowledgments and financial support:** CAPES, CNPq and FAPESP. The Ethics Committee in Animal Research of Ribeirão Preto Medical School approved all protocols (012/2015-1).

06.064 Role of CGMP in early sepsis. Oliveira JG[1], Kovalski V[1], Prestes AP[1], Alves GF[1], Colarites DFR[1], Mattos JEL[1], Vellosa JCR[2], Fernandes D[1] [1]UEPG – Ciências Farmacêuticas, [2]UEPG – Análises Clínicas e Toxicológicas

Introduction: Sepsis and septic shock are the major cause of morbidity and mortality in critically ill patients and is usually associated with hypotension and loss of vascular responsiveness to vasoconstrictors. Nitric oxide (NO) is an important player in the pathogenesis of sepsis and septic shock. However, NOS inhibitors tested so far in the clinical setting causes excessive vasoconstriction, which was the most likely reason for the interruption of a Phase III study with a NOS inhibitor in human sepsis. Therefore, the vasodilation mediated by NO-sGC-cGMP could be critical to preserve a proper blood perfusion and organ failure mainly in the early sepsis. Thus, we used a phosphodiesterase inhibitor as strategies to increase cGMP bioavailability and protected organs from injury in sepsis. **Methods**: Sepsis was induced by cecal ligation and puncture (CLP) procedure in male Wistar rats (n=32). Sildenafil (10 mg/kg, vo, n=16) or vehicle (n=16) was injected 8 h after the surgery and 24 h later of CLP procedure, blood pressure, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate, creatine kinase and nitrite/nitrate (NOx) levels and hematological analyzes were performed. Vasoconstrictive response to phenylephrine (Phe) and angiotensin II (AII) were also evaluated. **Results**: The CLP procedure induced a hypotension, hyporesponsiveness to vasoconstrictos (Phe and AII), leukopenia, thrombocytopenia and increase serum levels of urea, AST, ALT, lactate, CK and NOx. The sildenafil increases de plasma cGMP (Sham 15.1 ± 3.7; CLP 11.1 ± 1.4; CLP+Sildenafil 23.2 ± 1.66 nM, $p<0.01$) and reduced lactate (CLP 33.9 ± 6.7; CLP+Sildenafil 19.5 ± 2.7 mg/dL, $p<0.01$) and CK levels (CLP 4.3 ± 1.2; CLP+Sildenafil 0.9 ± 0.3 µg/dL, $p<0.001$). The CLP-induced hypotension, hyporesponsiveness to vasoconstrictors, leukopenia, thrombocytopenia and increase serum levels of urea, AST, ALT, and NOx were note changed by sildenafil ($P>0.05$). **Conclusion**: These results indicate that elevation of cGMP levels in the early stages of sepsis can lead to a better blood perfusion of tissues, avoiding hypoperfusion and reducing organ injury. Thus, the results suggest that the use of phosphodiesterase inhibitors such as sildenafil may represent a useful tool in the treatment of sepsis, since administered in a proper time. **Financial support**: This work was supported by Fundação Araucária, National Council for Scientific and Technological Development (CNPq, Brazil) and CAPES Foundation (Ministry of Education of Brazil). The procedures were approved by the University Institutional Ethics Committee (Protocol number 019/2013).

06.065 Components of renin-angiotensin system in perivascular adipose tissue in thoracic aorta and mesenteric bed: Alterations promoted by high-fat diet obesity. Inada AC[1], Hashimoto CM, Silva RNO, Costa TJ, Santos-Eichler RA, Carvalho MHC, Akamine EH
USP – Farmacologia

Introduction: Obesity is characterized by inflammation in adipose tissue. Angiotensin II/AT1 receptor pathway induces oxidative stress and vascular inflammation. Perivascular adipose tissue (PVAT) surrounds blood vessels and has paracrine actions in the vascular wall. It is classified as brown (thoracic aorta - TA) and white (mesenteric bed - MB) being the last one more willing to inflammation. **Methods**: Four-week old C57BL/6 male mice were divided in Control (CT) and Obese (OB) groups, fed with standard chow diet (SCD) and high-fat diet (HFD), respectively, for 16 weeks. We evaluated the components of renin angiotensin-system (RAS) in brown (TA-PVAT) and white (MB-PVAT) PVAT of CT and OB groups. The expression of mRNA of angiotensinogen (ANGT) and CCR2 receptor were analyzed by real-time PCR (RT-PCR). Receptors of angiotensin II (Ang II), AT1 (AT1R) and AT2 (AT2R) receptors and monocyte chemoattractant protein -1 (MCP-1) cytokine were analyzed by western blot. Angiotensin converser enzyme 1 (ACE1) activity was analyzed by ACE1 enzyme assay on spectrofluorometer. CT (CT/LOS) and OB (OB/LOS) groups were treated with losartan (10 mg/kg) for 30 days. **Results** are shown as mean ± standard error. Significance level accepted was $p<0.05$ in Student t-test and ANOVA-one way. **Results**: mRNA content of ANGT in brown PVAT (CT: 1.3 ± 0.3 n=8; OB: 0.5 ± 0.1 n=8) and white PVAT (CT: 1.5 ± 0.2 n=5; OB: 0.5 ± 0.2 n=5) was reduced in OB mice. ACE1 activity was lower in brown PVAT of OB group (OB: 14.9 ± 2.3 n=7) in comparison to CT group (CT: 31.2 ± 3.6 n=7) and without differences in white PVAT between groups. In brown PVAT, protein content of AT1 receptor (AT1R) was decreased in OB group (CT: 1.1 ± 0.1 n=7; OB: 0.6 ± 0.2 n=7) and AT2 receptor (AT2R) was not detected in both groups. In white PVAT, protein content of AT1R (CT: 1.0 ± 0.1 n=8; OB: 1.6 ± 0.2 n=8) and AT2R (CT: 0.9 ± 0.1 n=8; OB: 2.3 ± 0.2, n=8) were increased in OB group. mRNA expression of CCR2 receptor from MCP-1 in brown PVAT was similar to both groups; however, it was increased in white PVAT in OB group (CT: 0.9 ± 0.08 n=4; OB: 1.6 ± 0.2 n=4). Treatment with angiotensin II receptor blocker (ARB) (losartan; 10 mg/kg) for 30 days did not diminish MCP-1 expression in white PVAT of OB group (CT: 56.2 ± 15.3 n=5; OB: 111.2 ± 11.9 n=6; CT/LOS: 97.1 ± 8.1 n=6; OB/LOS: 120.6 ± 6.7 n=6). **Conclusion**: In relation to the components of RAS, brown and white PVAT responded differently to HFD obesity and treatment with losartan (10mg/kg) for 30 days did not modify inflammatory parameters presented in obesity. **Financial support**: CNPq, FAPESP, CAPES - DGU. Animal Research Ethical Committee: Protocol 94, page 3, book 9 (2013.09.06).

06.066 Modulation of leptin signaling in rats with cardiac dysfunction induced by hyperleptinaemia neonatal. Marques EB[1], Fernandes I[1], Fernandes-Santos C[2], Macedo FS[2], Scaramello CBV[1] [1]UFF – Fisiologia e Farmacologia, [2]UFF – Neurociências

Introduction: Previous data of our research group showed that hyperleptinaemia neonatal has induced cardiac dysfunction in rats (Marques *et al*., Int J Cardiol, 181C:141, 2015). Leptin determines its effects through the binding to specific receptors known as Ob-R which intracellular signaling in cardiomyocytes comprehends the Janus Kinase Signal Transduction and Translation System ($Jak_2/STAT_3$) (Purdham *et al*., Am J Physiol Heart Circ Physiol, 2876:H2877, 2004) as well as the activation of other kinases including AMP-activated protein kinase (AMPK) (Minokoshi *et al*., Nature, 415(6869):339, 2002). Studies have shown that these pathways play a critical role in the modulation of myocardial injury (Lee *et al*., Proc Natl Acad Sci, 101(37):13624, 2004; Elschami *et al*., Eur J Cell Biol, 92(1):21, 2013). So the aim of this work was to investigate if leptin signaling through $Jak_2/STAT_3$ and AMPK pathways is altered in cardiac dysfunction induced by hyperleptinaemia neonatal. **Methods:** Male Wistar rats were treated with leptin (L) at a daily dose of 8µg/100g body weight or saline (C) subcutaneously for the first 10 days of lactation and assessed at 150 days-old. Blood and cardiac tissue were collected to evaluate serum levels of leptin, quantification of cardiomyocyte hypertrophy by histopathological assays and cardiac lipid peroxidation. Cardiac homogenates were obtained (Bambrick *et al*., J Pharmacol Meth, 20: 313, 1988) to evaluate protein expression of Ob-R and other proteins related to leptin signaling (Jak_2, $STAT_3$, p-$STAT_3$, AMPK and p-AMPK) (Laemmli, Nature. 227:680, 1970). Data were presented as mean and standard error of the mean, analyzed by Student *t* test and considered statistically different if $p<0.05$(*). **Results**: Hyperleptinemia (ng/mL) was observed at the end of treatment (10^{th} day of lactation) (C=2,9 ± 0,9 vs L=11,7 ± 1,2*) and at 150 days-old (C=9,5 ± 0,8 vs L=14,4 ± 0,3*). Histopathological measurement of cardiomyocyte diameter (µm) showed left ventricular hypertrophy in L group (C=16,2 ± 0,3 vs L=20,3 ± 1,1*). Cardiac lipid peroxidation, related to malondialdehyde concentration (nmol/mL), were similar between groups (C=138,0 ± 12,9 vs L=148,0 ± 16,8). Hyperleptinemia led to the upregulation of Ob-R (C=1,9 ± 0,3 vs L=3,5 ± 0,4*), AMPK (C=1,9 ± 0,2 vs L=2,7 ± 0,3*) and p-AMPK (C=0,4 ± 0,1 vs L=0,9 ± 0,1*). However p-$STAT_3$ (C=0,6 ± 0,1 vs L=0,2 ± 0,05*) were downregulated. **Conclusions**: Hyperleptinemia and Ob-R upregulation observed are usually present in heart failure and seem to constitute a compensatory mechanism related to cardiac injury (Schulze et al. Eur J Heart Fail.5:33, 2003; McGaffin et al. Cardiovasc Res. 77: 54, 2008). However p-$STAT_3$ downregulation here described suggest that the cardioprotective role of leptin was disadvantaged, justifying cardiac injuries and hypertrophy noticed (Leifheit-Nestlen et al. J Transl Med. 11:170, 2013). Despite of this, AMPK pathway is upregulated, which in accordance to literature, reflects heart protection from lipid overload (Palanivel *et al*., Metabolism. 55(8):1067, 2006), explaining the absence of cardiac lipotoxicity. **Financial Support**: FAPERJ, CNPq, CAPES, PROPPI/UFF. The use of animals was approved by Ethics Committee (CEUA/ UFF389-13).

06.067 TP receptors activation induces hydrogen peroxide production in the vascular smooth muscle cells from normotensive but not from renal hypertensive rat aorta. Santos JD[1], Grando MD[2], Bendhack LM[2] [1]FMRP-USP – Farmacologia, [2]FCFRP-USP – Física e Química

Introduction: Vascular endothelial and smooth muscle cells produce reactive oxygen species (ROS) as superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). ROS can be produced by enzymes such as NADPH oxidase, uncoupled NO-synthase, and cyclooxygenase (COX). The endothelial cells play important role on the vascular tone control by the release of contractile agents like thromboxane A_2 (TXA_2) TP receptors agonist in the endothelial and vascular smooth muscle cells. TXA_2 contributes to increase the ROS production and activation of the COX causing prostanoids production. H_2O_2 stimulates production of TXA_2 and prostacyclin that cause vasoconstriction in hypertension. Hence, ROS are upstream and downstream of the COX-prostanoid system. The hypothesis of this study was that the contractile response caused by activation of TP receptors is modulated by ROS in renal hypertensive rat (2K-1C) aorta. This work aimed to evaluate the effects of TP receptors activation and the role of ROS inhibition on the contractile response induced by TP receptors agonist (U46619) in 2K-1C and normotensive (2K) rat aortas. **Methods**: Intact endothelium (E+) or denuded (E-) aortas isolated from 2K-1C and sham-operated rats 2K were used. Six weeks after the surgeries, 2K-1C presented systolic pressure ≥160 mmHg. Contraction was induced by U46619 in the absence (control) or in the presence of Tiron (superoxide scavenger, 100 µM), Catalase (300 U/mL), the enzyme that degrades H_2O_2 to water and molecular oxygen. **Results** are expressed as grams of tension presented as the mean ± SEM. The level of statistical significance was defined as $p<0.05$. Aortas were used also to evaluate the protein expression of COX-1 and COX-2. **Results**: There are no differences between 2K-1C and 2K in the U46619-induced contraction. The endothelium removal increased the potency (pD_2) of U46619 in 2K-1C (E+: 8.13 ± 0.08, n=6; E-: 8.69 ± 0.12, n=5; p=0.003) and 2K (E+: 8.13 ± 0.10, n=6; E-: 8.61 ± 0.12, n=6, p=0.015) but it did not change the maximum effect (ME) in both groups. Tiron increased the ME only in E+ aortas from 2K-1C (control: 2.6 ± 0.2, n=6 and Tiron 3.5 ± 0.2, n=6; p=0.01) but Catalase did not change the ME induced by U46619 (2.4 ± 0.2, n=6). The pD_2 values were not changed by Tiron or Catalase. In E+ aortas from 2K rats, Tiron or Catalase did not alter the ME or pD_2 values. However, in E- aortas, Catalase decreased the ME (control: 3.4 ± 0.0, n=6; Catalase: 2.7 ± 0.1, n=6; p=0.03) whereas Tiron had no effect. The pD_2 values were not altered by Tiron or Catalase. Both COX-1 and COX-2 were expressed in 2K and 2K-1C aortas which were greater in 2K-1C than in 2K aortas ($p<0.001$). **Conclusion**: Contractile response induced by TP receptors activation should involve endothelium formation of superoxide in 2K-1C rat aortas. On the other hand, hydrogen peroxide is produced in vascular smooth muscle cells in 2K rat aortas. These ROS production could be due to COX activation. **Supported** by CAPES, CNPq and FAPESP. All the procedures were performed in accordance with the standards and policies of the Ethics Committee on Animal Care and Use of the University of São Paulo (027/2015-1).

