06. Cardiovascular and Renal

06.001

Venous return related-pharmacotherapy is more prevalent in wheelchair non-athletes than athletes. Paiva RCA, Garbeloti EJR, Restini CBA UNAERP

Venous return is a hemodynamic mechanism that involves the transport of venous blood to the right atrium through veins focusing the right atrial pressure variation in relation to the rate of flow through the peripheral vasculature. Elevated cardiac output is regulated by the increase in skeletal muscle blood flow and venous return to the heart. It is widely established that the practice of physical activities improves the muscular tonus and the physiologic hypertrophy of skeletal muscles increases the efficiency of venous return. The symptoms and clinical signs are important tools; on the other hand, investigative studies based on biochemical markers as well as by inquiries to define the profile of the patients are useful to improve the knowledge and to clarify ways to deal with the venous diseases and their complications. One of the complications is the excessive use of the pharmacotherapy. Venous diseases and its intercurrences have been extensively studied through clinical and observational studies. Studies like these, whose subjects are wheelchair users, are rarely found. **Aims**: Evaluate the impact of the physical activity in individuals with muscular atrophy on the inferior members (wheelchair users) considering two main aspects: the quality of life and the self-perception of the venous return symptoms. **Method:** Data of handicapped non-athletes was collected from a university physiotherapy clinic at the University of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil. Data of the athletes' sample (basketball players) was obtained from Cava do Bosque, Ribeirão Preto, São Paulo, Brazil, between September 2013 and December 2013. This is a cross-sectional study, with a convenience sampling of wheelchair users: non-athletes (n=12) and athletes (n=13). A socio-demographic questionnaire was applied. Self-perception of functional performance and of venous symptoms was evaluated by the VEINES-QOL/Sym, and the quality of life was evaluated by the WHO-QOL/Bref. The statistical analysis was performed using Chi-square test for VEINES-QOL/Sym and Student's *t*-test for WHO-QOL/Bref. The study was previously approved by the Ethics Committee of The National Ministry of Health/University of Ribeirão Preto (CAAE: 18388513.7.0000.5498/ protocol: 462.531/2013) **Results:** Twenty-three subjects were men (92%). Data of physical conditions, environmental and psychological domains from WHO-QOL/Bref questionnaire demonstrated no significant differences between the groups. On the other hand, social relations domain was higher in athletes compared to non-athletes ($P<0.05$). The most results from VEINES-QOL/Sym questionnaire showed no statistically significance between the groups. On the other hand, leisure activity and time spent for daily activities showed higher values for the athletes ($P<0.05$). The continuous consume of medicines was more prevalent in the non-athletes group (100%) than among the athletes (53.85%). **Conclusion:** Considering the data obtained by WHO-QOL/Bref survey, physical exercise improves the quality of life of wheelchair individuals. However, it has not been possible to establish the relationship between physical exercise and the improvement of self-perception on venous symptoms in wheelchair individuals.

06.002

Antihypertensive effect of the methanolic fraction of the essential oil of *Alpinia zerumbet* in Wistar rats. Cunha GH[1], Marques LARV[2], Fechine FV[2], Moraes MO[2], Moraes MEA[2] [1]UFC – Enfermagem, [2]UFC – Farmacologia Clínica

Introduction: *Alpinia zerumbet* (Zingiberaceae Family) is known popularly as ?colonia? in the Northeast of Brazil, where it?s widely used in folk medicine, predominantly in the treatment of hypertension and anxiety (LAHLOU *et al.*, 2003; SANTOS *et al.*, 2011). The objective was to evaluate the antihypertensive effect of methanolic fraction of the essential oil of *Alpinia zerumbet* (MFEOAz) in hypertensive rats. **Methods:** This study was approved by the Ethics Committee on Animal Research of Federal University of Ceará, approval protocol number 18/2011. In the experiments, male Wistar rats were used, weighing between 250 and 330 grams and aged from 10 to 12 weeks. The model used involved hypertension chronic inhibition of nitric oxide for administration of L-NAME (30 mg/kg/day) diluted in the drinking water. The induction phase of hypertension corresponded to the first 30 days. After 31 days, the animals were divided into three treatment groups. Control Group: 9 hypertensive rats that received distilled water; Nifedipine Group: 10 hypertensive rats that received nifedipine 10 mg/kg body weight per day; FMOEAz Group: 8 hypertensive rats, which received FMOEAz 100 mg/kg of body weight per day. Treatments were administered by gavage once daily, starting on the 31st day, and lasting for 30 days. Blood pressure was measured by tail cuff plethysmography. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) parameters were measured. In the induction phase parameters were measured every 6 days, and the treatment phase measurements took place every 3 days. Comparisons between groups were performed by using analysis of variance (ANOVA) associated with the Tukey test. Results: The analysis of the temporal behavior of the treated group with MFEOAz showed that the hypotensive effect began on the 36th day of treatment ($P < 0.001$) compared to the control group and remained constant until the 60th day. However, the hypotensive effect in the positive control group (nifedipine) was observed from 33th day and remained constant until the end of the experiment. The hypotensive activity of the positive control was significantly higher than that MFEOAz on all days evaluated ($P < 0.05$). The MFEOAz at a dose of 100 mg/kg, significantly reduced blood pressure in hypertensive rats by chronic inhibition of nitric oxide (. The hypotensive effect intensified over the time, but it was inferior to the one provided by nifedipine. Thus, the dose of FMOEAz 100 mg/kg/day, has antihypertensive effect in rats with chronic hypertension. Discussion: This model of hypertension is widely accepted and validated (FAZAN *et al.*, 2001). In this study, it was found that the group treated with L-NAME had a statistically significant increase in the PAS when compared to the baseline time (day 0) and the control group, treated drinking water. Hypotensive effect was also observed in other pre-clinical studies that evaluated the essential oil of A. zerumbet (BARCELOS *et al.*, 2010; SANTOS *et al.*, 2011). It has been found that hypotension in rats occurs independently of the operational presence of the sympathetic nervous system, suggesting that EOAz is a direct vasodilator (LAHLOU *et al.*, 2002a; LAHLOU *et al.*, 2002b). Acknowledgments: National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Level -or Education- Personnel (Capes).

06.003

Loss of platelet antiaggregation activity in senescent endothelial cells and its recovery by polyphenols. Silva GC[2,1], Abbas M[2], Ribeiro TP[2], Burban M[2], Toti F[2], Braga FC[3], Côrtes SF[1], Schini-Kerth VB[2] [1]UFMG – Farmacologia, [2]UMR-CNRS, [3]UFMG – Farmácia

Objective The present study investigated loss of the platelet antiaggregation activity of senescent endothelial cells, and its recovery after treatment with a standard fraction of *Hancornia speciosa* (SFH) and its polyphenols. **Methods and Results** Replicative senescence was induced by sequential passaging of primary cultures of endothelial cells up to the fourth passage (P4). Senescence was assessed through β-galactosidase activity, the formation of reactive oxygen species (ROS) by dihydroethidine signal in flow cytometry, the protein expression by western blot, nitric oxide (NO) formation by electron spin resonance spectroscopy, and platelet aggregation by aggregometry and approved by ethics committee of UFMG with protocol 227/08. Results were considered statistically significant when $p<0.05$. Compared to P1, the SA-β-gal activity was increased by 90 ± 11%, 220 ± 4 % and 316 ± 14 % in cells at P2, P3 and P4, respectively, and this effect was associated with respective increase of 73 ± 0.03, 77 ± 0.02 % and 87 ± 0.01 on expression of p53, p21 and p16. Senescent endothelial cells (P3) had lower (30 ± 4.7%) antiaggregation activity than non-senescent cells (P1; 100 ± 0.8%). Compared to P1, the amount of NO and the expression of eNOS were strongly reduced 39 ± 5.6% and 50 ± 0.05 % at P3 cells, respectively. In addition, cells at P3 had increased ROS (67 ± 9%), expression of NADPH oxidase subunits gp91 phox (62.3 ± 0.2 %) and p47phox (39 ± 0.3 %), as well as COX-1 (62 ± 0.02 %) and tissue factor (TF, 106 ± 0.01%). Indomethacin (30 µmol/L) and VAS 2870 (5 µmol/L), respective inhibitors of COX-1 and NADPH oxidase, reduced the β-galactosidase activity (78 ± 9 % and 65 ± 8 %, respectively) and restored the ROS production to P1 cells level. Treatment of P3 cells with SFH (10 µg/L) and its polyphenols [quinic acid (QA), chlorogenic acid (CA) and rutin (RT); 3 µmol/L] reduced the β-galactosidase activity (42 ± 9 %, 49 ± 8 % 37 ± 9 %, 46 ± 6 %), ROS level (29 ± 8 %, 21 ± 6 %, 15 ± 8 % and 19 ± 6 %) and this effect was associated with recovery of platelet antiaggregation activity of senescent endothelial cell (85 ± 6 %, 82 ± 4%, 88 ± 5%, and 86 ± 3 treated with SFH, QA, CA and RT, respectively). Moreover, SFH reduced the up regulation of senescent markers p53 (47 ± 0.01 %), p21 (51 ± 0.02 %) and p16 (38 ± 0.02 %), the source of ROS gp91 phox (29 ± 0.03 %), p47 phox (46 ± 0.01 %) and COX-1(24 ± 0.02 %), coagulation protein TF (46 ± 0.01 %) and improved eNOS expression (115 ± 0.03 %) and NO production (118 ± 11 %). **Conclusion** The replicative senescence of endothelial cells induces reduction on their platelet antiaggregation activity through a mechanism dependent on decrease of eNOS expression and NO formation/bioavailability and on increase oxidative stress and TF expression. This senescent phenotype was reversed by SFH and its polyphenolic compounds. **Financial support:** CNPq and CNRS UMR 7213