06.068 Effects of exercise on cardiovascular response to acute restraint stress in rats. Matsubara NK[1], Volpini VL[1], Veríssimo LF[1], Marques LAC[1], dos Santos DC[1], Estrada VB[1], Ceravolo GS[1], Gomes MV[2], Martins-Pinge M[1], Pelosi GG[1] [1]UEL – Ciências Fisiológicas, [2]UNOPAR – Ciências da Saúde

Introduction: Stressful events may activate several systems to maintain homeostasis during real or potential threats (RADLEY, J. J.; Stress, v. 14, p. 481, 2011). Stress can modulate supramedullar areas, which interacts with arterial pressure (AP) control centers, interfering on cardiovascular system responses (FURLONG, T. M.; Eur J Neurosci, v. 39, p. 1429, 2014). Thus, it increases cardiac sympathetic activity by cathecolamines releasing. Physical exercise also induces systemic adaptations on locomotor, respiratory and cardiovascular control. On cardiovascular system, it may induce beneficial adjustments on sympathetic and parasympathetic tonus such as sympathetic activity increase, which leads an increase of heart rate (HR) and cardiac output (THOMPSON, P. D.; Med Sci Sports Exerc, v. 33, p. S438, 2001). Moreover, physical exercise may reduce renin-angiotensin-aldosterone system (RAAS) activity, being an important tool to hypertension management (MARTINS-PINGE, M. C.; Braz J Med Biol Res, v. 44, p. 848, 2011). Exercise improves parasympathetic component, which can counterbalance stress effects (MICHELINI, L. C.; Exp Physiol, v. 94, p. 947, 2009). Then, this study aimed to evaluate the exercise effects on cardiovascular responses during and after acute stress. **Methods**: Male Wistar rats (50-90 days) were trained in a glass tank for 20 swimming sessions of 1 h/day (acute stress trained group; AST) or maintained in control acute stress (AS) group. One day before AP recording, a catheter was implanted into the femoral artery for blood pressure recording. On the next day, basal AP was recorded for 20 min, animals were introduced in a cylindrical tube for acute restraint stress protocol, and AP was recorded during 1 h. AP of AST group was recorded 24 h after the last swimming session end. For temporal analysis of restraint stress, two-way ANOVA was used, and for recovery analysis, one-way ANOVA was used. Data showed as mean ± mean standard error and $P< 0,05$. **Results**: Exercise caused no change on mean arterial pressure (MAP) (Time: $p< 0,0001$, $F_{(15, 240)}= 14,05$; Treatment: $p= 0,6044$, $F_{(1, 16)}= 0,2793$; Interaction: $p= 0,4428$, $F_{(15, 240)}= 1,012$; n= AS: 9, AST: 9) and tachycardia response (Time: $p< 0.0001$, $F_{(15, 240)}= 12,18$; Treatment: $p= 0,7484$, $F_{(1, 16)}= 0,1065$; Interaction: $p= 0,3889$, $F_{(15, 240)}= 1,066$; n= AS: 9, AST: 9) during acute stress restriction. Exercise did not affect recovery of MAP after acute restraint stress (before stress: AS= 100,9 ± 2,96 mmHg, n= 20; AST= 96,8 ± 2,68 mmHg, n= 16; after stress: AS= 105,4 ± 2,58 mmHg, n= 18; AST= 99,20 ± 1,61 mmHg, n= 15; $p= 0,1347$; $F_{(3, 65)}= 1,922$). AS and AST animals showed an increase on HR after stress, but values did not differ between groups (before stress: AS= 374,3 ± 9,32 bpm, n= 20; AST= 360,7 ± 12,84 bpm, n= 16; after stress: AS= 433,1 ± 12,43 bpm, n= 18; AST= 418,1 ± 17,75 bpm, n= 15; $p= 0,0003$; $F_{(3, 65)}= 7,155$). **Conclusion**: The data suggest that previous physical exercise did not interfere on cardiovascular responses caused by acute restraint stress in rats. **Financial Support**: CAPES and CNPq (proc. 478566/2013-1). CEUA Number: 14441.2013.18/ UEL.

06.069 Mechanisms of action of metformin plus insulin treatment on high-fat diet/streptozotocin-induced diabetes in rats. Pereira ENGS, Silvares RR, Flores EEI, Estato V, Reis PA, Silva IJ, Machado MP, Neto HCCF, Tibiriçá E, Daliry A Fiocruz,

Introduction: Several studies have suggested the use of metformin adjunct therapy to overcome many of the deficiencies of insulin monotherapy; however the exact mechanism of action of metformin in type-1 diabetes (T1D) is poorly explored. As several studies have shown a link between T1D and liver disease we explored the liver alterations associated to T1D and its modulation by the hypoglycemic agent metformin in association with insulin. **Methods:** Sixty Wistar rats were divided into four groups: untreated animals (CTL, n=20), high-fat diet/streptozotocin induced diabetes (DM, n=20), treated animals which received only insulin (DM+Ins, n=15) and diabetes treated with metformin plus insulin (DM+Met+Ins, n=15). The biochemical parameters (%HbA1c, cholesterol, fructosamine, triglycerides, total proteins, bilirubin, urea, uric acid, and ALT, AST, ALP, GGT, and amylase activities) were analyzed by spectrophotometry. Intravital microscopy and laser speckle flowmetry were used to study the microcirculatory parameters. In the liver tissue, real-time PCR was used to analyze oxidative stress enzymes, the inflammatory marker MCP-1 and RAGE gene expression. Lipid peroxidation was assessed by TBARs. AGE deposition and RAGE protein expression were studied by fluorescence spectrophotometry and western blot, respectively. **Results:** Body weight, %HbA1c, urea, total proteins and oxidative stress parameters were found to be similarly improved by insulin or Met + Ins treatments. On the other hand, Met + Ins treatment showed a more pronounced effect on fasting blood glucose level than insulin monotherapy. Fructosamine, uric acid, creatinine, albumin and amylase levels and daily insulin dose requirements were found to be only improved by Met + Ins treatment. Liver, renal and pancreatic dysfunction markers were found to be more positively affected by metformin adjunct therapy when compared to insulin treatment. Liver microcirculation damage was found to be completely protected by Met + Ins treatment, while insulin monotherapy showed no effect. **Conclusion:** Our results suggest that oxidative stress, microcirculatory damage and glycated proteins could be involved in the etiology of liver disease due to diabetes. Additionally, metformin adjunct treatment improved systemic and liver injury in HFD-/STZ-induced diabetes and showed a more pronounced effect than insulin monotherapy. This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnologico (CNPq 455,384/2014-2) and PAPES/Fiocruz (401,803/2015-5). All of the experimental procedures were conducted in accordance with the internationally accepted principles for the International Journal of Experimental Pathology Care and Use of Laboratory Animals and were approved by the Oswaldo Cruz Foundation Animal Welfare Committee (CEUA licence # LW-52/13).

06.070 Atheroprotective effects of the enriched fraction obtained from *Ilex paraguariensis* A. St.-Hill. (n-FBIP) in rabbits. Gasparotto Júnior A, Gebara KS, Santiago PG UFGD – Toxicologia e Farmacologia Cardiovascular

Introduction: Dyslipidemia and atherosclerosis are the leading causes of death and disability in Western countries. The process consists of chronic and progressive alterations in arterial wall characterized by inflammatory and fibroproliferative response. Endothelial dysfunction appears to be the initial step in atherogenesis, and the dyslipidemia, often caused by a high fat diet is one of its main triggers. Considering the impact of this disease to humans, in recent decades there was a great interest in the research of medicinal plants and their extracts in medication therapy. Recent data (Balzan et al., 2013) have shown that an enriched fraction (n-FBIP) obtained from *Ilex paraguariensis* A. St.-Hill. (Aquifoliaceae) presents significant antioxidant and lipid-lowering effects in rats. **Aim:** Evaluate the hypolipemiant and antiatherogenic effects of n-FBIP in New Zealand rabbits submitted to high fat diet (HFD). **Methods:** n-FBIP was obtained and standardized according to previously described methods (Balzan et al., 2013). Dyslipidemia and atherogenesis were induced by the administration of HFD (1% cholesterol) for 8 weeks. The n-FBIP was administered orally at doses of 10, 30 and 100 mg/kg, once a day, for four weeks, starting from the 5nd week of HFD. The gain in body weight were measured weekly over the eight week study. Blood was collected and samples were analyzed at time zero and at the end of each month to measure the levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C). At the end of the experiments it was withdrawn the aorta and its direct branches to perform pathological study. **Results:** The HFD induced dyslipidemia and major structural changes in the aortic wall. The treatment with n-FBIP was unable to prevent the increase of TC, LDL-C, VLDL-C, and triglycerides levels in New Zealand rabbits. On the other hand, atherosclerotic lesions were significantly reduced in n-FBIP -treated rabbits. In the aortic arc, the average area of atherosclerotic lesions in n-FBIP-treated rabbits (10 mg/kg) were 1,83 ± 0.31 Sq µm, while on animals that received no treatment was 2,83 ± 0.17 Sq µm ($p<0.05$). Likewise, there were also significant reductions in the formation of atherosclerotic lesions in the abdominal branches of the aorta (n-FBIP 10 mg/kg: 0.66 ± 0.50 Sq µm; control: 2.33 ± 0.22 Sq µm; $p<0.05$). **Conclusion:** This study demonstrated that n-FBIP was able to prevent arterial thickening induced by HFD in New Zealand rabbits. Further studies should clarify the molecular mechanisms involved in these effects, including possible antioxidant effects. **Acknowledgements:** We are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Diretoria Executiva de Gestão da Pesquisa e Pós-Graduação (DEGPP/UNIPAR) for financial support. **Animal Research Ethical Committee**: Process 09/2015- CEUA/UFGD **References:** Balzan, S. et al. Fitoterapia, v. 81, p. 115, 2013.

06.071 Increased activity of matrix metalloproteinase (MMP)-2 in two kidney-one clip (2K-1C) hypertension-induced hypertrophic and dilated cardiac remodeling. Pereira SC, Sanchez ER, Tanus-Santos JE, Castro MM USP – Farmacologia

Aims: Persistent increased blood pressure is intrinsically related to transition from cardiac hypertrophy to heart failure. Increased MMP-2 activity contributes to hypertension-induced compensated cardiac hypertrophy, an effect mitigated after treatment with an MMP inhibitor. Increased MMP-2 activity also contributes to the progression of hypertrophic remodeling to heart failure, and MMP inhibition attenuates this process (1-2). We first examined whether renovascular hypertensive rats develop dilated cardiac remodeling at the chronic stages of hypertension, and whether MMP-2 may participate in this process. **Methods**: Male Wistar rats were subjected to the 2K-1C surgery and were studied after 16 weeks. MMP-2 activity was analyzed in the left ventricle by gel and *situ* zymography. The cardiac left ventricles were stained with hematoxylin and eosin to evaluate the structure, and picrosirius red staining was used to quantify interstitial collagen. **Results**: Systolic blood pressure started to increase at one week after surgery ($p<0.05$ vs. Sham) and was maintained elevated until 16 weeks. After 16 weeks, 30% of 2K-1C rats showed dilated cardiac chambers (HD-2K-1C), while 70% of them presented hypertrophic profile (HH-2K-1C). The diameter of left cardiac chamber increased in HD-2K-1C when compared to HH-2K-1C rats ($p<0.05$). However, both HH- and HD-2K-1C rats showed increased heart weight/body ratio and wall thickness ($p<0.05$ versus Sham). Furthermore, increased interstitial collagen and cardiomyocyte hypertrophy were observed in left ventricles of HH- and HD-2K-1C ($p<0.05$ versus Sham) without significant differences between them. We also showed that MMP-2 activity increased in the hypertrophic and dilated hearts 16 weeks after surgery ($p<0.05$ versus Sham). **Conclusion**: Increased MMP-2 activity may be associated with renovascular hypertension-induced hypertrophy and dilated cardiac remodeling. References: 1- J. Thomas Peterson *et al*. *Circulation*. 103:2303-2309.2001. 2- Yoshitaka Iwanaga, MD *et al.* J Am Coll Cardiol. 39:1384–91. 2002. **Financial support**: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Approved by the committee of ethics in Animal Research of Medical School of Ribeirão, University of São Paulo, SP, Brazil (Protocol no. 023/2015-1).

06.072 Simvastatin reduces endothelial adhesion molecules through 15-epi-lipoxin A4 production on a murine model of chronic Chagas cardiomyopathy. González-Herrera F[1], Pimentel P[2], Cramer A[2], Liempi A[3], Castillo C[3], Guzmán-Rivera D[1], Machado FS[2], Kemmerling U[3], Maya JD[1] [1]University of Chile – Clinical and Molecular Pharmacology, [2]UFMG – Biochemistry and Immunology, [3]University of Chile – Anatomy and Developmental Biology

Introduction: Chagas disease, caused by the parasite Trypanosoma cruzi, evolves to Chronic Chagas Cardiomyopathy (CCC) in 30% of patients, being the main cause of mortality[1]. In CCC, there is endothelial dysfunction caused by the increased expression of pro-inflammatory cytokines, activation of nuclear factor kappa B (NFκB) and the consequent increase in the expression of endothelial adhesion molecules (ECAMs), increasing endothelial permeability and inflammation, with subsequent cardiac tissue fibrosis[2]. Currently, the treatment for Chagas disease is Benznidazole but is not able to reduce the pathophysiological factors of CCC[3]. On the other hand, statins (cholesterol lowering agents) are also able to decrease inflammation. It has been shown in vitro that statins reduce NFκB activation and the ECAMs increase induced by *T. cruzi*[4]. Furthermore, it has been reported that these effects are mediated by 15-epi-lipoxin A4, an eicosanoid produced by 5-lipoxgenasa (5-LO) in the presence of statins[5]. 15-epi-lipoxin A4 is able of reducing cardiac inflammation[6]. Therefore, in this study we evaluated the ability of statins to decrease the expression of adhesion molecules in T. cruzi-infected murine serum and heart tissue, and the involvement of 15-epi-lipoxin A4 on these effects, in a murine model of CCC. **Methods**: Sv/129 WT and 5-LO-/- mice infected with 1000 trypomastigotes of Dm28c strain of T. cruzi were treated with benznidazole 100 mg/kg, simvastatin 40 mg/kg, or 15-epi-lipoxin A4 25 ug/kg/day for 20 days, starting at the 30th day post infection (p.i.). Animals were euthanized at day 80 p.i. to obtain blood and cardiac tissue for parasite load, soluble, and tissue ECAMs (VCAM-1, ICAM-1 and E-selectin) analysis, assayed qPCR, ELISA, and Immunohistochemistry, respectively. **Results**: On WT and 5-LO-/- mice, 100 mg/Kg/day Benznidazole and on WT 40 mg/kg/day simvastatin, parasite load and ECAMs were decreased. However, in 5-LO-/- mice, simvastatin 40 mg did not decrease the parasite load and ECAMs levels completely, but it did occur when exogenous 15-epi-lipoxinA_4 was administered. **Conclusion**: Simvastatin, through the production of 15-epi-lipoxin A4, can reduce T. cruzi-induced endothelial dysfunction, positioning it as a possible drug for treatment against CCC. **Financial support**: FONDECYT project N° 1130189, and Grant for short stays of the Department of Postgraduate of the University of Chile Ethical Committee: Animals handling and experimental procedures were authorized by the bioethics committee of the Faculty of Medicine of the University of Chile (protocol #FMUCH 0528). **References**: 1. Healy, C. Card Electrophysiol Clin. Vol 2, 251 (2015). 2. Rossi, MA. PLoS Negl Trop Dis. Vol 4, 674 (2010). 3. Morillo, CA. N Engl J Med. Vol 373, 1295 (2015). 4. Campos-Estrada, C. PLoS Negl Trop Dis. Vol 9, 5 (2015). 5. Spite, M. Circ Res. Vol 107, 1170 (2010). 6. Molina-Berríos, A. PLoS Negl Trop Dis. Vol 7, 4 (2013).