06.004

Effect of the NAD(P)H oxidase inhibitor apocynin on oxidative stress induced by chronic ethanol consumption in the rats corpus cavernosum. Leite LN[1], Hipolito UV[2], Tirapelli CR[2] [1]FMRP-USP, [2]EERP-USP

Introduction: The vascular damage caused by ethanol involves the generation of reactive oxygen species (ROS) and reduced bioavailability of nitric oxide (NO). The NAD(P)H oxidase plays a key role in generating ROS, including superoxide anion and hydrogen peroxide (H_2O_2) in vascular smooth muscle cells and endothelial cells. Therefore, the aim of this study is to evaluate the involvement of NAD(P)H oxidase in the effects induced by chronic ethanol consumption in the rat cavernosal smooth muscle (CSM) through its inhibition by apocynin (APO). **Methods:** The experimental protocols were approved by the Ethical Committee from USP (13.1.471.53.9). Male Wistar rats (200-250g) were divided into 4 groups: Control group (C): received drinking water by oral gavage; Control + APO group (AC): APO (30 mg/kg/day, oral gavage); Ethanol group (E): ethanol 20% (v/v) for 6 weeks and drinking water by oral gavage; Ethanol + APO group (AE): ethanol 20% and APO (30 mg/kg/day, oral gavage). Reactivity experiments were performed on isolated CSM. Systemic oxidative stress was evaluated by measuring plasma thiobarbituric acid-reacting substances (TBARS) and superoxide anion levels in CSM homogenates evaluated by lucigenin-derived chemiluminescence assay. Nitrate levels were measured in plasma and supernatants from total CSM homogenates. **Results**: Blood ethanol levels in the ethanol-treated rats averaged (E: 1.21 ± 0.21 mg/ml;n=8 AE: 1.39 ± 0.11 mg/ml;n=6). Chronic ethanol consumption reduced the relaxation induced by acetylcholine (C: 41.6 ± 1.5%;n=5 E: 29.7 ± 1.0%;n=7). APO treatment prevented the reduction of acetylcholine-induced relaxation (AC: 42.7% ± 1.1;n=4 AE: 41.8 ± 0.7%;n=6)($p<0.05$, ANOVA). Sodium nitroprusside-induced relaxation was not affected by ethanol consumption (C: 99.9 ± 4.3%;n=5 E: 91.6 ± 2.4%;n=5 AC: 91.5 ± 1.87%;n=5 AE: 100.6 ± 2.5%;n=5). Plasma TBARS levels (nmol/mL) were increased in ethanol-treated rats (34.6 ± 1.5, n=10) compared with control rats (19.7 ± 2.0, n=12). Treatment with APO prevented this response (AC: 18.7 ± 1.9, n=11 AE: 21.2 ± 1.6, n= 12). ($p<0.05$, ANOVA). Superoxide anion levels (RLU/ mg protein) were higher in CSM from ethanol-treated rats (241.9 ± 13.7, n=8) when compared with control (96.4 ± 16.0, n=7). APO prevented the increase in superoxide anion levels induced by ethanol (AC: 75.8 ± 12.1, n=8 AE: 106.7 ± 17.1, n=8)($p<0.05$, ANOVA). Finally, ethanol consumption reduced plasma and CSM nitrate levels. Treatment with APO prevented this response. **Discussion:** Chronic ethanol consumption induced oxidative stress, reduced acetylcholine-induced relaxation and nitrate levels in CSM from ethanol-treated rats, being these response mediated by NAD(P)H oxidase. **Financial Support:** CNPq.

06.005

Volume replacement during septic shock: are there significant differences in the CLP model? Guarido KL, da Silva Santos JE UFSC – Farmacologia

Introduction: Septic shock is a condition characterized by vascular refractoriness to vasoconstrictors and fluid loading. In the last decades, the polymicrobial model of sepsis induced by cecal ligation and puncture (CLP) has been used with several different protocols of fluid replacement. In this study, we investigated the impact of fluid replacement in the cardiovascular parameters of animals subjected to the CLP model. **Methods:** This study was approved by the Animal Experimentation Ethic Commit of UFSC (Protocol P00566). Male Wistar rats (3-4 months old), separated in Sham-operated and CLP groups, were anesthetized with oxygen/isoflurane (2%, inhalation) and had the peritoneal cavity opened. In CLP groups, the cecum was exposed and perforated (four holes; 16G needle) in order to release fecal content into the peritoneal cavity. Uninterrupted sutures were used to close the wound. The sham-operated animals were subjected for the same procedures, without perforation and further manipulation of the cecum. In both sham-operated and CLP groups the following protocols were conducted: i) fluid replacement with a single administration (30 ml/kg, subcutaneous) of sterile phosphate buffer saline (PBS) after surgery (0 hour) or; ii) fluid replacement with PBS repeated four times after surgery (0, 12, 24 and 36 hours). All groups received tramadol (5 mg/kg, subcutaneous) as a pain reliever at 12, 24 and 36 hours after the surgery. All groups had their systolic blood pressure (SBP) and heart rate (HR) measured by tail cuff; body weight (BW) and mortality rate were evaluated until 240 hours after the surgical procedures. We also analyzed diuresis, urinary electrolytes excretion and hematological parameters. **Results:** There were no deaths in sham-operated groups. Statistical analysis demonstrated a similar survival percentage in CLP group that received a single administration of PBS, when compared with the group subjected to 4 episodes of fluid replacement (40-60% during 120 hours of evaluation). Until 72 hours after CLP or sham protocol, neither the group with one nor the group with four fluid replacements presented statistically significant loss of weigh body. The HR and the SBP were changed from 319.4 bpm and 117.1 mm Hg to 388 bpm and 76.3 mm Hg at 3 hours after CLP in animals treated with four fluid replacements. In these animals, a similar pattern of changes remained until 72 h after CLP. Interestingly, the CLP group subjected to a single fluid replacement presented the same pattern and intensity of hypotension and changes in HR. There were no significant changes in the HR and SBP of the animals from sham-operated groups. A reduction in diuresis and urine pH, as well as in the excretion of $Na^{+,}$ K^{+} and urea was found in rats from CLP groups when compared with the sham-operated groups, mainly at 48 h after the surgery. The blood analyses revealed thrombocytopenia, leukopenia, and reduced levels of urea and creatinine in the CLP groups, besides increased levels of nitric oxide metabolites, which also did not different between the groups. **Conclusion:** In spite of the wide number of different protocols found in the current literature, the frequency of fluid replacement does not impact the mortality rate, the development of hypotension and changes in the heart rate, the acute renal failure and hematological disturbances usually associated with the polymicrobial sepsis induced by the CLP model. **Research support:** CNPq and FAPESC (2012000367 and 201200078).

06.006

Bufalin induces vasoconstriction at pharmacological concentration in rabbit aorta and mesenteric rings by stimulation of the α-adrenergic-PKC pathway. Oliveira IMB, Freire MSS, Farias VX, Santos CF, Nascimento NRF, Fonteles MC ISCB-UECE

Cardiotonic steroids (CE) such as bufalin are classical Na^+ -K^+ -ATPase (NKA) inhibitors. These compounds are potential vasoconstrictors and some investigators argue that such compounds may have a role in the development of certain types of hypertension. Nevertheless, it is not clear if this vasoconstrictor effect occurs in the physiological concentration range. Furthermore, the mechanism of action of these compounds as vasoconstrictors is not fully understood. In addition, NKA has been show to behave as a signaling molecule, since it is structurally related to Src-kinase and inhibition of NKA activity leads to Src-kinase activation. The aim of this study was to evaluate the *in vitro* effects of bufalin (BUF; 0,1 – 100μM) in rabbit aortic and mesenteric rings both in the presence or absence of a Src-kinase inhibitor (PP2; 30 μM), a non-selective endothelin receptor antagonist (tezosentan; 10 μM), a non-selective PKC inhibitor (staurosporine-STAU; 0.1 μM) and a non-selective α-adrenergic blocker (prazosin; 1 μM). The tissues were mounted horizontally in 5ml organ baths for isometric recordings and bufalin was added cumulatively in intervals of 3 minutes and the effects expressed as percentage of the maximal contraction attained by phenylephrine. The experimental protocols were approved by the Ceara State University Ethics Committee for the Use of Animals under the # 12781206-7. In aortic rings, bufalin, only at 100 μM, induced a slow onset contraction that attained its maximal after 58 minutes reaching 55 ± 10.5%. Neither PP2 nor tezosentan blunted the contractile response promoted by BUF, being the maximal contractile response in the presence of these drugs 59.4 ± 10.9% and 97 ± 21%, respectively. On the other hand, the inhibition of PKC completely blunted the contractile response evoke by BUF (BUF + STAU- 3.1 ± 0.4%). The blockade of α-adrenergic receptors completely prevented BUF-induced contraction. In mesenteric rings, BUF induced a qualitatively similar slow onset contraction with a maximal response 76.8% ± 15.6. This contraction was not affect by incubating the tissues during 30 minutes with PP2. The inhibition of PKC completely blunted the contraction (76.8 vs. 7.4%, $p<0.05$). The rabbit vascular segments studied had low sensitivity to bufalin-induced contraction. This slow onset contraction was blunted both by blocking α-adrenergic receptors or protein kinase C. Therefore we hypothesize that bufalin inhibits the NKA α3 isoform increasing noradrenaline release from adrenergic nerves leading to PKC activation. **Financial support**: Capes, FUNCAP, CNPq, FINEP.

06.007

Influence of perivascular adipose tissue (pvat) on vascular contractility in mice aortas. Nobrega N, Reis D, Facine LM, Miranda CAS, Bonaventura D UFMG – Farmacologia

Introduction: Perivascular Adipose Tissue (PVAT) was initially characterized as an important tissue with vascular structural function. Recently, PVAT was characterized as a biologically active tissue with an important role on vascular tone. PVAT acts as a source of reactive oxygen species (ROS) directly influencing the vasodilator response. However, PVAT also reduces vascular contraction due to a PVAT derived relaxing factor (PVRF), not yet identified. Based on this information, the aim of this study is to evaluate whether PVAT influences the contraction in mice aortas, as well as, whether this action is dependent on the vascular endothelium. **Methods:** This study was approved by Ethics Committee of Federal University of Minas Gerais (n° 225/2013). Vascular reactivity study was performed in thoracic aortas isolated from Balb/C mice. We analyzed the maximal effect (Emax) and potency (pD_2) for phenylephrine (Phe) and potassium chloride (KCl). Mice aorta was isolated and in some preparations PVAT were dissected and in others PVAT remained intact. Cumulative concentration-effect curves for Phe and KCl were performed in preparations with and without vascular endothelium. **Results:** Results showed that in preparations with intact endothelium, PVAT did not alter significantly the contractile response induced by Phe (pD_2: 7.25 ± 0.2, n=5 and Emax: 0.24 ± 0.01, n=5) when compared to aortas without PVAT (pD_2: 7.20 ± 0.1, n = 4 and Emax: 0.23 ± 0.03, n = 4). The same response was observed in aortas contracted with KCl (with PVAT: pD_2: 1.55 ± 0.03, n = 5 and Emax: 0.34 ± 0.02, n=5; and without PVAT: pD_2: 1.61 ± 0.02, n = 10). However, in denuded aortas, the presence of PVAT reduced, potency and maximum effect induced by Phe and maximum effect verified for KCl (Phe $\rightarrow pD_2$: 7.32 ± 0.07, n = 10 and Emax: 0.25 ± 0.02, n = 10; and KCl $\rightarrow$ pD_2: 1.65 ± 0.02, n = 7 and Emax: 0.31 ± 0.02, n=7) when compared to aortas without PVAT (Phe $\rightarrow pD_2$: 7.71 ± 0.12, n = 15 and Emax: 0.35 ± 0.02, n = 15, and KCl$\rightarrow pD_2$: 1.67 ± 0.02, n = 8 and Emax: 0.41 ± 0.02, n=8). Analyzing aortas with PVAT, the presence (pD_2: 7.15 ± 0.02, n = 8 and Emax: 0.24 ± 0.01, n = 8) or absence of endothelium (pD_2: 7.32 ± 0.07, n = 10 and Emax: 0.26 ± 0.02, n = 10) did not alter the contractile response induced by Phe. In the same way, vascular contraction induced by KCl in aortas with PVAT, the presence (pD_2: 1.55 ± 0.03, n = 5 and Emax: 0.34 ± 0.02, n = 5) or absence of endothelium (pD_2: 1.65 ± 0.04, n = 7 and Emax: 0.31 ± 0.02, n = 7) did not modulate contractile response. **Conclusion:** These results suggest that in mice aortas, PVAT negatively modulates vasoconstrictor responses induced by receptor-dependent or independent agents, since this modulation was observed for contraction induced by Phe and KCl, respectively. Furthermore, this negative modulation induced by PVAT occurs independently of the presence of vascular endothelium. **Financial Support:** FAPEMIG and CNPq.

06.008

Vasorelaxant, hypotensive and radical-scavenging activities of Jabuticaba (*Myrciaria cauliflora*). Andrade DML[1], Reis CF[1], Castro PFS[1], Amaral NO[2], Borges LL[1], Stefany GR[1], Gil ES[1], Pedrino GR[2], Conceição EC[1], Rocha ML[1] [1]FF-UFG, [2]ICB-UFG

Introduction: Jabuticaba berry presents high antioxidant activity due to the presence of phenolic compounds, which display an important role in the prevention of cardiovascular illness. This study's aim was to determine the effect of a hidroalcoholic extract of *Myrciaria cauliflora* (HEMC) on blood pressure and vascular tension in rats. Moreover, we have analyzed its antioxidant property by electroanalytical (differential pulse voltammetry) and DPPH radical scavenging assays. **Methods:** Catheters were inserted into the right femoral vein and artery of anesthetized rats for HEMC infusion and the measurement of blood pressure, heart rate and aortic blood flow. Moreover, the vasodilator effect of HEMC in rat aortas isola Ted in organ bath was examined. All experiments were carried out in accordance with the Animal Research Ethical Committee of the UFG (protocol: 056/2013). **Results:** The intravenous infusion of HEMC produced hypotension and increased aortic blood flow with no changes in heart rate. In pre-contracted isolated aortas, HEMC (0-120 µg/mL) induced relaxation only in vessels with endothelium. Pre-treatment with L-NAME (NO synthase inhibitor) or ODQ (soluble guanylyl cyclase (sGC) inhibitor) abolished the HEMC-induced relaxation. The treatment with MDL-12,330A (adenylyl cyclase (AC) inhibitor) or diclofenac (COX inhibitor) reduced HEMC-induced vasorelaxation. The blockade of muscarinic and β-adrenergic receptors (by atropine and propranolol, respectively) did not promote changes in HEMC-induced vasorelaxation. Electroanalytical and DPPH radical scavenging assays showed high antioxidant property for HEMC. **Discussion:** These findings showed that HEMC possess high antioxidant property, induces endothelium-dependent vascular relaxation and hypotension with no alteration in heart rate. The NO/sGC/cGMP pathway seems to be the main cellular route involved in the vascular responsiveness. **Financial Support:** FAPEG, CNPq

06.009

Analysis of renal disorders *in vivo* and *in vitro* on diabetic rats. Costa LLM[1], Alves RS[2], Seabra-Filho FT[1], Cancio KS[1], Marques KF[1], Pereira JM[1], Monteiro HSA[1], Alves NTQ[1]
[1]UFC – Fisiologia e Farmacologia, [2]UFC – Análises Clínicas e Toxicológicas

Diabetes mellitus is a chronic disease characterized by permanent hyperglycemia resulting from defective insulin secretion, insulin action, or both. It is associated with kidney long-term damage, such as diabetic nephropathy. The induction of experimental diabetes in rats aimed to study the renal effects *in vivo* and *in vitro* involved in the development of this disease. To induce experimental diabetes, we injected 50 mg/kg of Streptozotocin intravenously in male adult rats (weighted 250–300 grams).This study was performed in regard to the recommendations of the Animal Research Ethic Committee under the Protocol numbered: 45/09-UFC. Body weight and urinary volume (UV) were measured in metabolic cages for 10 days preceding the experiments of renal function. One and three months after diabetes were induced (n=6 per group), isolated kidney perfusion was realized with modified Krebs-Henseleit solution. The urinary flow (UF), perfusion pressure (PP), glomerular filtration rate(GFR), renal vascular resistance (RVR), the percentage of sodium (%TNa^{+}), potassium (%$TK+^{)}$ and chloride (% Cl^{-}) tubular transport were determined. Differences between groups were compared by One-way ANOVA and Student's t-test with significance of 95%. The perfused right kidneys were taken for histological analysis. UV increased after three months (UV_{CT} = 20,6 ± 2,4 vs UV_{1MONTH}= 23,49 ± 2,5 vs UV_{3MONTH} = 28,24 ± 4,5mL*,*p<0,05). The body weight was not affected. Only three months after diabetes induction significant changes on kidney perfusion were observed: increased PP ($PP_{60'}$ = 108,8 ± 6,4 vs $PP_{90'}$= 121,7 ± 7,9 mmHg*, *p<0,05), decreased GFR ($GFR_{30'}$=0,843 ± 0,036 vs $GFR_{60'}$=0,495 ± 0,026 mL g^{-1} min^{-1}*,*p<0,05), decreased of %TNa^{+} (%$TNa^{+}{}_{30'}$=75,88 ± 5,3 vs %$TNa^{+}{}_{120'}$ = 33,48 ± 22,61 µEq.g^{-1}. min^{-1*},*p<0,05) and %TK^{+} (%$TK^{+}{}_{30'}$= 54,84 ± 7,81 vs %$TK^{+}{}_{120'}$=6,30 ± 22,5 µEq.g^{-1}. min^{-1*},*p<0,05) and mild to extreme histologic changes on tubular dilation. Increased arterial stiffness have been proposed as a factor of increase blood pressure in diabetes, in addition to diabetic nephropathy, and it can cause a state of pressure diuresis, neurohormonal activation and compensatory changes in glomerular hemodynamics. Although the results showed evidence of a possible kidney injury, the diabetic nephropathy was not evidenced. Other studies are needed with longer periods of time for a better understanding about kidney damage arising to diabetes. **Financial Support**: FUNCAP. **Acknowledgments**: Laboratório de Farmacologia Venenos e Toxinas (LAFAVET) – UFC.