06.073 G Protein-Coupled Receptor Kinase 2 (GRK2) levels are NO-dependent in septic kidney. Rosales TO, Horewicz VV, Assreuy J UFSC – Farmacologia

Introduction: Septic shock is characterized by hypotension, decreased systemic vascular resistance and impaired vascular reactivity to vasoconstrictors in all of which nitric oxide (NO) has a relevant role. Distinctly from the generalized systemic vasodilation, the kidney and the lung exhibit marked vasoconstriction during sepsis. G protein-coupled receptor kinases (GRKs) phosphorylate several receptors, including the adrenergic receptors, which results in their labeling for internalization. Previous data of our laboratory suggest that sepsis activates GRK in the aorta, leading to the decrease in alpha-1 adrenergic receptor density. Considering the opposite vascular status between the kidney and the systemic circulation during sepsis, the present study aimed to evaluate the putative role of NOS-2-derived NO on GRK2 levels in the septic renal tissue. **Methods**: Female Swiss mice were treated with a NOS-2 inhibitor (1400W; 1 mg/kg) 30 min before and 12 hours after the induction of sepsis by cecal ligation and puncture. Twenty-four hours after sepsis induction, kidney and liver were removed for Western blotting analysis of GRK2 and inducible nitric oxide synthase (NOS-2). **Results**: Sepsis induced an increase in NOS-2 protein expression in liver and kidney. The level of GRK2 protein was unchanged in septic liver but rather surprisingly, a substantial (50 %) decrease was observed in the kidney. Treatment of septic animals with 1400W did not change GRK2 in the liver but the levels of this kinase were reduced to almost none in the kidney. **Conclusions**: Our results indicate that i) sepsis induced increased expression of NOS-2 in both tissues; ii) sepsis induces a reduction in kidney GRK2 levels but not in the liver; iii) results with 1400W show that the reduction of NOS-2-derived NO caused the almost total disappearance of GRK2 protein in the kidney but not in the liver. Therefore, it seems that during sepsis, the levels of GRK2 in the kidney are strictly dependent on NO production. The consequences of these findings to the understanding of septic vascular dysfunction remain to be studied. **Financial support**: CNPq, CAPES, FINEP and FAPESC. All procedures were approved by the institutional Animal Ethics Committee (CEUA PP0790).

06.074 Combined therapy with an adenosine A_{2A} receptor agonist and a phosphodiesterase 5 inhibitor ameliorates monocrotaline-induced pulmonary hypertension in rats. Carvalho FIS[1], Silva A[1], Alencar AKN[1], Martinez ST[2], Fraga AM[1], Barreiro EJ[1], Zapata-Sudo G[1], Sudo RT[1] [1]UFRJ – Farmacologia, [2]UFRJ – Química

Introduction: Current therapy for patients with pulmonary hypertension (PH) has failed to reverse this life-threatening disease and shows high indices of adverse effects. **Objectives:** Aiming to provide a novel insight into the therapy strategy of PH, we decided to combine the effects of two new drugs in rats with monocrotaline (MCT)-induced PH: LASSBio-1359, a selective agonist of the adenosine A_{2A} receptor, and lodenafil citrate (LodC), a compound that belongs to a new generation of PDE5 inhibitors. **Methods:** Male Wistar rats received a single intraperitoneal injection of MCT (60 mg/kg) for PH induction. Experimental groups were: control, MCT + vehicle, MCT + LodC, MCT + LASSBio-1359, and MCT + combined drugs (MCT + mix). Rats were treated with vehicle (DMSO), or LodC alone (34 and 170 µmoles/kg/day, p.o., n = 6 rats), LASSBio-1359 alone (34 and 170 µmoles/kg/day, p.o., n = 6 rats), and combination of LodC (34 µmoles/kg/day p.o., n = 6) plus LASSBio-1359 (34 µmoles/kg/day p.o.) during 14 days after disease onset. Pulmonary acceleration time (PAT) was measured by transthoracic echocardiography. Exercise capacity was investigated using a treadmill test to observe the walk time to exhaustion (TE). Right ventricular systolic pressure (RVSP) was measured by cardiac catheterization. **Results:** PAT was reduced after MCT injection from 44.2 ± 0.9 ms (control) to 24.8 ± 1.4 ms (MCT + vehicle; $P < 0.05$ *vs.* control). Both drugs normalized PAT when administrated alone at a dose of 170 µmoles/kg/day [(42.3 ± 1.2 ms; MCT + LodC *vs.* MCT + vehicle; $P < 0.05$); (42.7 ± 0.9 ms; MCT + LASSBio-1359 *vs.* MCT + vehicle; $P < 0.05$)]. Administration of 34 µmoles/kg/day of LodC or LASSBio-1359 alone did not change PAT, while their combination significantly increased it at a lower dose (43.8 ± 0.7 ms; MCT + mix *vs.* MCT + vehicle; $P < 0.05$). TE (s) was reduced from 1188 ± 43.4 (control) to 188.8 ± 26.17 (MCT + vehicle; $P < 0.05$) and recovered to 853.0 ± 64.5 (MCT + LodC at 170 µmoles/kg/day; $P < 0.05$ *vs.* MCT + vehicle) and to 778.5 ± 56.3 (MCT + LASSBio-1359 at 170 µmoles/kg/day; $P < 0.05$ *vs.* MCT + vehicle). Exercise intolerance was only partially reversed when LodC or LASSBio-1359 were administrated at a dose of 34 µmols/kg/day, respectively. MCT-injected rats which received a combined therapy with lower doses of LodC and LASSBio-1359 exhibited exercise tolerance times equivalent to the control rats (979.7 ± 43.4 s). High pulmonary arterial pressure induced an increase of RVSP in MCT-challenged rats (82.5 ± 4.1 mmHg; MCT + vehicle *vs.* 23.8 ± 0.9 mmHg; control; $P < 0.05$) and monotherapy with LodC or LASSBio-1359 at a dose of 170 µmoles/kg/day beneficially reduced RVSP [(31.0 ± 1.0 mmHg; MCT + LodC *vs.* MCT + vehicle); (36.3 ± 0.9 mmHg; MCT + LASSBio-1359 *vs.* MCT + vehicle); $P < 0.05$]. Lower doses of both substances alone (34 µmoles/kg/day) have no effect on RVSP. However, combined therapy with LodC and LASSBio-1359 significantly reversed RV overload in MCT-induced PH rats (20.1 ± 0.3 mmHg; MCT + mix *vs.* MCT + vehicle; $P < 0.05$). **Conclusions:** Compared with the administration of high doses of LodC or LASSBio-1359 alone, the combination of both drugs in a lower dose provided equivalent response in the treatment of MCT-induced PH rats, a finding that may represent an option for reducing the adverse effects and reversing the disease in the future.

06.075 Tumor necrosis factor-alpha modulates thrombocytopenia, platelet aggregation and adhesion in experimental model of sepsis. Bueno PI, Naime ACA, Abreu A, Bonfitto PHL, Marcondes S FCM-Unicamp – Farmacologia

Introduction: Platelets are important and primary haemostasis and thrombosis, but their role in inflammation needs to be better elucidated. Tumor necrosis factor-alpha (TNF-α) is a cytokine largely produced in different inflammatory condition, including sepsis. Sepsis is a serious problem in worldwide and can be studied using lipopolysaccharide (LPS), a constituent of gram-negative bacteria wall. Therefore, we decided to study the effect of TNF-α on the number of circulating platelets, platelet aggregation and adhesion of LPS-injected rats. **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – UNICAMP, protocol number 3533-1). Wistar male rats were injected with saline or LPS (1 mg/kg, i.p.) and after 30 min they were treated with the antibody anti-TNF-α infliximab (10 mg/kg, s.c.). After 6h or 48h the arterial blood was collected. Platelet number was determined in peripheral blood. ADP (10 μM)-induced platelet aggregation was evaluated using a two-channel aggregometer. Washed platelet adhesion was evaluated using fibrinogen-coated 96-well microtiter plates. Platelets were maintained in the plate for 30 min. After that, the plate was washed and adherent platelets were incubated with the acid phosphatase substrate for 1h. The plate was read by a microplate reader set at 405nm. **Results**: Treatment of rats with LPS significantly reduced the number of circulating platelets either at 6h or at 48h (reduction about 73%). LPS inhibited ADP-induced platelet aggregation by 38% and 85% after 6h and 48h, respectively. Adhesion of stimulated platelets was not modified by LPS, however, adhesion of non-activated platelets to fibrinogen was increased about 64% at 6 h after LPS injection and about 80% after 48h. Treatment of LPS-injected rats with infliximab (antibody anti- TNF-α) prevented the thrombocytopenia in 54% and 63% at 6h and 48h after LPS injection, respectively. Infliximab practically abolished the LPS effect in ADP-induced platelet aggregation and in the non-activated platelet adhesion to fibrinogen either at 6h or at 48h. Infliximab did not modify any evaluated parameters in platelets of saline injected rats. **Conclusion**: TNF-α plays an important role in the thrombocytopenia and in the modulation of platelet reactivity (aggregation and adhesion) in experimental model of sepsis. **Supported by**: CNPq / FAPESP

06.076 Acute and chronic effects of northeastern Brazilian red wine on platelet aggregation Vieira RLP[1], Machado-Calzerra NT[1], Bezerra LS[1], Maciel PMP[1], Melo PM[1], Assis KS[1], Rezende MSAR[1], Azevedo FLAA[1], Medeiros FA[1], Veras RC[1], Medeiros IA[1 1]UFPB

Introduction: Platelets play a pivotal role in the physiology of hemostasis and pathophysiologic processes such as arterial thrombosis and diseases as angina or acute coronary syndromes. The red wine consumption, a rich source of polyphenols, has been associated with antiplatelet activity and cardioprotective effects. Previous studies have shown that alcohol-free (Cabernet-Sauvignon) lyophilized northeastern Brazilian red wine – RIO SOL (LRW-RSCS) is rich in polyphenolic compounds (4.2 g GAE/L of polyphenols). Therefore, the aim of the present study was to evaluate the acute and chronic effect of the LRW-RSCS on platelet aggregation induced by different agonists. **Methods**: All protocols were approved by CEUA/UFPB nº 0612/12. In the present work, two different strategies were adopted to evaluate "*in vitro*" platelet aggregation: direct effect and chronic treatment of LRW-RSCS. Platelets were isolated from venous blood obtained from rats anesthetized with ketamine (75 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Platelet-rich plasma (PRP) was prepared by venous blood centrifugation at 120 *g* for 15 minutes and adjusted to $2x10^7$ platelets/mL with HEPES solution (pH 7.4). To evaluate the direct effect of LRW-RSCS on platelet activity, PRP was incubated with LRW-RSCS (300 µg/mL) at 37°C for 15 minutes in aggregometer (AgreGO, São Paulo, Brazil) with continuous stirring at 1000 rpm. The sample was then stimulated with adenosine diphosphate (ADP; 10 µM) or phorbol myristate acetate (PMA; 0.1 µg/mL) for 10 minutes. Furthermore, the effect of chronic treatment of LRW-RSCS on platelet aggregation was determined. Wistar rats were treated with LRW-RSCS (100 mg/kg) or vehicle by oral gavage once daily for 4 weeks. After this period, PRP was isolated as described above and stimulated with ADP (10 µM) or collagen (25 µg/mL) for 10 minutes. The percentage of platelet aggregation was expressed as the maximum light transmission obtained in the sample. The baseline aggregation values were determined by using a platelet-poor plasma sample. **Results**: The platelet aggregation induced by ADP (57 ± 3%, n=4) was significantly reduced by pre-incubation with LRW-RSCS at 300 µg/mL (34 ± 5%, n=4, $p<0.05$). Similar results were obtained by stimulation with 0.1 µg/mL PMA (64 ± 1%, n=4), which was accompanied by a marked reduction in the degree of platelet aggregation in the presence of 300 µg/mL LRW-RSCS (38 ± 6%, n=4; $p<0.05$). Chronic treatment of 100 mg/kg/day of LRW-RSCS for 4 weeks significantly inhibited ADP (57 ± 3, n=4 to 40 ± 2%, n=4, $p<0.05$) and collagen-induced platelet aggregation (61 ± 2%, n=6, to and 28 ± 6%, n=6, $p<0.05$) when compared with the controls. **Conclusion**: The results obtained so far indicate that LRW-RSCS reduced platelet aggregation in rats and therefore suggests an additional beneficial effect induced by LRW-RSCS, in cardiovascular diseases prevention. **Financial support:** CNPq and CAPES

06.077 Action of PDE5 Inhibitors (Tadalafil) in the Treatment of Lower Urinary Tract Symptoms in Heart Failure Rats. Mora AG, Tartarotti SP, Andrade DR, Barbosa JWP, Gonçalves TT, Janussi SC, Claudino MA

Introduction: Chronic heart failure (CHF) affects 6.4 million people in Brazil and has become a challenge to public health policies. Studies have shown a strong association of CHF with the development of lower urinary tract symptoms (LUTS). Epidemiological data CHF patients showed that 34% of men and 62% of women have reported LUTS. Recent epidemiological studies have shown that Tadalafil treatment has been effective in reducing LUTS. Thus, the aim of study was evaluated the contractile mechanism of detrusor smooth muscle (DSM) in CHF rats and the contribution of chronic treatment with PDE5 inhibitors (Tadalafil). **Methods**: Heart failure (HF) was induced by surgical creation of an arteriovenous fistula in rats. After 8 weeks, the animals were divided into 4 groups: Sham and HF groups (which received 0.9% saline solution, daily), and Sham/TADA and HF/TADA groups (treated with Tadalafil 5mg/daily for 4 weeks). The HF validation was evaluated by transthoracic echocardiography. Cumulative concentration-response curves to muscarinic agonist, charbacol (CCh; 1 nM-100 μM) and chloride potassium (KCl; 1-300mM), and purinergic agonist, α-β-metil ATP (ATP; 10 μM) was obtained in DMS rats. Neurogenic contractile responses induced by electrical-field stimulation (EFS) were also obtained in all groups. Oxidative stress was evaluated by Thiobarbituric Acid activity (TBARS). **Results**: After 12 weeks, the left ventricular heart mass was higher (1.47 ± 0.4 g; $p<0.05$) and ejection fraction decreased in the HF group (64.5 ± 9.8% $p<0.05$), when compared to Sham group (1.05 ± 0.08 g; 80.2 ± 6.9%, respect.). Tadalafil treatment restored the cardiac function mediated by HF/TADA group (1.16 ± 0.09 g; 69.2 ± 7.5%, respect.). Cumulative addition of CCh and KCl produced concentration-dependent contractile responses in DSM rats. Both the potency (pEC_{50}) of CCh and KCl did not changed in any group, however, maximal response (E_{max}) of both, were significantly increased in HF group (1.56 ± 0.08 $mN.mg^{-1}$ and 2.44 ± 0.19 $mN.mg^{-1}$, respect.), compared with Sham group (0.89 ± 0.1 $mN.mg^{-1}$ and 1.63 ± 0.19 $mN.mg^{-1}$, respect). Tadalafil treatment restored the increase in the contractile mechanism in the HF/TADA group (1.0 ± 0.18 $mN.mg^{-1}$ and 1.74 ± 0.07 $mN.mg^{-1}$, respect.). Similarly, the ATP contractions was increased in the HF group ($p<0.05$) when compared to Sham group, whereas in the HF/TADA this effect was restored ($P<0.05$). Neurogenic contractile response induced by EFS was significantly increased in all frequencies (1-32Hz; $p <0.05$) in HF group, however, this increased was prevented in the HF/TADA group. The TBARS activity was increased in the HF group ($p<0.05$) compared to Sham group, but was restored in the HF/TADA group ($p<0.05$). **Conclusion**: Tadalafil chronic treatment was able to restore, both the cardiac function as the increased of contractile mechanism and oxidative stress in the DSM of rats with heart failure. **Financial Support**: FAPESP/CNPq. Approval: Ethics Committee for Experimental Research of the São Francisco University. Protocol Nº 001.06.11 **References**: Palmer M. H. Urinary incontinence and overactive bladder in patients with heart failure. J Urol. v. 182, 196, 2009.