06.010

Role of TNF-α in chronic ethanol consumption-induced oxidative stress: involvement of perivascular adipose tissue. Simplicio JA[1], Cunha TM[1], Tirapelli CR[2] [1]FMRP-USP – Pharmacology, [2]EERP-USP – Pharmacology

Introduction: Chronic ethanol consumption induces increase in blood pressure, oxidative stress and vascular inflammation with increased production of TNF-α. It is known that activation of the TNFR1 receptor induces activation of several cellular pathways that lead to oxidative stress. Moreover, inflammation may involve participation of PVAT (*Perivascular adipose tissue* – PVAT), known as a major source of adipokines and pro-inflammatory cytokines. However, the role of TNF-α in the induction of oxidative stress associated with chronic ethanol consumption and the involvement of PVAT In such response remains elusive. **Objectives:** Evaluate the role of TNF-α in the induction of oxidative stress caused by chronic ethanol consumption and the involvement of PVAT. **Methods**: C57/BL6 mice (wild type – WT) and TNFR1 knockout mice ($TNFR1^{-/-}$) were treated with ethanol (20 % v/v) for 9 weeks. At the end of treatment, animals were sacrificed and plasma and thoracic aorta were used in biochemical experiments: detection of superoxide anion (O_2^-) by chemiluminescence of lucigenin; determination of thiobarbituric acid reactive species (TBARS); evaluation of nitrate levels and tissue cytokines levels (CEUA protocol: 12.1.1654.53.9). **Results:** Treatment with ethanol increasedof O_2^- generation (Control:149 ± 8 URL, n=5; ethanol: URL 235 ± 13, n=5) in the aorta without PVAT in WT mice. This increase was not observed in $TNFR1^{-/-}$ mice (control: 156 ± 30 URL, n=4; ethanol: URL 184 ± 36, n=4). Ethanol treatment increased plasma TBARS levels (control: 16.24 ± 1.5 nmol /ml, n=7; ethanol 23.9 ± 1.7 nmol/ml, n=6) in WT mice and this increase was not observed in $TNFR1^{-/-}$ mice (control: 18.05 ± 1.9 nmol/ml, n = 6; ethanol: 16.9 ± 2.3 nmol/ml, n = 6). Ethanol treatment increased levels of TBARS in the aorta without PVAT (control: 12.06 ± 0.8 μM/ mg protein, n=5; ethanol: 18.58 ± 2.0 μM/ mg protein, n=6) and with PVAT (control: 9.5 ± 0.8 μM/ mg protein, n = 5; ethanol:16.9 ± 2.5 μM/ mg protein, n=5) in WT mice. Nitrate levels were reduced in the aorta without PVAT (control: 16.6 ± 1.6 μmol/l/ mg protein, n=5; ethanol: 7.7 ± 0.8 μmol/l/ mg protein, n=6) and with PVAT (control: 15.0 ± 3.5 μmol/l/ mg protein, n=5; ethanol:1.9 ± 0.3 μmol/l/ mg protein, n=5). Finally, ethanol treatment increased the tissue levels of cytokines (TNF-α, IL-6, IL-1β and IL-18) in the aorta with PVAT in WT mice. **Conclusions:** Our data show that TNF-α appears to modulate oxidative stress caused by chronic ethanol consumption and apparently the PVAT does not a beneficial or protective role in reducing ethanol-induced inflammation and oxidative damage. **Financial Support:** Capes, NAP- DIN, Fapesp.

06.011

Reactivity of rat, guinea-pig, rabbits and human aortic rings to ouabain, bufalin and digoxin. Martins ICMT, Oliveira IMB, Freire MSS, Santos CF, Nascimento NRF, Fonteles MC ISCB-UECE

The cardiotonic steroids (CE), such as cardenolides and bufodienolides, are classically known as Na^+-K^+ -ATPase (NKA) inhibitors. This mechanism is the basis for its cardiotonic, vasoconstrictor, nerve stimulant and putative diuretic effects. Recently, some cardiotonic steroids have been identified in the plasma, urine and other biological samples of several mammalian species and have been denominated endogenous cardiotonic steroids. In addition, it has been postulated that NKA is also a receptor for cardiotonic steroids and is linked to Src-kinase signaling pathway. These compounds may have a role in both physiological and pathological conditions. In fact, some studies have shown that the plasma and urine levels of such compounds raises in the course of preeclampsia, nephrotic syndrome, renal failure, hypertension, cardiac failure, for example. Nevertheless, most of the pharmacological data concerning these compounds were obtained from rats as models despite its known low sensitivity to CE. The aim of the present study was to evaluate the response of aortic rings (n=4-5) from rats, guinea-pig, rabbits and humans to ouabain (OUA), digoxin (DIG) and bufalin (BUF). The experimental protocols were approved by the Animal Care and Ethics Committee of the Ceara State University under the number 12781206-7 and by the Human Research Ethics Committees of the UFC under the protocol #191.528. The aortic rings (4 mm) were mounted horizontally in 5 ml organ baths for isometric recordings of contractions in response to cumulative addition of OUA, BUF, DIG (0.1-100 μM) or the vehicle added isovolumetrically. The contractions were expressed as percentage of the maximal contraction elicited by phenylephrine. Ouabain, up to 100 μM, promoted no contraction in rat aorta but promoted a slow onset contraction that attained a maximum of 71.5 ± 14.8% and 101.2 ± 8.5% in guinea-pig and rabbit tissue, respectively. Bufalin promoted slow onset contractions after 30 and 100 μM that reached a maximum of 20.6 ± 5.2%, 55.0 ± 10.5% and 94 ± 19.8% in tissues gathered from rats, guinea-pigs and rabbits, respectively). On the other hand, digoxin did not elicit contraction in rabbit or rat aortic rings but elicited a similar slow onset that attained its maximum after more than 50 minutes and reached 79.7 ± 15.6% in guinea-pig tissues. In human aortic rings bufalin only promoted contraction of the vessel in response to 100 μM with a lag of 15-20 minutes and reached a maximum at 96.7 ± 24.4% with bufalin and 140 ± 26.7% with ouabain. The contractions elicited by both compounds were qualitatively similar in regard to require concentrations above 30 μM, beginning after a lag of 15-20 minutes and achieving a maximal after 50-60 minutes after incubation. The tissue sensitivity to CE was similar for rabbit and human rings, smaller for guinea-pig and even smaller for rat tissues. So the best animal model to study the vascular reactivity to cardiotonic steroids is the rabbit. **Financial support**: FUNCAP, Capes, FINEP and CNPq.

06.012

Pinitol attenuates experimental diabetic nephropathy. Cortez LUAS, Paz IA[1] Sousa LGF, Santos FR, Nascimento NRF, Fonteles MC ISCB-UECE

Diabetes nephropathy (DN) is one of the most common cause of glomerulopathy and kidney failure. In Brazil, approximately 25% of type 1 and 5-10% of type 2 diabetic patients develop endstage renal failure. On the other hand, pressure natriuresis is a protective phenomenon that was shown in our lab to be blunted during the course of diabetes. Pinitol (3-O-Methyl-D-chiro-inositol) is a naturally occurring substance that has antidiabetic, antioxidant and anti-inflammatory properties. We have previously shown that pinitol prevents both autonomic and somatic neuropathy and endothelial dysfunction in experimental diabetes. In the present study we aimed to evaluate whether pinitol would prevent diabetic nephropathy, in a 2-month STZ-diabetic rat model. Diabetic nephropathy was evaluated by decreased GFR, increased urinary protein/creatinine excretion and blunted natriuresis. The experimental protocols were approved by the Animal Care and Ethics Committee of the Ceara State University under the number 12237146-1. Diabetes was induced by intraperitoneal injection of streptozotocin (65 mg/kg) in Wistar rats weighing 200-250 g and animals with glycemia higher than 200 mg/dL were randomly divided in 3 groups, euglycemic (E), diabetic (D) and diabetic treated with pinitol (DP; 20 mg/kg/12h p.o during 60 days). 24-hour urine samples were collected in metabolic cages in order to measure the creatinine clearance. 1 week-after evaluation in the metabolic cage, animals were anesthetized and instrumentalized for renal hemodynamics studies. Pinitol at this dosage regime did not affect glycemia after 2-months when compared to control values (before treatment) or when compared to the control non-treated diabetic group. The GFR was reduced from 1.22 ± 0.11 mL/min in the Euglycemic group to 0.309 ± 0.058 mL/min in the Diabetic group. The GFR of rats treated with pinitol was 0.98 ± 0.12 mL/min ($p<0.05$, compared to group D). The urine protein to creatinine ratio was higher in the D group (38.9 ± 4.5 µg/mL/24h) compared to the E group (13.6 ± 2.9 µg/mL/24h; $p<0.05$) and the DP group had a lower ratio (25.03 ± 4.5 µg/mL/24h) when compared to the D group. The pressure natriuresis was evaluated in animals submitted to the same increment in renal perfusion pressure. The fractional excretion of sodium (FE_{Na}) during pressure natriuresis increased 7.4-fold in the E group (0.14 ± 0.06 to 1.0 ± 0.17%), but only 2.6-fold in the D group (0.24 ± 0.07 to 0.62 ± 0.11%; $p<0.05$). The group treated with pinitol had a 4-fold increase in FE_{Na} during pressure natriuresis (0.45 ± 0.4 to 1.80 ± 0.36%). Pinitol ameliorates kidney function in an experimental model of diabetic nephropathy independently of glycemic control. **Financial support**: Capes, CNPq, FUNCAP and FINEP.

06.013

Treatment with doxycycline prevents the renal function impairment in rats subjected to kidney ischemia-reperfusion. Cortês AL, Gonsalez SR, Melo PA, Lara LS ICB-UFRJ

Introduction: Renal injury caused by ischemia is a major cause of acute renal failure. After renal ischemia-reperfusion, activity and expression of matrix metalloproteinases (MMPs) is increased due to an accumulation of inflammatory cells that increase the levels of reactive oxygen species (ROS) and pro-inflammatory cytokines. Doxycycline is an antibiotic derived from tetracycline that in subclinical doses have unveiled various pharmacological effects, such as reduction of inflammation, inhibition of ROS and MMPs. These observations suggest that doxycycline may have a protective role against the damage caused by renal ischemia-reperfusion (I/R). **Objective:** To determine the effect of doxycycline on the reduced renal function of Wistar rats subjected to kidney I/R. **Methods:** Male Wistar rats (Ethical committee approval number: CEUA 137-13) were divided into 3 groups (n = 5, each group and sub-group) (a) Sham-operated (control); (b) I/R: ischemia was induced by applying a non-traumatic vascular clamp in the two renal arteries for 30 min, then the clip was removed for reperfusion occurs and wound was closed;(c) I/R + doxycycline at doses of 1, 3 or 10 mg/kg of body weight (Dc1, Dc3 and DC10, respectively)administered intraperitoneally 2 h before surgery so that during ischemia to reach their plasma concentration peak. After surgery the animals were placed in metabolic cages for 24 hours, at the end of the urine samples and blood were collected. **Results:** The I/R resulted in an elevated proteinuria (2 times greater than control) and doxycycline treatment prevented this increase independent of the dose. Treatment with Dc3 partially prevented: (1) an increase of 268% in blood urea nitrogen; (2) the decrease in glomerular filtration rate (control: 448 ± 62, I/R: 189 ± 33, Dc3: 346 ± 107µl/min). The reduced urinary Na+ concentration observed in I/R, corresponding to 26% of the load was recovered in control with Dc 3 and DC10. The increase of 170% of the $(Na^+ + K^+)$-ATPase activity observed in I/R was blocked by treatment with Dc1 and Dc3. **Discussion:** The results obtained so far indicate that doxycycline prevents renal injury caused by I/R. The administration of doxycycline 3 mg/kg prevented both glomerular as tubular damage, because prevented the fall in glomerular filtration rate, urinary sodium excretion and increase in the $(Na^+ + K^+)$-ATPase activity, being the most effective dose. **Financial support:** CNPq, Faperj, INBEB

06.014

The vascular effects of d-pinitol in mouse small mesenteric arteries. Moreira LN[1], Côrtes SF[1], Lemos VS[2] [1]UFMG – Pharmacology, [2]UFMG – Physiology and Biophysics

Introduction: D-pinitol (3-O-methyl-D-chiro-inositol) is a cyclitol present in several plant species (citrus fruits and vegetables) and has structural and biochemical actions similar to D-chiro-inositol. Recent studies demonstrated the ability of this cyclitol in reversing the endothelial dysfunction and to inhibit the release of pro-inflammatory cytokines in animal models of diabetes mellitus. In humans, D-pinitol shows hypoglycemic activity and reduces systolic and diastolic blood pressure in individuals with type II diabetes mellitus. Considering the importance of cardiovascular diseases in the morbidity and mortality associated with diabetes mellitus, the effects described for the D-pinitol suggest a protective effect of this drug in the cardiovascular system. The present work aimed at investigating the mechanisms involved in the vascular effects of D-pinitol in mouse mesenteric artery. **Methods**: Male mice C57BL/6 with 8-14 weeks were used (Animal Ethics Committees: Protocol 170/2014). Mesenteric arteries 2nd branch were dissected and sectioned at 1.6 to 2.0 mm rings. These segments were mounted in a wire myograph (DMT, Aarhus, Denmark). The rings were kept in physiological saline solution (PSS) of the following composition (in mmol/l): NaCl, 119; KCl, 4.7; KH_2PO_4, 0.4; $NaHCO_3$, 14.9; $MgSO_4$, 1.17; $CaCl_2$, 2.5; glucose, 5.5 at 37 ˚C and aerated with carbogen. **Results and discussion:** D-pinitol induced a concentration-dependent vasodilation with maximum effect (E_{max}) of 20.3 ± 1.8 %, in the presence of a functional endothelium. This vasodilator effect was abolished in the absence of a functional endothelium and in the presence of L-NAME (300 μM), a non-selective inhibitor of nitric oxide synthase (NOS). These results suggest the participation of the endothelium and NOS in the vasodilator effect of D-pinitol. In arteries treated with apocynin (100 μM), a non-selective inhibitor of NADPH oxidase, the concentration-response curve was shifted to the left (pCI_{50} = 7.2 ± 0.5), with a strong increase in E_{max} (72.3 ± 4.9 %), suggesting an inhibition of the vasodilator effect of D-pinitol by NADPH oxidase-derived reactive oxygen species (ROS). These results suggest that the D-pinitol has an endothelium- and NOS-dependent vasodilator effect, modulated by the activation of NADPH oxidase in mouse mesenteric artery. **Financial Support:** Capes and FAPEMIG.

06.015

Increased resting tension abolishes the endothelial anti-contractile effect induced by phenylephrine in renal hypertensive (2K-1C) rat aorta. Silva BR, Grando MD, Bendhack LM FCFRP-USP

The role of mechanical stress for contraction is yet unclear in hypertensive animals. This study aimed to evaluate the role of the rest tension of 1.5 g and 3.0 g on the endothelial nitric oxide synthase (eNOS) activity for contraction induced by phenylephrine (PE) in isolated intact-endothelium aorta (E+) from 2K-1C as compared to normotensive rat aorta (2K). Concentration-effect curves for PE were constructed in E + under rest tension of 1.5 g or 3.0 g, in the absence or after incubation with the NOS inhibitor (L-NAME, 100 μM) for 30 min. The potency (pD_2) and efficacy (ME) of PE were evaluated. eNOS residue Ser^{1177} phosphorylation activates its enzyme. Phosphorylated Ser^{1177} (P-Ser) expression was evaluated by Western Blot. This study was approved by the Ethics Committee of the University of São Paulo (156/2009). ME induced by PE on rest tension of 1.5 g was lower in 2K-1C (1.2 ± 0.2g, n=7; $p<0.001$) than in 2K (2.2 ± 0.1g; pD_2:7.44 ± 0.03; n=5), which was normalized by L-NAME. PE induced higher P-Ser of eNOS in 2K-1C than in 2K aorta, but under rest tension of 3.0 g the PE-induced contraction was similar between 2K-1C and 2K aorta. Our results indicate that the anti-contractile effect induced by PE in 2K-1C E+ aorta under rest tension of 1.5 g is due to NO through eNOS-Ser^{1177} phosphorylation and it is abolished by the increase in tension to 3.0 g. Supported by Fapesp and Capes.

06.016

Are the chronotropic effects of A1 adenosine receptors altered in hypertension?