06.078 Evaluation of the toxicological and renal effects caused by oncocalyxone isolated from *Auxemma oncocalyx* **Taub.** Nogueira Júnior FA[1], Costa LLM[1], Costa PHS[1], Silveira JAM[1], Alves NTQ[1], Silva PLB[1], Pessoa ODL[2], Evangelista JSAM[3], Alves RS[4], Monteiro HSA[1] [1]UFC – Physiology and Pharmacology, [2]UFC, [3]UECE –Veterinary, [4]UFC –Clinical and Toxicological Analysis

Introduction: *Auxemma oncocalyx* Taub, from Boraginaceae family, popularly known as "white wood", is a tree characteristic of the Brazilian northeast. Chemical investigation of the stem hydroalcoholic extract allows the isolation of several quinones, among which stands out the Oncocalixona A (Onco-A). Pharmacological studies for this quinone showed antiproliferative, antimitotic, analgesic, antiedematogenic, antiplatelet and antidiabetic. However, quinone can cause considerable toxic reactions resulting from its chemical property oxirredutora. The absence of in vivo toxicological data and the possibility of using this substance in anticancer therapy motivated the execution of this work. This study aimed to investigate the acute toxicity of Onco-A, and assess the effect of quinone in the perfusion of isolated kidney, possible target organ of toxicity. **Methods:** Swiss female mice were used, weighing between 20 and 25g, for testing the acute oral toxicity and male Wistar rats, weighing between 250-300g, for renal perfusion method. For evaluation of acute toxicity was followed by 425 guide OECD (2008), which consists in administering a single dose of orderly progression, in which the substance is administered to the animal, one at a time at a minimal interval of 48 hours. The body weight change was registered, plasma samples were collected for subsequent biochemical analysis and the kidney, heart and lung were removed for later histological analysis. For the analysis of kidney function, the kidneys of male Wistar rats were surgically excised and perfused with Krebs-Henseleit solution modified with 6% w/v of pre-dialysed bovine albumin. the effects of Onco-A have been investigated in three concentrations (1 g / ml, 3µg / ml and 10mg / ml). **Results**: In Acute Oral Toxicity test using the Up and Down method, Onco-A showed an LD50> 2000mg / kg, indicating that a substance of low lethality. Histological analysis of heart tissue, lung and kidney of animals submitted to the treatment after 14 days, indicated pronounced signs of toxicity caused by Onco-A in these organs. Biochemical parameters of urea, creatinine and uric acid showed no changes. The isolated kidney perfusion front Onco-A resulted in an increase in perfusion pressure (PP) in all three concentrations studied (1, 3 and 10 ug/ml) with a proportional increase in renal vascular resistance (RVR). The glomerular filtration rate (GFR) decreased and then reestablished itself in the group 1/ml, showed an increase in the group of 3 mg/mL, and the 10 mg/mL group remained low until the end of the experiments. The Urinary Flow (FU) increased in three concentrations. The percentage of the total tubular transport of sodium (% TNA) chloride (TCl-%) and potassium (TK +%) showed reductions in three concentrations. **Conclusion:** The results show that the Onco-A has low lethality in acute exposure, but showed pronounced effects on morphological changes indicative of tissue damage and changes in all kidney function parameters, indicating the toxic potential of quinone. **Financial support:** CNPQ, CAPES and FUNCAP. Keywords: Oncocalyxone A. Acute toxicity. Renal perfusion.

06.079 Evaluation of cardiovascular and renal parameters in a recovery model of hemorrhagic shock. Sordi R, Alves GF, Paiva NH, Vellosa JCR, Santos FA, Fernandes D, Gomes JR

Introduction: Hemorrhagic shock (HS) is a common cause of death in severely injured patients. It is associated with impairment of organ perfusion, systemic inflammatory response and multiple organ failure. However, there are few studies focused on the comprehension of cardiovascular and renal dysfunction that occurs over time in HS. Thus, the aim of the present study is to provide a better comprehension of the HS through temporal evaluation of cardiovascular and renal parameters using a recovery model of HS. **Methods**: Male Wistar rats (250g) were subjected to HS. Briefly, rats were anesthetized (sodium thiopental; 100 mg/kg; i.p.) and the femoral artery and vein were cannulated. The mean arterial pressure (MAP) was reduced to 30 mmHg (through removal of total blood) and maintained for 90 min, followed by resuscitation with shed blood. Four and 24 h later, basal MAP, heart rate (HR), renal blood flow (RBF) and vascular reactivity were evaluated. Blood was obtained for determination of urea and creatinine levels. Data were analyzed by one or two-way ANOVA followed by Dunnet's or Bonferroni post hoc test. **Results**: The induction of HS model in rats caused a ~70% reduction in RBF (at 90 min of hemorrhage: Sham = 398.2 ± 26.73 and HS = 125.6 ± 94.43 AU; $p<0.05$). Four hours after resuscitation, RBF was still lower in HS (269.3 ± 106.8 AU) when compared with Sham animals (424.9 ± 13.4 AU) $p<0.05$). Twenty-four hours later, the RBF of HS rats was partially recovered (339.1 ± 71.7 AU). Plasma levels of urea and creatinine of HS animals (4 and 24 h after surgery) were higher when compared to Sham, and we have found a moderate negative correlation ($-0.75 < r < -0.50$) between RBF and both urea and creatinine. When compared to Sham animals, the response to the vasoconstrictors phenylephrine (Phe) and angiotensin II (AII) was reduced in HS animals 4 and 24 h after surgery. However, basal MAP and HR, as well the reactivity to acetylcholine and sodium nitroprusside of HS rats were not different among the groups. **Conclusion**: Hemorrhagic shock causes an expressive reduction of RBF of rats during the model induction as well as after resuscitation. Interestingly, the RBF was negatively correlated with creatinine and urea levels (low RBF was associated with high creatinine and urea values). HS rats developed vascular hyporeactivity to vasoconstrictors 4 and 24 h after resuscitation, but the response to vasodilators was not affected. Taken together, HS model causes cardiovascular and renal dysfunction which persists for at least 24 h after resuscitation. Thus, the recovery HS model is an interesting model to investigate therapeutic agents to treat cardiovascular dysfunction associated with this condition. **Financial support**: CAPES, CNPq and Araucaria Foundation. This work was approved by Animal Research Ethical Committee from UEPG, protocol number 048/2015.

06.080 LASSBio-788 inhibits iNOS-induced NF-κB expression through enos dependent signaling in aortas of hypercholesterolemic rats. Motta NAV[1], Lima GF[1], Barreiro EJ[2], Kummerle AE[3], Brito FCF[1] [1]LAFE-UFF – Fisiologia e Farmacologia, [2]LASSBio-UFRJ, [3]UFRRJ – Química

Introduction: Atherosclerosis is a chronic inflammatory disease and is closely associated with inflammation, thrombogenesis and oxidative stress. The LASSBio-788 compound is a thienylacylhydrazone derivative with antiplatelet, vasodilatory and anti-atherogenic properties in an animal model of hypercholesterolemia, however the mechanisms involved in vasodilatory effects remain unknown. The objective of this work was the investigation of the vasodilatory mechanism underlying the effect of LASSBio-788. **Methods:** The animal protocols were approved by the Ethics Committee for Experimental Research of the Federal Fluminense University (CEPA/UFF 287/12). Adult male Wistar rats (150-200g) were randomly divided into four groups (n= 10, for each group): control group (C) and positive control (C+788) fed standard chow diet, hypercholesterolemic diet group (HC) and diet group + compound LASSBio-788 (HC+788) fed a hypercholesterolemic diet. At 31º diet day, was performed the chronic treatment with LASSBio-788 once daily, totalizing 15 days of treatment. The animals were euthanized by cervical dislocation and decapitated under anesthesia. Blood samples were collected, the thoracic aorta were excised for vascular reactivity, ELISA and western blot analysis. Data were analyzed using one-way ANOVA followed by a post-hoc Bonferroni Multiple Comparison Test, $p<0.05^{*}$. **Results:** The hypercholesterolemic diet increased serum lipids levels and reduces HDL levels in the HC group ($p\leq 0.05$). The HC group showed an increase of blood pressure (systolic 170.40 ± 4.70 x 130.80 ± 4.80 mm/Hg), besides increased the lipid peroxidation in the aortas (63.41 ± 5.70 x 33.20 ± 0.50 nmol/mg protein) of HC group when compared to C group. The HC group showed an increase of TNF-α (74.22 ± 0.38 x 61.62 ± 0.58 pg/mg), TXA_2 (400.30 ± 3.83 x 345.50 ± 7.00 pg/mg) and a decrease of cyclic nucleotide levels in aortas (cAMP: 84.0 ± 1.23 x 102.70 ± 0.64; cGMP: 21.95 ± 1.32 x 63.47 ± 1.14 pmol/mg protein). The hypercholesterolemic diet promoted an increase of VCAM-1 protein expression. Hypercolesterolemic diet increased PKC-α, NF-kB, iNOS and inhibited eNOS, IκB-α, PKA and PKG protein expression. The chronic treatment with LASSBio-788 (100µmol/kg) reduced serum lipids and increased HDL levels ($p\leq 0.05$). LASSBio-788 reduced the blood pressure induced by hypercholesterolemic diet (systolic 118.60 ± 1.30 mm/Hg). LASSBio-788 also inhibited TNF-α (58.32 ± 1.1pg/mg), TXA_2 (354.60 ± 6.14 pg/mg) and cyclic nucleotide levels in aortas (cAMP: 102.40 ± 1.97, GMPc: 115.10 ± 1.48 pmol/mg protein) when compared to HC group. LASSBio-788 inhibited VCAM-1, PKC-α, NF-kB and iNOS protein expression. LASSBio-788 also increased the eNOS, IκB-α and PKG protein expression in aortas. **Conclusion** - The vasodilatory mechanism underlying the effect of LASSBio-788 is associated to activation of eNOS/NO/GC/cGMP/PKG pathway, resulting in inhibition of TXA_2/PKC-α/NF-kB/iNOS pathway. Our results indicate the LASSBio-788 compound as a potential drug for the treatment of cardiovascular disorders such as atherosclerosis. **Financial support:** CNPq, CAPES, PROPPI-UFF, FAPERJ. CEPA/UFF 287/12

06.081 FPR2/ALX receptor activation is beneficial in pneumonia-induced sepsis. Horewicz VV, Assreuy J UFSC – Farmacologia

Introduction: Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Sepsis-induced myocardial dysfunction is common and directly related to the sepsis severity. Mechanisms of sepsis-induced cardiac dysfunction include attenuation of the cardiomyocyte adrenergic response, alterations of intracellular calcium trafficking and blunted calcium sensitivity of contractile proteins. Notwithstanding the involvement of other mediators, cytokines and nitric oxide (NO) play a role in these mechanisms. The formyl peptide receptor 2 (FPR2/ALX) belongs to the G protein-coupled receptors family. Its activation result in powerful pro- or anti-inflammatory responses. We have shown that FPR2/ALX stimulation with a peptide agonist (WKYMVm) decreases lipopolysaccharide-induced NO production, substantially reduces vascular dysfunction and improves the survival of mice bearing pneumosepsis. Thus, the aim of this study was to investigate in greater detail the role of FPR2/ALX activation in the cardiovascular dysfunction caused by pneumonia-induced sepsis. **Methods**: *Klebsiella pneumoniae* was inoculated intratracheally in anesthetized Swiss male mice. Animals were treated with WKYMVm (4 mg/kg; s.c.) or vehicle (PBS) 2 h and 14 h after infection. Organ damage markers, local (heart) and systemic inflammatory parameters, and cardiac proteins expression were evaluated 6 h and/or 24 h after bacteria inoculation. **Results**: Pneumosepsis resulted in renal, liver and cardiac dysfunction, all of which was prevented by the FPR2/ALX stimulation. FPR2/ALX and nitric oxide synthase-2 (NOS-2) protein expression was increased in septic animals whereas levels of NOS-1 and NOS-3 were unchanged. The NOS-2 expression coincided with increased levels of NO metabolites in the plasma. Activation of FPR2/ALX carried out by WKYMVm decreased sepsis-induced NO production and prevented vascular hyporeactivity in aorta rings. Sepsis increased plasma levels of TNF, IL-6 and MCP-1. Interestingly, high IL-6 levels were detected in heart homogenates of septic animals. WKYMVm inhibited IL-6 production in the heart as well as decreased its plasma levels. Finally, FPR2/ALX-mediated IL-6 reduction in the septic heart was independent of nuclear factor kappa-B activation. **Conclusion**: These data demonstrate that exogenous activation of FPR2/ALX receptor plays a major role in dampening dysfunction of distinct organs during pneumonia-induced sepsis. Regarding septic cardiomyopathy, FPR2/ALX activation seems to be beneficial for the heart preserving its contractile function both by reducing the local inflammatory response and via a mechanism involving the reduced bioavailability of NO due to superoxide anion formation. **Financial support**: CNPq; CAPES; FINEP. All procedures were approved by the institutional Animal Ethics Committee (CEUA PP0790).