Câmara H, Rodrigues JQD, Silva Junior ED, Godinho RO, Jurkiewicz A Unifesp – Farmacologia

Introduction: Primary hypertension has high incidence and is the main risk factor for cardiovascular disease. It occurs concomitantly with various metabolic disorders and is associated with many molecular alterations, including changes in central and peripheral purinergic receptors. Adenosine acts in the purinergic system regulating cardioprotection and cardiac automatism. These chronotropic effects of adenosine are mainly due to activation of A_1 receptors. Therefore, this study aimed at evaluating the chronotropic effects induced by adenosine and cyclopentyladenosine in right atria (RA) from normotensive (NWR) and hypertensive rats (SHR). **Methods:** RA was isolated from NWR and SHR (4-6 months old, 350-450 g). After an equilibration period time, cumulative concentration response curves for adenosine (non-specific agonist of adenosine receptors) or cyclopentyladenosine (specific agonist of A_1 receptors) were constructed. The pharmacological parameters pD_2 (potency) and E_{max} (maximum effect) were measured for comparisons between groups. The results were analyzed by unpaired t test and one-way ANOVA. All experiments procedures were approved by the Ethics Committee of Unifesp (n° 5001120214). **Results**: Adenosine (ADO) and cyclopentyladenosine (CPA) exerted a decrease in the chronotropism in a concentration-dependent manner. The maximum effect exerted by ADO and CPA was the cardiac arrest in both groups (0 bpm). The ADO were more potent in RA from SHR (pD_2= 4,40 ± 0,04; n=7) when compared with NWR (pD_2=4,00 ± 0,03; n=3) Similarly, potency of CPA was higher in SHR (pD_2=7,66 ± 0,05; n=4) when compared with NWR (pD_2=7,25 ± 0,04; n=2) . **Conclusion:** The response of adenosine was increased in RA from SHR when compared with NWR. This may indicate changes in the density of A_1 receptors or potentiation of the purinergic signaling pathway in hypertension. **Financial support** (Fapesp and CNPq)

06.017

Cardioprotection evaluation of ipriflavone in spontaneously hypertensive rats. Castro QJT[1], Mosqueira VCF[1], Pereira SC[1], Souza ACM[1], Guimarães HN[2], Leite R[1], Grabe-Guimarães A[1] [1]CiPharma-UFOP, [2]DEE-UFMG

Introduction: Ipriflavone (7-isopropoxy-3-phenyl-4H-1-benzopyran- 4-one) is a semi-synthetic soy derivative, used in several countries for prevention and treatment of osteoporosis. Its cardioprotective effect administered orally was demonstrated in isolated heart of rabbits (Feuer *et al*, 1981). Self-emulsifying drug delivery systems (SEDDS) have gained great importance as a promising technology to improve the bioavailability of poorly water-soluble drugs like ipriflavone. **Objective:** Evaluate the potential cardioprotective effect of SEDDS containing ipriflavone in spontaneously hypertensive rats (SHR) submitted to stimulation with norepinephrine. **Methods**: All the procedures were approved by The CEUA/UFOP (2013/02). Female SHR rats (180 to 250 g) received vehicle or ipriflavone (30 mg/kg) in SEDDS by oral route. The cardioprotective activity of ipriflavone was evaluated *in vivo* in rats for its ability to prevent cardiovascular disorders, demonstrated in signs of arterial pressure (AP) and ECG induced by IV administration of norepinephrine (NE), doses of 3 and 10 µg/kg. **Results:** The ipriflavone did not cause changes of baseline AP (145.1 ± 5.56 mmHg and 135.7 ± 8.51 mmHg, respectively for systolic AP of control and treated groups) and ECG signals of SHR. It was observed a significant increase of the AP after NE administration in both doses in the experimental groups. The PR and QRS intervals of ECG were not altered by NE. The ipriflavone in SEDDS was able to reduce prolongation of QT (16.1 % and 19.1 %) and QTc (17.4 % and 21.2 %) intervals after 3 and 10 µg/kg of NE, IV, compared to animals that received vehicle, showing a significant reduction after administration of 10 µg/kg of NE. It was observed QT values of 93.5 ± 7.45 ms and 101.3 ± 5.20 ms for control group and 79.6 ± 2.68 ms and 79.9 ± 2.91 ms for treated group. For QTc, it was observed values of 149.4 ± 13.42 ms and 167.2 ± 10.66 ms for control group and 120.0 ± 5.27 ms and 130.0 ± 5.06 ms for treated group intervals after 3 and 10 µg/kg of NE. **Conclusion:** Ipriflavone in SEDDS given for single dose presents cardioprotective effect demonstrated by the ability to inhibit the QT interval prolongation. **Funding agencies:** FAPEMIG (Rede Nanobio mg; PPM-00481-13; CDS – APQ-02346-11) and UFOP **Acknowledgments:** CNPq; UFOP; Capes, FAPEMIG

06.018

Treatment with enalapril improves renal function in nephrectomized rats. Pereira JM, Costa PHS, Rodrigues FAP, Alves NTQ, Silva PLB, Coelho YP, Bona MD, Vasconcelos MJS, Alves RS, Monteiro HSA UFC – Farmacologia

Introduction: Chronic Kidney Disease (CKD) is characterized by a slowly progressive and irreversible loss of renal function, requiring dialysis and renal replacement therapy in end-stage disease. Angiotensin II (AgII) appears to play a fundamental role in CKD, contributing to activation of inflammation and increase of oxidative stress, leading to renal fibrosis. Some drugs, such as those that inhibit the renin -angiotensin-aldosterone system, have been used to prevent the progression of the disease. This study aims to evaluate the effects of enalapril, a potent inhibitor of angiotensin converting enzyme (IACE) in an experimental model of CKD in rats. **Methods:** Wistar rats weighting between 250-300 g were used. All procedures were approved by the Ethics Committee in Animal Research of the UFC (protocol 33/12). The animals were divided into 3 groups: sham operated (SHAM), 5/6 nephrectomy (Nx) and 5/6 nephrectomy treated with enalapril (10 mg/kg/day) diluted in drinking water during 6 weeks (Nx + E). Nx and Nx + E underwent surgery for removal of the right kidney and occlusion of 2 or 3 branches of the left renal artery. SHAM group underwent only laparotomy and handling of both kidneys. Before surgery all animals were anesthetized by intraperitoneal administration of ketamine (50 mg/kg) and xylazine (10 mg/kg). After 10 weeks of the surgical procedure, all groups were placed in metabolic cages for collection of 24-hours urine and water intake evaluation. Then, rats were sacrificed for blood collection. Some parameters of renal function were evaluated, such as glomerular filtration rate (GFR) based on creatinine clearance, serum urea (U) and proteinuria (Prot). Left kidney weight (W) in each group was determined. Results were considered significant at $P<0.05$. **Results and Discussion:** Nx showed lower GFR compared to SHAM (GRF_{SHAM} = 0.8552 ± 0.1497 vs GRF_{Nx} = 0.4353 ± 0.07010 * mg/24hrs). There was an increase in the levels of Prot, U and W in Nx group compared to SHAM ($Prot_{SHAM}$ = 9.2216 ± 1.566 vs $Prot_{Nx}$ = 111.6350 ± 16.60 *** mg/24hrs; U_{SHAM} = 49.63 ± 3.615 vs U_{Nx} = 89.43 ± 5.131*** mg/dL; W_{SHAM} = 1.159 ± 1.793 vs W_{Nx} = 1.793 ± 0.09679***g). Nx + E increased GFR compared to Nx (GRF_{Nx} = 0.4353 ± 0.07010 vs $GRF_{Nx + E}$ = 0.8208 ± 0.04665* mL/min). Nx was able to reduce Prot and W, but not Up ($Prot_{Nx}$ = 111.6350 ± 16.60 vs $Prot_{Nx + E}$ = 31.94 ± 6.464 *** mg/24hrs; W_{Nx} = 1.793 ± 0.09679 $W_{nx + E}$ = 1.337 ± 0.06156 N = 9 g **). These results show that the experimental model was effective in inducing CKD. GFR increase in Nx + E may be due to decreased intraglomerular pressure and release of pro-inflammatory cytokines induced by increased AgII in CKD progression. Reduced kidney weight Nx + E confirm the ability of ACE inhibitors to attenuate glomerular hypertrophy. Improvement in proteinuria can be explained by enhanced selectivity of enalapril at the glomerular basement membrane, as described in other studies with ACE inhibitors. Therefore, this study showed that enalapril was effective in improving renal function in nephrectomized rats, confirming its nephroprotective role in CKD.

06.019

The Ca^{2+} ionophore-induced relaxation involves NO-synthase and cyclooxygenase (COX) activation in renal hypertensive (2K-1C) but only NO-synthase in 2K rat aortas. Feitoza PR[1], Silva BR, Bendhack LM FCFRP-USP – Física e Química

Introduction: The vascular endothelium plays physiological role in the control of vascular tone by releasing endothelium-derived relaxing factors (EDRFs) and endothelium-derived contractile factors (EDCFs). The major EDRF is nitric oxide (NO). Cytosolic Ca^{2+} concentration is important for the activation of enzymes such as NOS3 and phospholipase A_2. The cation-specific ionophore (A23187) is a useful tool to study the role of the cytosolic Ca^{2+}. **Aim**: To verify the A23187-induced effect on the renal hypertensive (2K-1C) rat aorta contraction and relaxation and activation of NOS3. **Methods**: In phenylephrine- contracted aortic rings, concentration-effect curves were constructed for the ionophore A23187 (0.1 nM-10 μM) in aortas with intact endothelium (E+) or denuded arteries (E-) isolated from 2K-1C and control normotensive rat (2K) to study the A23187-induced relaxation and at the basal tone to study A23187-induced contractile response. The responses were evaluated in the absence or after incubation with the non-selective inhibitors of NOS3 (L-NAME), COX (Ibuprofen) and L-NAME and Ibuprofen together. We analyzed the maximum effect (ME, g tension). It was quantified [NO]c in endothelial cells isolated from 2K and 2K-1C rat aorta in the presence and absence of A23187. The experimental procedures were approved by the Ethics Committee (No. CEUA 2012.1.120.53.0). **Results**: The A23187 promoted lower relaxation in of 2K-1C rat aorta (E+: 64.6 ± 5.3%, n=6, $p<0.05$) than in 2K (E+: 84.1 ± 6.1%, n=5). The relaxation was impaired in E- aortas in both 2K (16.9 ± 7.0%, n = 4, $P<0.001$), 2K-1C (21.7 ± 5.5 %, n=9, $p<0.001$) or after incubation with L-NAME, 2K (5.6 ± 1.9%, n=4, $p<0.001$) and 2K1C (20.9 ± 3.4% n=3, $p<0.001$). Ibuprofen did not alter the A23187-induced relaxation in 2K aorta, but it was normalized in 2K-1C (83.0 ± 3.6%, n=3, $P<0.05$), whereas the combination of L-NAME + Ibuprofen abolished the relaxation in 2K (:3.0 ± 4.4%, n=4) and 2K-1C (5.4 ± 4.6%, n=5). A23187-induced contraction was similar in 2K E + (0.62 ± 0.19g, n=4) and E- (0.55 ± 0.21g, n=4), but in 2K-1C it was lower in E + (0.11 ± 0.02g, n=3) than in E- (0.74 ± 0.08g, n=8, $p<0.01$). L-NAME did not change the A23187-induced contractile effect in 2K, but it was potentiated in 2K-1C (1.80 ± 0.18g, n=4, $p<0.01$) and (L-NAME + Ibuprofen: 0.71 ± 0.21g, n=5). Ibuprofen decreased the contraction in 2K (0.1 ± 0.04%, n=5, $p<0.05$), but had no effect in 2K-1C. A23187 increased [NO]c only in 2K aorta endothelial cells (from 1207.2 ± 10.3 to 1696.2 ± 46.6, n=4, $p<0.001$). **Discussion**: The Ca2+ ionophore- induced relaxation involves NO-synthase activation in 2K and 2K-1C. In addition, the relaxation is also due to COX activation in 2K-1C rat aortas. At high concentrations, A23187 induces contraction, which is impaired in 2K-1C E + aorta due to NOS3 activation. Supported by Fapesp and CNPq.

06.020

Nitroxyl donor Angeli's salt-induced relaxation of rat veins compared to nitric oxide donors. Zuchi FC, Paulo M, Bendhack LM FCFRP-USP – Physics and Chemistry

Introduction: Nitric oxide (NO) is the most studied of the endogenously generated nitrogen oxides and is well known to mediate many cardiovascular functions. Recently, nitroxyl (HNO) that is the one electron reduced and protonated form of NO, has received significant interest as a cardiovascular agent, with distinct pharmacological actions and therapeutic advantages over NO. Furthermore, HNO is resistant to scavenging by superoxide anion and it does not induce tolerance development. As such, the vascular actions of HNO may be preserved under disease conditions, which make HNO donors an alternative to traditional vasodilators for the treatment of cardiovascular diseases. **Aim**: This study aimed to verify the relaxant effect of the nitroxyl released by Angeli's Salt (AS) compound in cava vein compared to traditional nitrovasodilators Nitroglycerin (GTN) and Sodium Nitroprusside (SNP), and also the new NO donor cis-[Ru(bpy)(2)(py)NO(2)](PF(6)) (RuBPY). We evaluated the maximal relaxation (ME) and potency (pEC_{50}) of the vasodilators in cava vein. **Methods:** Male rats (220-250 g) were killed under anesthesia and vein rings (4 mm length) were removed and mounted in organ chambers for isometric tension measurements. The integrity of the endothelium was tested with acetylcholine (100 µmol/L) after pre-contraction with norepinephrine (10 µmol/L). Cumulative concentration-effect curves were constructed for the vasodilators after pre-contraction with EC_{50} of endothelin-1 (20 µmol/L). When the contractions reached a plateau, the NO donors were added cumulatively (AS: 10 nmol/L –1mmol/L; GTN: 1 nmol/L–10 µmol/L; SNP: 0.1 nmol/L–1 µmol/L; RuBPY: 0.1 nmol/L–10 µmol/L). In addition, cumulative concentration-effect curves were constructed for the compounds in the presence of L-NAME (100 µmol/L), NO-synthase inhibitor, to study the participation of this enzyme in HNO and NO-induced relaxation. All these procedures were in accordance with the guidelines of the Animal Ethics Committee, University of São Paulo, Brazil (CEUA 13.1.993.53.5). **Results:** Our results demonstrated that the ME induced by AS was lower (28.8 ± 2.9%, n=6, $P<0.05$) than the NO donors (GTN: 72.4 ± 3.3%, n=6; SNP: 87.2 ± 5.3%, n=7; RuBPY: 88.5 ± 4.2%, n=6). Since the concentration-effect curves for AS relaxation does not fit a sigmoidal equation, it was not possible to evaluate its pEC_{50}. However, it is possible to classify the potency (pEC_{50}) of the NO donors as follows: SNP (8.07 ± 0.05, n=7, $P<0.05$) > RuBPY (7.21 ± 0.07, n=6) > GTN (6.98 ± 0.07, n=6). The incubation with L-NAME did not change the relaxation induced by HNO (34.9 ± 3.7%; n=5) or NO-donors SNP (82.3 ± 6.0%, n=6) and RuBPY (80.4 ± 5.2%, n=7). But it decreased the ME induced by GTN (52.6 ± 2.1%, n=6, $P<0.05$). **Discussion**: Taken together, these results indicate that AS is less effective to produce vasodilation as compared to the NO donors studied. Previous exposure to L-NAME did not change the relaxation induced by AS, SNP or RuBPY, but it reduced the maximal relaxation induced by GTN. This indicates that NO-synthase modulates the relaxation produced by this compound or even that NO released from GTN activates the NO-synthase enzyme in rat cava vein. Supported by Fapesp and CNPq.

06.021

Effects of sildenafil treatment in experimental renovascular hypertension. Gomes IBS[1], Dias AT[2], Froussard J[3], Cintra A[3], Freitas FP[2], Balarini CM[4], Gava AL[5], Meyrelles SS[5], Vasquez EC[6] [1]UFES – Ciências Farmacêuticas, [2]UFES, [3]EMESCAM, [4]UFPB, [5]UFES – Ciências Fisiológicas, [6]UVV – Ciências Farmacêuticas

Hypertension is a major worldwide health issue. The aim of the present study was to evaluate the beneficial cardiovascular effects of sildenafil treatment on experimental hypertension. Male C57BL/6 mice were subjected to 2K1C hypertension. After 14 days, sildenafil (40 mg/kg/day) or vehicle was administrated, orally, for 14 days. At the end of experimental period, 28 days after the clipping of a renal artery, animals were anesthetized and catheterized for direct arterial pressure measurements, the mesenteric arteriolar bed (MAB) was removed to vascular function studies and intrarenal levels of angiotensin II were determined. All experimental procedures were previously approved by Intitutional Animal Care Committee (CEUA-Emescam, Protocol #02/2013). Data are mean ± SEM. One-way ANOVA was performed followed by Bonferroni post hoc test. $^{*}p<0.05$ *vs*. 2K1C + vehicle. Sildenafil treatment reduced mean arterial blood pressure (125 ± 2 vs. 112 ± 3* mmHg) and heart rate (516 ± 2 vs. 471 ± 12* bpm) in hypertensive-treated animals. Sildenafil treatment prevented the elevation of renal levels of angiotensin II in hypertensive group (179 ± 32 vs. 94 ± 6**p*mol/g). Endothelial function was impaired in hypertensive animals and was successfully restored by sildenafil (Rmax: 48,7 ± 1,8 vs. 67,48 ± 4%**).Blockage of NO production with L-NAME revealed that the participation of this molecule in ACh-induced vasodilation was augmented by sildenafil (dAUC: 51 ± 5 vs. 74 ± 6*a.u). Similarly, blockage with indomethacin showed the increased participation of COX-derived prostanoids in vasodilation of treated animals (dAUC: 9.0 ± 3,8 vs. 29,6 ± 6,5*a.u). The participation of ROS was evaluated by blockage of NAPH oxidase and was greater the participation of ROS in 2K1C group and sildenafil caused a significant reduction of oxidative stress. Therefore, sildenafil can restore endothelial function, reduce blood pressure and intrarenal angiotensin II levels in experimental hypertension and could be suggested as a novel therapeutic approach while treating hypertensive patients. **Financial support**: FAPES (Proc. 54498465/2011 Edital Universal), Capes, CNPq (Proc. 476525/2012-8)