06.082 A new look into hypertension: A1 adenosine receptor function is potentiated in the right atrium of spontaneous hypertensive rats. Rodrigues JQD, Camara H, Jurkiewicz A, Godinho RO Unifesp-EPM – Farmacologia, [2]Unicamp

Introduction: Classically primary hypertension is associated with sympathetic and parasympathetic neurotransmission dysfunction. ATP works as a co-transmitter of autonomic neurotransmitters noradrenalin and acetylcholine. Once released, ATP is converted into adenosine in the synaptic cleft by ectonucleotidases. Although extracellular adenosine is able to modulate the cardiac inotropism and chronotropism through activation of G protein coupled receptors, it is not clear which adenosine receptor (AR) subtypes (A_1, A_{2A}, A_{2B} and/or A_3) are involved in the regulation of cardiac function of hypertensive rats. Aims: In the present study, we investigated the subtypes of adenosine receptor and signaling pathways involved in the chronotropic effects of adenosine in the right atrium of normotensive Wistar rats (NWR) and spontaneously hypertensive rats (SHR). **Methods:** The right atrium from 5-month-old male NWR and SHR, which contains the primary pacemaker of the heart (the sinoatrial node), was mounted in an organ bath and used to investigate the cardiac chronotropism. After 60 min stabilization, cumulative concentration-response curves (CCE) for adenosine (a non-specific agonist of AR) and for CPA (selective agonist of A_1AR) were constructed in the absence and presence of DPCPX (a selective antagonist of A_1AR), MRS1523 and ZM241385 (antagonists of A_2AR and A_3AR, respectively). Also, we tested the influence of pertussis toxin, which inactivates Gi protein subunit α, on the adenosine chronotropic effect. The pharmacological parameters pD_2, pA_2 and E_{max} were measured and analyzed by unpaired t test and one-way ANOVA (Bonferroni post-hoc; $p<0.05$). **Results:** In the right atrium, adenosine and CPA decreased the chronotropism in a concentration-dependent manner, culminating in cardiac arrest (0 bpm) in NWR and SHR at 1mM and 300 µM adenosine and 300 nM and 100 nM CPA, respectively. Adenosine was 3-fold more potent in SHR right atrium when compared to NWR (NWR pD_2=3.9 ± 0.10, n=16). Similarly, potency of CPA was 2.8-fold higher in SHR (NWR pD_2=7.5 ± 0.1, n=18). In atria of SHR and NWR, the chronotropic effects of adenosine were not modified by A_2 and A_3 adenosine receptor antagonists. On the other hand, DPCPX shifted the CPA CCE to the right in both strains, with a Hill slope equal to unity, indicating that A_1AR is the AR subtype involved in these effects. We also investigated if differential effects of adenosine and CPA on SHR and NWR atria were due to changes in Gi protein signaling pathways. Pre-incubation of the NWR right atrium with pertussis toxin, which inactivates Gαi subunit reduced by 20% and 40% the negative chronotropic effects of adenosine and CPA, respectively. This inhibitory effect of PTX was not observed in SHR atrium, suggesting that there is a dysfunction in the Gi protein signaling pathway in SHR right atrium. **Conclusion:** Our results show that the negative chronotropic response of adenosine is enhanced on the right atrium of SHR, probably due to an increase in signal pathway of Gi protein. **Financial support:** (FAPESP, Capes and CNPq).Ethics Committee of UNIFESP (n° 6269070414).

06.083 G-protein coupled estrogen receptor activation reduces cardiac, vascular and skeletal muscle dysfunction in female rats with pulmonary hypertension. Alencar AKN[1], Gabriel G[1], Silva A[1], Montes GC[1], Martinez ST[2], Fraga A[3], Wang H[4], Groban L[4], Sudo RT[1], Zapata-Sudo G[1] [1]UFRJ – Farmacologia e Química Medicinal, [2]UFRJ – Química, [3]UFRJ – Ciências Farmacêuticas, [4]Wake Forest University

Introduction: Pulmonary hypertension (PH) is primarily a disease of women (female-to-male ratio 4:1), and is associated with cardiac and skeletal muscle dysfunction. **Aim:** Herein, the activation of a new estrogen (E_2) receptor (GPER) by its agonist G1 was evaluated in monocrotaline (MCT)-induced PH female rats. **Methods:** Depletion of E_2 was induced by bilateral oophorectomy (OVX; n = 18) in female Wistar rats (12 weeks old). Experimental groups included SHAM or OVX rats that received a single intraperitoneal injection of MCT (60 mg/kg) for PH induction followed by administration of vehicle or G1 (400 µg/kg/day s.c.) for 14 days after the onset of disease (n=7 per group). Cardiovascular parameters were determined by transthoracic echocardiography. The effects of G1 in the maintenance of exercise capacity and skeletal muscle function were investigated using a treadmill test to observe the time to exhaustion (TE) and evaluating the contractile properties of electrically stimulated soleus (SOL) muscle. **Results:** MCT injection and E_2 loss (interaction effect; $P < 0.05$) led to a significant decrease in pulmonary artery acceleration time (PAAT; ms) [(33.2 ± 1.2; SHAM + MCT *vs.* 45.0 ± 0.8; SHAM; $P < 0.05$); (20.7 ± 0.6; OVX + MCT *vs.* 44.3 ± 1.3; OVX; $P < 0.05$)] and an increase in RV free wall thickness (mm) [(1.0 ± 0.03; SHAM + MCT *vs.* 0.5 ± 0.03; SHAM; $P < 0.05$); (1.8 ± 0.09; OVX + MCT *vs.* 0.5 ± 0.02; OVX; $P < 0.05$)] in both SHAM and OVX groups. Treatment with G1 attenuated those PH-related changes in PAAT [(43.2 ± 0.7; SHAM + MCT + G1 *vs.* SHAM + MCT; $P < 0.05$); (38.1 ± 1.0; OVX + MCT + G1 *vs.* OVX + MCT; $P < 0.05$)] and reduced RV wall hypertrophy [(0.6 ± 0.03 mm; SHAM + MCT + G1 *vs.* SHAM + MCT; $P < 0.05$); (0.7 ± 0.03 mm; OVX + MCT + G1 *vs.* OVX + MCT; $P < 0.05$)]. RV systolic pressure (RVSP) was increased in MCT-injected rats and the magnitude of this increase in OVX + MCT rats (60.2 ± 4.9 mmHg *vs.* 33.1 ± 0.8 mmHg; OVX; $P < 0.05$) was significantly higher (interaction between E_2 and MCT effects; $P < 0.05$) than that in SHAM + MCT group (46.9 ± 3.9 mmHg *vs*. 30,7 ± 2.3 mmHg; SHAM; $P < 0.05$). G1 significantly normalized RVSP in both SHAM and OVX PH rats [(29.8 ± 1.3 mmHg; SHAM + MCT + G1; *vs.* SHAM + MCT; $P < 0.05$); (29.1 ± 1.9 mmHg; OVX + MCT + G1 *vs.* OVX + MCT; $P < 0.05$)]. Expression of calcium handling proteins as SERCA2a and phospholambam was altered in cardiomyocytes from MCT- injected rats and G1 treatment normalized it. Reductions of TE (min) were greater in OVX group (13.4 ± 3.3; OVX + MCT *vs*. 16.7 ± 1.7; OVX group; $P < 0.05$) than that in SHAM animals (17.0 ± 2.6; SHAM + MCT *vs.* 36.7 ± 1.0; SHAM; $P < 0.05$). This reduction in exercise performance was further related to a lower amplitude of twitches of electrically stimulated SOL muscles from both MCT-challenged SHAM and OVX rats. PH female rats treated with G1 exhibited exercise tolerance times and muscle strength equivalent to their corresponding controls ($P < 0.05$). **Conclusion:** G1 reversed PH-related cardiopulmonary dysfunction and exercise intolerance in female rats with and without endogenous E_2, a finding that may have important implications for the ongoing clinical evaluation of new drugs for the treatment of the disease in women.

06.084 Sodium nitrite antihypertensive effects in renovascular hypertensive rats are independent of oral bacteria Pinheiro LC, Ferreira GC, Amaral JH, Passo MA, Portela RL, Tanus-Santos JE [1]FMRP-USP – Farmacologia

Introduction: Nitrate and nitrite exert antihypertensive effects in humans and many animal models. Several works suggest that the nitrate-nitrite-NO cycle is central to the effects of nitrate and nitrite. However, the role of oral bacteria in the chronic antihypertensive effects of sodium nitrite remains to be proved. **Objective**: This study aimed at examining whether suppression of oral microflora with antiseptic mouthwash prevents the chronic antihypertensive effects of sodium nitrite in renovascular hypertension. **Methods:** Renovascular (two kidney, one clip; 2K1C) hypertensive and sham operated control rats were treated with sodium nitrite (15 mg/Kg by gavage, daily) or saline for four weeks and treated with chlorhexidine-based antiseptic mouthwash or vehicle (once a day). Systolic BP (SBP) was measured weekly by tail plethysmography. Plasma nitrite and S-nitrosothiols concentrations were measured by chemiluminescence NO detection. The total of nitrosylated proteins are measured by SNO-RAC, followed by protein quantification. The number of bacteria in oral saliva was assessed with basis on the number of growing colonies counted on a Petri dish. The results were analyzed by two-way ANOVA. The results are shown as mean ± standard error of the mean. **Results:** After two weeks of surgery, 2K1C rats were hypertensive (SBP=173 ± 10 versus 125 ± 4 mmHg in controls, $P<0.05$). In the first week of treatment, sodium nitrite decreased blood pressure independently of treatment with mouthwash. This decrease in blood pressure persisted throughout the four weeks of nitrite treatment compared to vehicle treatment (SBP=147 ± 15 mmHg in 2K1C+nitrite and 149 ± 11 mmHg in 2K1C+nitrite+mouthwash *versus* 186 ± 9 mmHg in 2K1C+saline, $P<0.05$) Mouthwash or nitrite alone had no significant effects on blood pressure in sham operated rats. Higher plasma nitrite concentrations were found in hypertensive rats treated with nitrite (1.7 ± 0.3 μM in 2K1C+nitrite *versus* 0.7 ± 0.07 μM in 2K1C+saline, $P<0.05$). Mouthwash treatment significantly decreased nitrite levels in vehicle groups ($P<0.05$) and slightly decreased nitrite levels in nitrite treated groups. Treatment with nitrite increased S-nitrosothiols plasmatic concentrations and total nitrosylated proteins in aorta, independently of mouthwash treatment (RSNO=7.5 ± 1.1 nM in 2K1C+nitrite and 10.2 ± 2.9 nM in 2K1C+nitrite+mouthwash *versus* 4.4 ± 1 nM in 2K1C+saline, $P<0.05$). Mouthwash decreased the number of oral bacteria by 70%. **Conclusion:** Nitrite decreases blood pressure in 2K1C hypertension independently of the nitrate-nitrite-NO cycle. The anti-hypertensive effect could be explained, in part, by formation of S-nitrosothiols and nitrosylation of proteins. **Financial Support**. FAPESP, CNPq e CAPES **Research approval:** Protocol # 42/2012

06.085 A novel role of LASSBio-788 in inhibiting NF-KB mediated signaling in platelet of hypercholesterolemic rats. Motta NAV, Lima GF, Oliveira AFR, Barreiro EJ, kummerle AE, Brito FCF LAFE-UFF – Fisiologia e Farmacologia

Introduction: Atherosclerosis is described as a chronic process closely related to inflammatory and proliferative responses of the endothelium after injury. Circulating platelets become hyperactive under hypercholesterolemic conditions, leading to the development and progression of atherosclerosis. LASSBio-788 is a thienylacylhydrazone derivative with anti-atherogenic properties in an animal model of hypercholesterolemia. However, the molecular mechanism underlying its antiplatelet effect remains unclear. The aim of this study was to investigate the signaling pathway involved in the antiplatelet effects of LASSBio-788. **Methods:** The animal protocols were approved by the Ethics Committee for Experimental Research of the Federal Fluminense University (CEPA/UFF 287/12). Adult male Wistar rats (150-200g) were randomly divided into four groups (n= 10, for each group): control group (C) and positive control (C+788) fed standard chow diet, hypercholesterolemic diet group (HC) and diet group + compound LASSBio-788 (HC+788) fed a hypercholesterolemic diet. At 31º diet day, was performed the chronic treatment with LASSBio-788 once daily, totalizing 15 days of treatment. The animals were euthanized by cervical dislocation and decapitated under anesthesia. Blood samples were collected from each animal and the platelets were isolated to biochemical and molecular analysis. Data were analyzed using one-way ANOVA followed by a post-hoc Bonferroni Multiple Comparison Test, $p<0.05$*. **Results:** The hypercholesterolemic diet increased serum lipids levels and reduces HDL levels in the HC group. LASSBio-788 reduced serum lipids and increased HDL levels ($p\leq 0.05$). The HC group showed an increase of malondialdehyde levels in platelets (63.90 ± 5.50 nmol/mg protein) when compared with C group (17.40 ± 1.60 nmol/mg protein). Furthermore, the hypercholesterolemic diet increased TXA_2 (HC: 360.20 ± 9.18 x C: 259.30 ± 20.44 pg/1.2 x 10^8 platelets) and a decrease cAMP (HC: 85.05 ± 3.19 x C: 95.43 ± 1.49 pmol/1.2 x 10^8) ; cGMP (HC: 20.34 ± 3.41 x C: 120.00 ± 9.73 pmol/1.2 x 10^8) levels. LASSBio-788 inhibited these effects promoted by diet: TXA_2 (294.50 ± 3.27 pg/1.2 x 10^8 platelets); cAMP (103.50 ± 1.51 x 10^8 platelets); cGMP (94.56 ± 4.42 pmol/1.2 x 10^8 platelets). NF-kB signaling events, including PKC-α, iNOS, P-selectin and CD40L increased protein expression, IκB-α degradation, eNOS, PKA and PKG decreased protein expression were observed in hypercholesterolemic rats. These signaling events were attenuated by chronic treatment with LASSBio-788 (100µmol/kg) $p<0.05$. **Conclusion:** The most important findings of this study demonstrate for the first time that LASSBio-788 possesses potent antiplatelet activity, which may involve activation of the eNOS/NO/GC/cGMP/PKG pathway, resulting in inhibition of the NF-κB/PLC/PKC/TXA_2 cascade, and, finally, inhibition of platelet aggregation. Our results indicate the LASSBio-788 compound as a potential drug for the treatment of cardiovascular disorders such as atherosclerosis. **Financial support:** CNPq, CAPES, PROPPI-UFF, FAPERJ. CEPA/UFF 287/12

06.086 Cilostazol exerts antiplatelet and anti-inflammatory effects through AMPK activation and NF-κB inhibition on hypercholesterolaemic rats. Motta NAV, Lima GF, Brito FCF UFF – Fisiologia e Farmacologia

Introduction - Cilostazol is a PDE3 (phosphodiesterase 3) inhibitor, approved for the treatment of intermittent claudication and has shown promising results in the treatment of cardiovascular disorders, because it presents anti-platelet, vasodilatory and anti-inflammatory properties. Some authors have shown that cilostazol inhibits the cytokine-induced expression of various pro-inflammatory and adhesion molecule genes through cAMP/PKA independent pathway. The present study was performed to evaluate the spectrum of molecular mechanisms by which a long-term oral administration of cilostazol, in rats fed a high cholesterol diet, acts through platelets signaling pathway to avoid atherosclerosis early development. **Methods** - The animal protocols were approved by the Ethics Committee for Experimental Research of the Federal Fluminense University (CEPA/UFF 287/12). Adult male Wistar rats (150-200g) were randomly divided into three groups (n= 10, for each group): control group (C) fed standard chow diet, hypercholesterolemic diet group (HCD) and diet group + cilostazol (HCD+CIL) fed a hypercholesterolemic diet. At 31º diet day, was performed the chronic treatment with cilostazol (30 mg/kg/p.o) once daily, totalizing 15 days of treatment. The animals were euthanized by cervical dislocation and decapitated under anesthesia. Blood samples were collected for ELISA, platelet aggregation and western blot analysis. Data were analyzed using one-way ANOVA followed by a post-hoc Bonferroni Multiple Comparison Test, $p<0.05$. **Results** - Cilostazol reduced the levels of total cholesterol (361.0 ± 12.8 vs. 111.5 ± 1.6 mg/dl), triglycerides (186.9 ± 17.7 vs. 55.4 ± 3.1 mg/dl), cLDL (330.9 ± 9.7 vs. 61.5 ± 3.5 mg/dl), cVLDL (45.0 ± 4.6 vs. 11.1 ± 0.6 mg/dl) and malondialdehyde (9.4 ± 0.5 vs. 3.2 ± 0.3 nmol/ml) compared to the HCD group. The platelet aggregation induced by ADP was increased in the HCD group (EC_{50} = 0.40 ± 0.05 µM) compared to C group (EC_{50} = 0.09 ± 0.02 µM). Cilostazol decreased the response to ADP with an EC_{50} of 0.42 ± 0.06 µM. The HCD group showed a significant increase of inflammatory cytokines levels. However, chronic treatment with cilostazol promoted a decreasing of these levels, when compared with the HCD group ($P<0.05$). The cyclic nucleotides levels were decreased to 21% for cAMP ($P<0.05$) and 83% for cGMP ($P<0.001$) in HCD when compared with C group. Cilostazol was able to restores cAMP and GMP levels when compared to HCD group. Western blot analysis revealed that HCD group showed an increase of PKC-a (1.2 fold), pNF-kB/NF-kB (1.6 fold), iNOS (1.7 fold), P-selectin (1.4 fold) and CD40L (1.6 fold) protein expression. In addition, HCD group decreased AMPK and eNOS activity in the platelets when compared to C group. These signaling pathways were attenuated by chronic treatment with cilostazol (30mg/kg) $p<0.05$. **Conclusion** - Cilostazol presented antiplatelet properties and decreased inflammatory markers levels. These effects seem to be related to AMPK and eNOS activation, resulting in inhibition of NF-kB pathway. **Financial support** – CNPq, CAPES, PROPPI-UFF, FAPERJ. CEPA/UFF 287/12

06.087 Glycosylation with N-acetylglucosamine in lymphomononuclear cells of type 2 diabetic patients undergoing caloric restriction and a hypoproteic diet Rassi DM[1], Zanotto C[1], Conceição R[2], Mestriner F[1], Barreto PA[1], Donadi EA[2], Foss-Freitas MC[2], Tostes RC[1]
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Introduction: O linked β-N-acetylglucosamine (O-GlcNAc) is an abundant, inducible and reversible post-translational modification that modulates several signaling pathways in response to nutrients and cellular stress. O-GlcNAc plays an important role in the development of insulin resistance and glucose toxicity and has been proposed as a potential marker of early metabolic dysfunction. Virtually, all metabolic pathways influence the cellular concentrations of UDP-GlcNAc, the substrate for protein modification with O-GlcNAc. Considering that 1) type II diabetes (T2DM), a metabolic disorder characterized by hyperglycemia and insulin resistance, affects the metabolism of carbohydrates, lipids and proteins; 2) several nutritional interventions, including caloric restriction, improve diabetes risk indicators, our objective is to evaluate how caloric restriction of 25% and a hypoproteic diet will affect biochemical and metabolic parameters, as well as levels of O-GlcNAc-modified proteins in lymphomononuclear cells and markers of cardiovascular diseases in T2DM patients. **Methodology*:*** The project is in its initial phase. 20 diabetic patients will be recruited and divided into 2 groups: patients undergoing caloric restriction and patients on a low protein-diet. Patients follow a prescribed diet for 15 days in their residence and, after this period, are admitted to the hospital (Hospital das Clínicas da Faculdade de Medicina de Ribeirao Preto) for 4 weeks to continue the diet protocols. Peripheral blood is collected when patients are admitted and after 7, 15, 21 and 30 days to evaluate glucose, lipid profile (cholesterol, triglycerides, LDL), creatine phosphokinase (CPK), sVCAM, ICAM-1, RECAM and VEGF. Lymphomononuclear cells (PBMC) are collected for protein extraction and evaluation of glycosylation by western blot. Body mass index (BMI) is also evaluated before and after the treatment. **Results**: To assess whether lymphomononuclear cells (PBMC) are good reporters for the study of glycosylation, cells were isolated from two healthy individuals and cultured with Thiamet-G, a potent inducer of glycosylation. Thiamet-G increased glycosylation in PBMC, which was not observed in vehicle-treated cells (110,8 ± 11,09, vehicle, *vs*. 225,9 ± 38,87 Thiamet-G). To date, two patients on the hypoproteic diet were recruited. The hypoproteic diet produced a significant decrease in blood glucose and creatine phosphokinase. Protocols to determine O-GlcNAc levels at the different time points are being performed, but preliminary data show decreased O-GlcNAc levels upon the hypoproteic diet. **Conclusion:** Short-time treatment with a hypoproteic diet alters metabolic parameters in diabetic patients and affects levels of O-GlcNAc-modified proteins in PBMC. The extent of protein modification by *O*-GlcNAcylation shows potential as a tool for the diagnosis of metabolic changes in T2DM patients. Modulatory effects of O-GlcNAc on PBMC function are implied. **Financial support:** CAPES and FAPESP. Ethics Committee Protocol # 50376415.0.0000.5440.

06.088 Cilostazol exerts vasodilatory and anti-inflammatory effects through cAMP independent signaling pathway on hypercholesterolemic rats. Motta NAV, Lopes RO, Oliveira AFR, Jappour LA, Brito FCF LAFE-UFF – Fisiologia e Farmacologia

Introduction: Atherosclerosis is one of the most important pathogenic mechanisms involved in ischemic stroke development. The central events of atherosclerosis include endothelial dysfunction and inflammation. Cilostazol is a PDE3 (phosphodiesterase 3) inhibitor that increases intracellular cyclic AMP levels and decreases intracellular calcium levels, inhibiting platelet aggregation and inducing vasodilatation. The molecular mechanism involved in vascular effects of cilostazol has not yet been established. In the present study we investigated whether the effects of cilostazol is associated with suppression of p38 mitogen-activated protein kinases (MAPK) and nuclear factor kappa B (NF-kB) signaling pathways in rats fed a high cholesterol diet. **Methods**: The animal protocols were approved by the Ethics Committee for Experimental Research of the Federal Fluminense University (CEPA/UFF 287/12). Adult male Wistar rats (150-200g) were randomly divided into three groups (n= 10, for each group): control group (C) fed standard chow diet, hypercholesterolemic diet group (HCD) and diet group + cilostazol (HCD+CIL) fed a hypercholesterolemic diet. At 31º diet day, was performed the chronic treatment with cilostazol (30 mg/kg/p.o) once daily, totalizing 15 days of treatment. The animals were euthanized by cervical dislocation and decapitated under anesthesia. Blood samples were collected for ELISA and biochemical analysis. Aortas were dissected for vascular reactivity and western blot analysis. Data were analyzed using one-way ANOVA followed by a post-hoc Bonferroni Multiple Comparison Test, $p<0.05$. **Results**: The hypercholesterolemic diet increased serum lipids levels ($P<0.05$ x C group), TNF-α (74.1 ± 2.0 x 62.3 ± 3.2 pg/mg protein^{-1}), TXA_2 (398.9 ± 3.3 x 347.0 ± 5.0 pg/mg protein^{-1}) and a decrease HDL serum levels, cyclic nucleotide levels in aortas (cAMP: 84.0 ± 1.2 x 102.7 ± 1.6; cGMP: 22.0 ± 1.0 x 63.2 ± 1.0 pmol/mg protein). However, chronic treatment with cilostazol promoted a decreasing of these levels, when compared with the HCD group ($P<0.05$). The HCD group showed a significant increase of contraction response to phenylephrine (CE_{50}: 8.2 x 10^{-8}M x 3.3 x 10^{-7} M) and a decrease of the endothelium-dependent relaxation induced by acethylcholine (CE_{50}:83.4 ± 1.4 x 94.7 ± 2.2 %). On the other hand, the chronic treatment with cilostazol produced a significant decrease of contraction response (CE_{50}: 1.0 x 10^{-6}) and increase of the maximal responses to acetylcholine (102.2 ± 2.2%), compared with HCD group. The HCD group also showed an increase of PLC-γ, PKC-α, NF-kB, p38, VCAM-1, iNOS and inhibited eNOS, IκB-α, PKA and PKG protein expression. These signaling pathways were attenuated by chronic treatment with cilostazol (30mg/kg) $p<0.05$. **Conclusion**: This study demonstrate for the first time that vasodilatory and anti-inflammatory effects of cilostazol may involve a cAMP independent signaling pathway through activation of the eNOS/NO/cGMP/PKG pathway, followed by inhibition of the PLCγ-PKC-p38 MAPK-TXA_2 cascade. Hypercholesterolemic patients usually are associated with a high incidence of atherosclerosis and thrombotic complications. This study provides a new insight of vascular mechanisms of cilostazol to explain its clinical protective effect in coronary artery diseases. **Financial support** – CNPq, CAPES, PROPPI-UFF, FAPERJ.

06.089 The Adipokine Soluble Dipeptidyl Peptidase-4 Induces Endothelial Dysfunction Via Proteinase-Activated Receptor 2. Peiro C[1], Romacho T[2], VallejoS[1], Villalobos LA[1], Wronkowitz N[2], Indrakusuma I[2], Sell H[2], Eckel J[2], Sanchez-Ferrer CF[1] [1]Universidad Autónoma de Madrid – Pharmacology, [2]German Diabetes Center – Integrative Physiology

Introduction: Vascular complications are the main cause of morbidity and mortality in patients suffering from type 2 diabetes. Dipeptidyl peptidase-4 (DPP4) is a key protein in glucose homeostasis and a pharmacological target in type 2 diabetes mellitus. The soluble form of DPP4 (sDPP4) has been recently identified as an adipokine, exhibiting high circulating levels in the context of metabolic diseases. This study explored whether sDPP4 can directly impair vascular reactivity, as an early marker of endothelial dysfunction. **Methods:** Microvascular reactivity was studied in mesenteric arteries from 3-month-old female mice, using a small vessel myograph. Thromboxane A_2 (TXA_2) release was explored in cultured human coronary artery endothelial cells by enzyme immunoassay. **Results:** Neither the contractility to noradrenaline (1 nM-3 µM) nor the endothelium-independent relaxations induced by sodium nitroprusside (1 nM-100 µM) in NA-precontracted vessels were modified by sDPP4 (20-500 ng/ml). However, sDPP4 impaired in a concentration-dependent manner the endothelium-dependent relaxation elicited by acetylcholine (1 nM-10 µM). The DPP4 inhibitors K579 (100 nM) and linagliptin (1-100 nM) prevented the defective relaxation induced by sDPP4. In the presence of the GLP1 receptor antagonist exendin-(9–39) (1 µM), linagliptin could still prevent the negative effect of sDPP4 on vasodilation, which suggested an incretin-independent action of linagliptin. Similarly to the DPP4 inhibitors, GB83 (10 µM), an inhibitor of membrane protease-activated receptor 2 (PAR2) prevented the deleterious action of sDPP4. Downstream of PAR2, the cyclooxygenase (COX) inhibitor indomethacin (10 µM), the COX2 inhibitor celecoxib (3 µM) or the thromboxane receptors blocker SQ29548 (100 nM) prevented the deleterious effects of sDPP4. Indeed, in cultured human endothelial cells sDPP4 triggered the release of TXA_2 by endothelial cells, which was prevented by pharmacologically inhibiting DPP4, PAR2 or COX. **Conclusion:** In summary, these findings identify sDPP4 as a direct mediator of endothelial dysfunction, acting through PAR2 activation and promoting the release of vasoconstrictor prostanoids. By interfering with these actions, DPP4 inhibitors might help preserving endothelial function in the context of cardiometabolic diseases exhibiting high levels of sDPP4. Supported by grants from Ministerio de Economía y Competitividad (SAF-2014-52762R and Spanish-German Concerted Action PRI-AIBD-2011-0811)

06.090 BK channel activation in chronic vasodilation by thiazide-like diuretics: role of the beta-1 auxiliary subunit.. Martín P[1], Ernique N[1], Rebolledo A[1], Asuaje A[1], Milesi V[1] [1]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP CONICET-UNLP), Argentina

Introduction: Arterial hypertension is one of the major risk factors for cardiovascular disease. Treatment with thiazide-like diuretics is based on the decrease in blood volume and direct vasodilation. The goal of this study was to determine the vasodilator mechanism of the diuretic hydrochlorothiazide (HCTZ), whose molecular target is still unknown. Previous studies indicated that activation of the large-conductance Ca^{2+}- and voltage-operated K^+ channel (BK) may be involved in this effect. This channel is formed by 4 alpha-subunits that may (or may not) be accompanied by accessory subunits, depending on the cell type, that confer specific pharmacological characteristics to the channel. In particular, the accessory beta-1 subunit is mainly expressed in smooth muscle cells (SMCs). **Methods**: Using the patch-clamp technique we analyzed the effect of HCTZ on BK channel activity expressed (with and without the beta-1 subunit) in HEK293 T (an heterologous expression system) and in SMCs from human umbilical artery (HUASMCs) as native system. **Results**: Using the whole-cell configuration in HEK cells transfected with the BK channel accompanied by beta-1 subunit we observed that addition of HCTZ to the extracellular solution increased the current amplitude in a concentration-dependent manner, with an EC_{50} of 28.4 µM (pD_2=4.546 ± 0.211, n: 5-8). However, HCTZ did not change the channel activity when it was evaluated in the same cells in the inside-out configuration, where cell integrity is lost (% increase in current at + 90mV: 6.3 ± 15.0, 12.8 ± 38.1, - 17.8 ± 12.7% to HCTZ 10, 30 and 100 µM, respectively, NS; n: 3-7), suggesting an indirect action of the diuretic in channel activation. Furthermore, we checked the effect of HCTZ in whole-cell currents in HEK cells transfected with the BK channel without the beta-1 subunit. In this condition HCTZ did not change the BK current (% increase in current at +40 mV: 13.9 ± 15.7 and 6.9 ± 5.1% to HCTZ 100 and 300 µM, respectively, NS; n: 3-4). Interestingly, these results indicate that, despite being an indirect activation, HCTZ requires the presence of beta-1 subunit to stimulate the BK channel. Finally, these experimental results were repeated in HUASMCs, where the channel is naturally expressed with the beta-1 subunit and is further modified by post-transcriptional regulation. Consistent with the results of the heterologous system, the extracellular application of 10 µM HCTZ caused significant activation of BK current recorded in whole-cell configuration (528 ± 215 to 1379 ± 132 pA at +40mV, n: 4, $p < 0.05$) while, when the diuretic was applied in the inside-out configuration, it did not produce any changes in BK channel unitary conductance and open probability (NPo at + 40mV: 0.0114 ± 0.0015 (control) vs 0.0135 ± 0.0037 (HCTZ), n: 4, $P > 0.05$). **Conclusion**: These results suggest that the vasodilatory effects of HCTZ could be due to an indirect activation of the BK channel which depends of the beta-1 subunit expression. **Financial support**: PICT-2014-0603, ANPCYT, Argentina. The use of HUA was approved by the ethics and research committee of the Hospital General Zonal de Agudos San Roque / Gonnet, and the written informed consent of each subject were obtained.

06.091 Vascular β-adrenoceptor desensitization in rats with blood pressure variability caused by sinoaortic denervation. Rocha ML[1], Silva BR[2], Lunardi CN[3], Ramalho LNZ[4], Bendhack LM[5] [1]UFG – Farmácia e Farmacologia, [2]FCF-USP, [3]UnB, [4]FMRP-USP, [5]FCFRP-USP

Introduction: Spontaneous variation in blood pressure is defined as 'blood pressure variability' (BPV). Sinoaortic denervation (SAD) is characterized by BPV without sustained hypertension. In the present study, we investigated whether BPV could be related to vascular β-adrenoceptor desensitization in rats. **Methods:** Three days after surgery (SAD and control), aortic rings were placed in an organ chamber and the relaxation stimulated by β-adrenoceptor agonists, isoprenaline, terbutaline, BRL37344 and cyanopindolol was verified. The participation of intracellular nucleotides signaling pathways was also verified using forskolin, sodium nitroprusside and acetylcholine to induce relaxation. The effects of BPV on the increase in endothelial citosolic Ca^{2+} concentration stimulated by the β_2-adrenoceptor agonist was examined by confocal microscopy. In addition, the vascular expression of the β_2-adrenoceptor was also examined by immunohistochemistry. **Results:** The results show that isoprenaline and terbutaline-induced relaxation was lower (58.6 ± 6.7% and 70.8 ± 5.3 %, respectively; $p<0.01$) in the aortas of rats with BPV when compared to the control (91.7 ± 7.9% and 92.2 ± 3.8%, respectively). Relaxation responses to other vasorelaxant compounds were similar in both groups of rats. Histological analysis revealed a lower level of β_2-adrenoceptor and confocal microscopy showed minor citosolic Ca^{2+} concentration in endothelial cells stimulated by the β_2-adrenoceptor agonist in rats with BPV. **Conclusion:** BPV leads to desensitization of the β_2-adrenoceptor, which could contribute to worse β-adrenoceptor agonist-induced relaxation in isolated arteries. **Financial support:** FAPEG/GO and FAPESP/SP. **Ethical Committee:** 07/56433-1.

06.092 Endothelium potentiates the relaxation induced by a nitric oxide donor Martinelli AM, Vatanabe IP, Rodrigues CNS, Rodrigues GJ UFSCar – Ciências Fisiológicas

Introduction: The endothelium plays an important role in the regulation of vascular tone by releasing vasodilator factors including nitric oxide (NO). In endothelial cells, the NO production is regulated by endothelial NO-synthase (eNOS) activation in response to the increase of $[Ca^{2+}]c$, phosphorylation, dimerization of proteins and protein interactions. Previously, we have verified that the relaxation to NO donor sodium nitroprusside is potentiated in the presence of endothelium, which is dependent of Ca^{2+} influx and activation of eNOS. Thus, the aim of this study was verify if the endothelium potentiate the relaxation induced by a spontaneous NO donor (DETA-NO), as well as identify the mechanism of this action. **Methods:** Wistar rats were used (400– 500 g). To vascular reactivity study, thoracic aortas were isolated cleaned and mounted in isolated organ bath. The relaxation was performed to DETA-NO (NO donor) in pré-contracted aortic rings with phenylephrine, in the presence or absence of endothelium. The potency (pD2) and maximum relaxant effect (ME) was measured. To investigate the mechanism of endothelium potentiation, we have measured intracellular NO (by DAF-2DA fluoresncence intensity FI) in Human Umbilical Vein Endothelial Cells (HUVEC) in culture. HUVECs were treated during 30 minutes with Ataciguat (0.1, 1.0 and 10 µM) that is an activator of soluble guanylate cyclase (sGC). The HUVECs treatment was performed in the absence or presence of non-selective NOS inhibitor (L-NAME), or sGC inhibitor (ODQ), or calcium channel blocker (verapamil). All experimental protocols with rats were approved by the Ethical Committee of the Federal University of São Carlos – UFSCAR (nº 012/2013). **Results**: In pré-contract aortic rings, the presence of endothelium potentiated the relaxation induced by DETA-NO (pD2 E+: 8.02 ± 1.72, n=6 > pD2 E-: 5.45 ± 0,20, n=7, $p<0.05$). In the presence of non-selective NOS inhibitor (L-NAME), the effect of endothelium was abolished (pD2 E+ L-NAME: 4.84 ± 0.45, n=6; pD2 E- L-NAME: 5.42 ± 0.03, n=8) and the relaxation was similar to without endothelium. The ME was similar to all conditions. To investigate the mechanism of endothelium potentiation, we have measured if the activation of soluble guanylate cyclase (sGC) induces the NO production in endothelial cells. The sGC activation by ataciguat induced a NO production in HUVECs cells (Basal detection: 5.36 ± 0.33 FI, n=3 < ataciguat 0.1µM: 10.55 ± 0.50 FI, n=3; ataciguat 1.0 µM: 9.13 ± 0.19 FI, n=3; ataciguat 10 µM: 8.52 ± 0.20 FI, n=3, $p<0.05$). No difference to NO production was verified between concentration of ataciguat, which indicates that 0.1µM should have reached the maximum effect to this response. In the presence of non-selective NOS inhibitor (L-NAME) or sGC inhibitor (ODQ) the NO production induced by ataciguat 0.1µM was abolished (L-NAME: 6.75 ± 0.14 FI, n=3; ODQ: 6.69 ± 0.10 FI, n=3; $p<0.05$). However, in the presence of calcium channel blocker (verapamil) no alteration was verified to ataciguat effect (verapamil: 8.54 ± 0.21 FI, n=3). **Conclusion:** Taken together our results indicate that the activation of sGC in endothelial cells can induces a NO production by a mechanism independent of calcium influx. **Financial support:** FAPESP and CNPq.

06.093 Mechanisms related to prostanoids cooperate with Nitric Oxide to maintain reduced the Angiotensin II (ANG II) responses in femoral veins of hypertensive rats (2K1C) during exercise. Ledo PBO, Oliveira PR, Chies AB FAMEMA – Farmacologia

Introduction: The angiotensin II (Ang II) responses in femoral veins are constantly modulated by NO in normotensive rats, not exposed to exercise. After a single bout of exercise or inasmuch as the animals are submited to a physical training, vasodilator prostanoids start to participate in a redundant fashion with NO in the modulation of the Ang II responses in femoral veins. A reduction in the Ang II-induced endothelin-1 production may also be involved in these modifications response induced by the exercise in the femoral vein. These exercise-induced vascular adaptations, however, are poorly known in hypertensive animals. Thus,the aim of the present study was to investigate the Ang II responses in rat femoral vein taken from 2K1C rats (4 weeks after clipping), as well as to verify the effects of exercise on these responses. **Methods:** Wistar rats (♂) 350-400g were divided in 4 groups: resting sedentary, exercised sedentary, resting trained and exercised trained, according to the exercise protocol. The exercise training was performed in treadmill (60% of maximal capacity of each animal), 1 hour per day, 5 day/week, for 10 weeks. To induce the hypertension, clips were implanted in the renal arteries after 5 weeks of exercise training. Five weeks later, in the 10th week of training, sedentary and trained animals were sacrificed at rest or after a single bout of exercise. Rings of their femoral veins were transferred to an organ bath system, where were challenged by cumulative concentrations of Ang II in presence of inhibitors of NOS or COX as well as antagonists of ETA/ETB receptors. Through concentration-response curves were obtained pEC50 and Rmax that were compared (mean ± SEM of 8-11 determinations) by two way ANOVA/Bonferroni (differences were considered significant whether $P<0.05$). This study was approved by CEUA-FAMEMA (protocol 300/14). **Results:** Ang II responses are discrete in femoral veins taken from 2K1C rats. These responses were increased by L-NAME (Rmax - from 0.04 ± 0.02 to 0.16 ± 0.05; $p<0.05$) in animals not exposed to exercise, but not in animals exposed to acute exercise or training. In presence of both L-NAME and indomethacin, the Ang II responses determined in femoral veins were not modified by the training. However, the sensitivity to Ang II was augmented in femoral veins taken from sedentary animals exposed to a single bout of exercise (pEC50 - from 8.44 ± 0.05 to 9.91 ± 0.33; $p<0.05$). When BQ-123 was added in the incubation, in addition to L-NAME/indomethacin, the Ang II responses were reduced in all assessed groups and exercise-induced differences were no longer observed. On the other hand, the treatment with BQ-788+L-NAME+ indomethacin elevated the Ang II Rmax in all groups of animals. This elevation of Rmax, however, was not able to mask the increase of Ang II pEC50 induced by exposure of sedentary animals to a single bout of exercise (pEC 50 - from 8.48 ± 0.09 to 9.05 ± 0.23; $p <0.05$). **Conclusion:** mechanisms related to prostanoids and those activated by endothelin-1, through ETB receptors, cooperate with NO to maintain controlled de Ang II responses in femoral veins of 2K1C rats. **Financial support:** FAPESP (Proc. 2013/22655-9).

06.094 Role for the pentose phosphate pathway in the vascular cell damage induced by high glucose Sanchez-Ferrer CF[1], Romacho T[1], Azcutia V[1], Villalobos L[1], Fernandez E[2], Bolaños JP[2], Moncada S[3], Peiro C[1] [1]Facultad de Medicina, Universidad Autónoma de Madrid – Farmacologia, [2]Universidad de Salamanca-CSIC – Instituto de Biología Funcional y Genómica, [3]University College London – Wolfson Institute for Biomedical Research

Background: Hyperglycemia is a risk factor for cardiovascular diseases, but the links between glucose metabolism and atherosclerosis still require elucidation. We have previously shown that normal vascular cells are not damaged by high glucose concentrations unless they are primed with an inflammatory stimulus like interleukin (IL)1β. Now, we identify the cellular mechanisms by which high glucose exacerbates the vascular inflammation induced by IL1β. **Methods:** Cultured human aortic smooth muscle cells (HASMC) and isolated rat mesenteric microvessels were treated with IL1β in medium containing 5.5 to 22 mmol/L glucose. Glucose utilization, lactate production, GLUT1 levels, NADPH oxidase activity, NF-kB activation, and iNOS expression were measured in HAMSC, while endothelium-dependent relaxations were determined in rat microvessels. Pharmacological inhibition of IL1 receptors, NADPH oxidase and glucose-6-phosphate dehydrogenase (G6PD), as well G6PD silencing, were also performed. Moreover, the pentose phosphate pathway (PPP) activity and the levels of reduced glutathione were determined. **Results:** In HASMC submitted to IL1β an excess glucose uptake and consumption occurred in a medium with high glucose concentrations, by using an IL1β-induced enhanced expression of GLUT1 glucose transporters. However, the simple entry of glucose was not deleterious to the cells, as the inhibition of mitochondrial respiration with sodium azide increased glucose uptake and consumption without enhancing NF-□B activation or iNOS expression. We found that, besides allowing glucose entry, IL1β enhances G6PD expression and activates the PPP in VSMC submitted to high glucose, thus permitting some of the excess glucose to be metabolized by this route. This provides additional substrate for enhancing the NADPH oxidase enzymatic activity, producing O_2 that is required for the activation of NF-□B and iNOS, and giving rise to an increased inflammatory condition which cannot be counterbalanced by the simultaneous regeneration of reduced glutathione. Moreover, in rat mesenteric microvessels high glucose incubation enhanced the endothelial dysfunction induced by IL1β by a mechanism which was abrogated by PPP inhibition. **Conclusions:** A pro-inflammatory stimulus like IL1β transforms excess glucose into a vascular deleterious agent by causing an increase in glucose uptake and its subsequent diversion into the PPP, promoting the pro-oxidant conditions required for the exacerbation of pro-oxidant and pro-inflammatory pathways. These pathways were blocked with the IL1 receptor antagonist anakinra, suggesting these anti-inflammatory agents can be effective for preventing diabetic vasculopathy. **Financial support**: Supported by grants from Plan Nacional de I+D (SAF2014-52762-R) and ISCIII (RETICEF-R2/0043/0021). **Ethical approval**: The Ethics Committees for Clinical Research from Hospital Universitario de Getafe (reference numbers 11-20 and 14-117) and Hospital Universitario La Paz (reference numbers PI-1111 and PI-1878) approved the protocol in human cell cultures. Animal studies were performed according to European guidelines (2010/63/EU), approved by the ethics committee of Universidad Autónoma de Madrid (reference CEI 27-670), and developed in registered animal facilities (ES-28079-000097).

06.095 Acute restraint stress increases carotid reactivity in Type-I diabetic rats by enhancing nox4/nadph oxidase functionality. Moreira JD, Moreira RP[1], Pernomian L[2], Gomes MS[3], Prado AF, Pernomian L[4], de Oliveira A[4] [1]UNIFAP, [2]FCFRP-USP – Pharmaceutical Sciences, [3]FCFRP-USP – Ciências Farmacêuticas, [4]FCFRP-USP

Introduction: The present study investigated the hypothesis that stress could exacerbate reactive oxygen species generation by activating NADPH oxidase via angiotensin AT1 receptors in type-I Diabetes. **Methods**: The animals were divided into Normoglycemic (N), Stressed normoglycemic (SN), Diabetic (D) and Stressed Diabetic (SD). Type-1 Diabetes was induced in eight-weeks-old male Wistar rats (350–400 g) by a single dose of streptozotocin (50 mg/kg). In the present study, 3 h-lasting restraint stress model was used as an acute stress intervention as previously validated in our laboratory. Corticosterone dosage was applied as the standard protocol to validate the acute stress. Cumulative concentration–response for angiotensin II (Ang II) (10 pmol/l –1 mmol/l) were obtained in endothelium intact (E^+) or endothelium-denuded (E^-) carotid rings. Cumulative concentration–response curves for Ang II were also obtained in the presence of (tiron, 100 µmol/l, 30 min), (PEG)- Catalase (250 U/ml, 30 min), Nox4 inhibitor (VAS2870 (5.0 µmol/l), Nox1 inhibitor ML171 (0.5 µmol/l), (COX-1) inhibitor SC560 (1 nmol/l) and (COX-2) inhibitor SC236 (0.1 nmol/l) added 30 min prior to Ang II. Nox1 and Nox4 expression and activity were assessed by Western blotting and lucigenin chemiluminescence. The role of Nox1 and Nox4 on reactive oxygen species generation was evaluated by flow cytometry and Amplex Red assays. Cyclooxygenases expression was assessed by real-time polymerase chain reaction. **Results:** In the analysis of reactivity Ang II, there was an increase in the E_{max} of D rings (E^+) (E_{max}: 0,70 ± 0,04) and compared with the N rings (E^+) (E_{max}: 0,48 ± 0,03). Acute Stress exacerbate the contraction of Ang II SD rings (E^+) (Emax: 0,88 ± 0,05) when compared D and SN (E^+) ($_{Emax}$ 0,49 ± 0,02). In carotid rings (E^-) there was an increase in the E_{max} SD (E_{max}: 1,38 ± 0,06), N (E_{max}: 0,99 ± 0,04) and SN (E_{max}: 1.00 ± 0,03). In the presence of Tiron, the E_{max} de D (E^+) (E_{max}: 0,51 ± 0,03), SD (E^+) (E_{max}: 0,51 ± 0,04) and SD (E^-) (E_{max}:1,17 ± 0,05) were reduced. In the presence of PEG- catalase E_{max} SD (E^+) (E_{max}: 0,64 ± 0,03), (E^-) (E_{max}: 1,21 ± 0,05) and D (E^+) (Emax: 0,51 ± 0,02) were reduced. Nox 1 reduced the Ang II emax in D (E^+) (E_{max}: 0,50 ± 0,04), SD (E^+), (E_{max}: 0,50 ± 0,05) and SD (E^-) (E_{max}: 0,99 ± 0,05). Nox4 reduced the Ang II E_{max} in D (E^+) (Emax: 0,54 ± 0,04), SD (E^+), (E_{max}: 0,66 ± 0,03) and SD ($E^{-)}$ (E_{max}:1,24 ± 0,02). Increased higher expression of Nox 4 in rings carotid arteries of SD compared to D and SN. In presence of SC 236 and SC560 it was also observed reduced of Emax Ang II D (E^+) SD (E^-) and increased gene expression of the enzyme COX-2 em D and SD. **Conclusion:** Taken together, our findings suggest that acute restraint stress exacerbates the contractile hyperreactivity to angiotensin II in diabetic rat carotid by enhancing Nox4-driven generation of hydrogen peroxide, which evokes contractile tone by cyclooxygenases-dependent mechanisms**.** **Financial Support:** FAPESP and CNPQ. **Ethics Committee number:** Ethics Committee on Animal (CEUA) from USP(protocol number: 13.1.441.53.2)

06.096 Clinical trial on resistant hypertension: pharmacometabolomic evaluations of antihypertensive drugs Bueno C, Faria H, Figueiredo E, Krieger JE, Krieger EM, Pereira AC, Santos PCJL

Introduction: Resistant hypertensive patients are individuals with uncontrolled blood pressure despite treatment with one diuretic and two antihypertensive drugs with different mechanisms of action in adequate doses. The field called pharmacometabolomic can be conceptualized as a set of changes on metabolome or drug metabolites. It can clarify mechanisms for variations to treatment responses, or even understand how genetic alterations influence the responses. In this scenario, the main aims were to understand the clinical variables of the protocol; to separate and quantify six antihypertensive drugs (chlorthalidone, enalapril, losartan, amlodipine, clonidine and spironolactone) and some of their metabolites in serum samples from hypertensive patients; and to observe the association of the metabolite measures with drug response or resistant hypertension. **Methods:** The clinical trial included 1,597 patients, being 238 resistant hypertensive patients. The serum samples were analyzed by ultra-performance liquid chromatography coupled to a mass spectrometer and using an internal standard to control for each of the analytes. **Results:** Clinical and demographic information of the patients showed that there was a significant difference between resistant and non-resistant patients, the systolic/diastolic blood pressure ($p<0.001$), the number of patients who have suffered stroke ($p<0.001$) and the number of patients with diabetes mellitus ($p<0,001$). The fragmentation of each analyte were respectively: chlorthalidone (176, 240 and 212m/z), enalapril (234, 117 and 160m/z), enalaprilat (117, 206 and 303m/z), losartan (207, 405 and 180m/z), losartan carboxylic acid (180, 235 and 207m/z), N-glucuronide 2 losartan (193, 554 and 207m/z), amlodipine (238, 294 and 206m/z), clonidine (213, 160 and 145m/z), spironolactone (107, 91 and 187m/z) and 7α thiomethyl spironolactone (149, 113 and 167m/z). Also, the column switching test was performed with success. **Conclusion:** We were able to measure the clinical variable from resistant and non-resistant patients and to standardize the proposed analytical methods. Our end findings may clarify mechanisms for variations to responses with antihypertensive drugs. The study was approved by the institutional review board InCor-HC (0758/09).

06.097 Angiotensin II-induced mononuclear cell arrest is CXCR6/CXCL16 mediated. implications in abdominal aortic aneurysm (AAA) Formation Sanz MJ[1], Collado A[1], Rius C[1], Marques P[1], Escudero P[1], Piqueras L[2 1]University of Valencia. Institute of Health Research INCLIVA – Pharmacology, [2]Institute of Health Research INCLIVA

Introduction: Abdominal aortic aneurysm (AAA) is a degenerative disease of the aorta that mainly affects elderly population over the age of 65. Nowadays the pathways involved in its onset and progression remain unknown and angiotensin-II (Ang-II) has been widely implicated. Therefore, the potential link between CXCR6/CXCL16 axis in AAA was investigated. **Methods and Results***:* Apolipoprotein E-deficient mice (apoE$^{-/-}$) were subjected or not to a high-fat diet and infused with Ang-II (500 ng/kg/min) for 28 days. Some of the animals were daily treated with losartan at 10 or 30 mg/kg/day. Flow cytometry and immunofluorescence were used to determine CXCL16 expression on human umbilical vein or artery endothelial cells (HUVEC and HUAEC, respectively). Parallel-plate flow chamber assay was employed to evaluate leukocyte adhesion to Ang-II (1 µM)-stimulated human endothelium. Mice subjected to a high-fat diet and infused with Ang-II showed higher incidence of AAA, increased macrophage, CD3^{+} lymphocyte and CXCR6^{+} cell infiltration and enhanced neovascularization than unchallenged animals. These effects were accompanied by increased MCP-1/CCL2, CXCL16, CXCR6 and VEGF mRNA expression within the lesion. These events were reduced when losartan was administered at 30 but not at 10 mg/kg/day. When HUVEC and HUAEC were stimulated with 1 µM Ang-II (24h), a significant increase in CXCL16 expression was detected by flow cytometry and immunofluorescence. However, neutralization of CXCL16 activity only significantly inhibited Ang-II-induced mononuclear leukocyte-HUAEC interaction by 49% without affecting their interaction with HUVEC. Ang-II-induced CXCL16 expression was found to be dependent on Nox5 expression and subsequent RhoA/p38-MAPK/NFκB activation. **Conclusion***:* These results suggest that the CXCR6/CXCL16 axis could constitute a new therapeutic strategy in the treatment of cardiovascular diseases associated with activation of the renin-angiotensin system (RAS). This study was supported by grants SAF2014-57845R, PI15/00082 and PIE15/00013 from the Spanish Ministry of Economy and Competiveness, Carlos III Health Institute and the European Regional Development Fund (FEDER). The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by the ethics review board of the Faculty of Medicine, University of Valencia. Procedure: A1296128251125.

06.098 Mast cell and testosterone interaction on kidney fibrosis induced by unilateral ureteral obstruction in rats. Oliveira-Silva GL, Morais IBM, Alvarez MMP, França-Silva N, Galo JA, Balbi APC, Hiraki KRN, Bispo-da-Silva LB ICB-UFU

Introduction: both mast cell activity and testosterone action have been associated with renal fibrosis after unilateral ureteral obstruction (UUO). Therefore, in the present study we evaluated possible interactions between mast cell and testosterone in determining renal fibrosis in rats submitted to UUO. **Methods**: Wistar rats (n=5-6) were subjected to orchiectomy (OQ) or sham-surgery (nonorchiectomized: NOQ) and 7 days after these procedures OQ and NOQ rats were submitted to UUO; a NOQ group was subjected to sham-surgery (control: CR). OQ-UUO and NOQ-UUO animals were divided into two groups: one was treated with saline (OQ-UUO-Sal and NOQ-UUO-Sal) and the other with mast cell stabilizer sodium cromoglycate (50 mg/kg/day, i.p.; OQ-UUO-CG and NOQ-UUO-CG). One group of OQ-UUO-Sal and one of OQ-UUO-CG was submitted to testosterone (propionate) replacement (TR: 0.5 mg/kg/day, s.c.), i.e., OQ-UUO-Sal-TR and OQ-UUO-CG-TR. Hormone replacement and treatment with CG/Sal were initiated 1 and 5 days after OQ, respectively, and they lasted until the end of the experiments; kidneys and blood were collected 14 days after UUO or sham-surgery in anesthetized rats. Kidneys were fixed, paraffin-embedded, and histological sections were stained with toluidine blue to quantify mast cells, and picrosirius red for analyzing collagen. Plasma testosterone level (ng/dL) was measured by eletroquimioluminescence. **Results**: OQ decreased testosterone plasma level and TR normalized it (156.40 ± 51.47^{a}, 4.92 ± 0.59^{b}, 208.80 ± 84.65^{a}; CR, OQ-UUO-Sal and OQ-UUO-Sal-TR, respectively. Means sharing the same superscript are not significantly different from each other, $P>0.05$, ANOVA/Newman-Keuls). UUO increased mast cell density (cell/mm^2) in the kidney pelvis (3.28 ± 1.14 vs. 10.51 ± 2.55, CR vs. NOQ-UUO-Sal, $P<0.05$, Student's t-test), but not in the kidney parenchyma (0.05 ± 0.01 vs. 0.02 ± 0.01, $P>0.05$, CR vs. NOQ-UUO-Sal, Student's t-test, $P>0.05$). UUO increased the picrosirius red-stained areas (%) of renal parenchyma, an alteration that was abolished by CG treatment or OQ; TR reverses the effects of OQ, and CG treatment partially inhibits the effects of hormone replacement (2.19 ± 0.5^{a}, 5.37 ± 0.61^{b}, 2.30 ± 1.06^{a}, 1.95 ± 0.35^{a}, 1.62 ± 0.23^{a}, 5.65 ± 1.33^{b}, 3.90 ± 0.75^{ab}; CR, NOQ-UUO-Sal, NOQ-UUO-CG, OQ-UUO-Sal, OQ-UUO-CG, OQ-UUO-Sal-TR and OQ-UUO-CG-TR, respectively. Means sharing the same superscript are not significantly different from each other, $P>0.05$, ANOVA/Newman-Keuls). **Conclusion**: The data suggest that mast cells and testosterone interact in the development of renal fibrosis induced by UUO. **Financial support**: This work was supported by a master fellowship by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for Oliveira-Silva, GL. CEUA/UFU: process n° 0132/13.

06.099 Vasorrelaxant effect of R(+)-pulegone in rats. Alustau Fernandes MC[1], Mendes-Neto JM[2], Santos-Vidal R[2], Correia NA[3], Albuquerque KLG[3], Capettini LAS[4], Lauton-Santos S[5] [1]CFP-ESTC-UFCG, [2]PROCFIS-UFS, [3]CCS-DFP-UFPB, [4]UFMG, [5]UFS

Introduction: R (+)-pulegone is a monoterpene ketone with blocker properties of calcium channels in the heart. The administration of substances that blocks calcium channels and nitric oxide production stimulates modify the vascular tone of the aorta and trigger reduction of pulsatile pressure, becoming essential for therapy in isolated systolic hypertension, which is the most common disease in the elderly population. **Methods/ Results**: vasorrelaxant effects of R (+)-pulegone were tested in normotensive rats using two different methodological approaches. In vivo, increasing doses (1, 3, 10, 20 and 30 mg / kg) were administered in the animals, i.v. bolus and selected randomly, then the parameters for mean arterial pressure (MAP) and heart rate (HR) were evaluated. In this situation the substance triggered a hypotensive effect and bradycardia. In the ex vivo approach, we used the thoracic aorta of these animals and isometric tension experiments, evaluated the vasorelaxant activity of the substance. The administration of R(+)-pulegone triggered vasorelaxant effect concentration-dependent in both rings with intact endothelium and with this removed, but the substance had the lowest pD2 value in the presence of the endothelium (-3.64 ± 0.06, n = -3.17 vs 5 ± 0.034, n = 6, respectively), no change in peak effect (98.2 ± 1.2%, n = 5 vs. 106.0 ± 8.1%, n = 6) indicating that the substance acts triggering vasodilation in aortic dependent manner and independent of the vascular endothelium. In rings with intact endothelium, the vasorelaxant activity of R (+) - pulegone was not altered in the presence of diclofenac and atropine, but was modified by L-NAME (-3.00 ± 0.016; n=5), HDX (hydroxocobalamin) (-3.07 ± 0.021; n=5), ODQ () (-3.17 ± 0.03; n = 5) and the red ruthenium (-3.14 ± 0.04; n=5) vs control: -3.64 ± 0.06; n = 5. These results suggest that the substance is probably stimulating NO production via the activation of TRP (transient receptor potential) channels. In smooth muscle vascular, R (+)-pulegone inhibited curve calcium concentration-dependent manner (Emax: 10-4 M: 68 9 ± 3.81%; 3x10-4 M: 40.97 ± 8.05%; 10-3 M: 24.79 ± 5.04% and 3x10-3 M: 0.29 ± 0.33%) via calcium channels type L, as in the presence of nifedipine, there was a reduction of the maximum effect (Emax: 93.3 ± 1.7% ; n = 6 vs Emax control: 106.8 ± 8.1%; n = 6). Additionally, it was surveyed the participation of for potassium channels, using 4-aminopyridine (-2.93 ± 0.012 - n = 5 vs control - 3.17 ± 0.034 - n = 6), since, in the presence of glybenclamide, the relaxant response to R (+)-pulegone was also inhibited (-2.94 vs. -3.17 ± 0.012 ± 0.03).**Conclusion** R (+)-pulegone, stimulates the production of NO (nitric oxide) in endothelial cells, probably by activating calcium influx via TRP channels. The effect independent of the endothelium is mediated by inhibition of calcium influx, likely through the CaVs and for opening potassium channels (K_{ATP} and Kv). **Financial Support**: CNPq Animal Research Ethical Committee: All approaches of this study was approved by CEPA/UFS (nº #4/2015)