06.022

NCX2121-induced relaxation is due to intracellular NO release and reduction in TXA_2 production. Paula TD[1], Silva BR[1], Grando M D[1], Pernomian L[2], Bendhack L M[1] [1]FCFRP-USP-USP – Física e Química, [2]FMRP-USP – Farmacologia

Introduction: The vascular endothelium plays important role on the tone control by the production and/or release of contractile factors (EDCFs) and relaxing factors (EDRFs). There is an imbalance between EDCFs and EDRFs in hypertension that is defined by endothelial dysfunction. In accordance to several authors, these alterations are due to increased production of reactive oxygen species (ROS) which can affect NO signaling, production and bioavailability. NO is considered the major EDRF. Moreover, the levels of prostaglandins and thromboxane A_2 (TX A_2) that are EDCFs products of COX can be increased. **Aim**: This work aimed to study the NCX2121 (a non-steroidal anti-inflammatory drug associated with NO donor) induced relaxation in 2K-1C rat aorta and to investigate the cellular mechanisms involved in this effect in comparison with normotensive 2K rat aortas. **Methods:** Renovascular hypertension (2K-1C) was induced in anesthetized rats (180–200 g). Another rat group was only submitted to sham-operation (normotensive 2K). After 6 weeks, the systolic arterial pressure (SAP) was measured and the rats were considered hypertensive rats those with SAP ≥ 160 mmHg. The rats were killed under anesthesia and aortic rings (4mm length) were removed and mounted in organ chambers for isometric tension measurements. The integrity of the endothelium was tested with acetylcholine (1 μmol/L).Vascular reactivity was conducted in intact-endothelium (E+) or denuded (E-) rat aortas. Extracellular NO was measured in the solution by amperometry, and in the cytosol ([NO]c) by confocal microscopy. ROS was measured in freshly isolated endothelial cells by flow citometry. The expression of P-NOS was measured by Western Blot and TXA_2 by Elisa immunoassay. The TXB_2 also was measured by kit ELISA immune assay in aorta homogenate. **Results:** NCX2121 induced similar relaxation in 2K-1C and 2K which was impaired by endothelium removal and NOS inhibition. NCX2121-induced relaxation is due to NO, since sGC inhibition by ODQ reduced its effect in (E-) 2K-1C (54.5 ± 5.5% *vs* 13.5 ± 5.1%, n=5, $P<0.05$) and in 2K aorta (70.1 ± 6.8% *vs* 12.9 ± 4.5%, n=5, $P<0.05$). In (E+) 2K aorta, the relaxation to NCX2121 was inhibited by ODQ (89.8 ± 4.4% *vs* 44.2 ± 8.3%, n=5, $P<0.05$) whereas in 2K-1C it was not changed. NCX2121 did not change the phosphorylation of NOS sites. The [NO]c was increased by NCX2121in a similar way in 2K-1C (Δ: 25.1 ± 6.7%) and 2K (Δ: 21.3 ± 6.7%). Extracellular NO was not detected by amperometric measurement. Relaxation was inhibited by ROS since the apocynin increased the relaxation in 2K-1C aorta.NCX2121 decreased ROS in isolated endothelial cells from hypertensive and normotensive rat aorta. NCX2121 reduced TXA_2 levels in 2K and 2K-1C rat aortas. **Discussion**: Our results indicate that the compound NCX2121 induces relaxation by intracellular NO release and COX inhibition due to reduced TXA_2 production. NCX2121-induced relaxation can be impaired by ROS. Number of Ethics Commitee 12.1.120.53.0. Supported by Fapesp, Capes and CNPq.

06.023

Endothelial NO-Synthase is involved in decreased epinephrine-induced contraction in renal hypertensive rat aorta by beta-adrenoceptor activation. Bocalon AC, Silva BR, Grando MD, Bendhack LM FCFRP-USP – Física e Química

Introduction: The sympathetic nervous system plays important role on the endothelium-dependent vascular tone control. Adrenoceptors (AR) agonists can activate endothelial NO-synthase (NOS3). This study aimed to evaluate the effect of NOS3 inhibition with L-NAME on epinephrine-induced contraction and investigate the NOS3 residue Serine^{1177} phosphorylation, in intact endothelium aortas isolated from renal hypertensive (2K-1C) and control normotensive 2K rats. **Methods**: Concentration-effect curves were constructed for epinephrine in 2K and 2K-1C aortas, in the absence or after incubation for 30 min with L-NAME (100 μM). Aorta rings submitted to tension in organ bath were incubated with the antagonists β-AR (propranolol, 10 μM) or α-AR (phenoxybenzamine, 10 μM), for 30 min. The samples were collected after stabilization of contraction and frozen in liquid nitrogen. The protein expression of phosphorylated NOS3 residue Serine^{1177} and total NOS3 were accessed in the samples by Western Blotting. All the procedures were approved by the Ethics Committee of the University of São Paulo (2012.1.1419.53.0). **Results**: Epinephrine-induced contraction was lower in 2K-1C (1.4 ± 0.1g, n=9, $p<0.05$) than in 2K rat aorta (2.6 ± 0.1g, n=11). L-NAME increased the maximum contractile effect of epinephrine in 2K-1C aortas (from 1.4 ± 0.1 n=9 to 2.0 ± 0.2 n=5, $p<0.05$) without changing its potency. L-NAME had no effect on 2K rat aorta. The NOS3 phosphorylation of the activation site Serine^{1177} expressed in densitometric units, was higher in 2K-1C rat aorta (0.80 ± 0.05, n=4, $p<0.05$) than in 2K (0.61 ± 0.03, n=5). Phenoxybenzamine increased Serine^{1177} phosphorylation in 2K (Control: 0.61 ± 0.03, n=5 to 0.99 ± 0.13, n=5, $p<0.05$) but had no effect in 2K-1C rat aorta. Propranolol decreased the Serine^{1177} phosphorylation in 2K-1C rat aorta (Control: 1.38 ± 0.20, n=4 to 0.74 ± 0.12, n=5, $p<0.05$) but it had no effect in 2K aorta. **Discussion**: Our results suggest that reduced epinephrine-induced contractile response in 2K-1C rat aorta is due to activation of NOS3 by Serine^{1177} phosphorylation stimulated by β-AR activation. Supported by Fapesp and CNPq.

06.024

The synergism between ischemic postconditioning and lysophosphatidic acid (LPA) on renal function of rats subjected to kidney ischemia-reperfusion. Gonsalez SR, Monteiro SH, Leal A C, Costa RS, Souza P H M, Einicker-Lamas M, Lara LS UFRJ – Farmacologia

Introduction: Acute renal failure (ARF) is defined as a decrease in renal function in hours or days, resulting in glomerular and tubular changes. Our group observed that LPA prevented glomerular damage from kidney I/R, but was ineffective on the rate of renal Na+ excretion and proteinuria. **Objective:** Using the ischemic postconditioning technique (IPC), that is known to protect the tubules from damage caused by I/R, we aimed to determine the synergistic effect when combined to the pharmacological treatment with LPA in preventing renal function. **Methods:** Adult male Wistar rats (170-200 g) were divided into four groups (n=xxx, each; Ethical committee approval: CEUA 137/13): (1) sham-operated (control); (2) I/R: ischemia was induced by applying a non-traumatic vascular clamp in both renal arteries for 30 minutes. Following, the clamp was removed to promote reperfusion and the wound was closed; (3) I/R + LPA: during the 30 minutes of ischemia related to the procedure, LPA was administered 1 mg/kg body weight into renal capsule; (4) IPC + LPA: After 30 minutes of ischemia and LPA treatment, six cycles of 10 seconds of I/R was applied early of the 24 hours of reperfusion. During the 24 hours of reperfusion, the rats were allocated individually in metabolic cages to collect biological materials (blood and urine) for biochemical analyzes. **Results:** The water intake and Na+ plasma concentration did not modify in all experimental groups. It was observed that the LPA-IPC combination prevented: (1) the blood urea nitrogen accumulation (from 73 ± 19 in the I/R to 44 ± 4.2 mg/dL in the IPC + LPA; value similar to control) and (2) proteinuria (control: 3.9 ± 0.6, I/R: 6.8 ± 0.7, I/R + LPA: 10.2 ± 2.1 and I/R + LPA + IPC: 3.9 ± 0.6 mg/24 h). LPA treatment prevented decrease in urinary creatinine concentration promoted by I/R independent of the association with PCI. Moreover, the reduced LPA plasma concentration found in the I/R (27%) was prevented in both treatments. **Discussion:** Ischemic postconditioning was an efficient method in preventing damage to the renal tubules caused by I/R. In addition, the PCI did not alter the profile of action of LPA on the glomeruli. Alltogether, these data suggest that the synergistic effect of the combination between IPC and LPA is mainly due to the IPC action on renal tubular function. **Financial support:** CNPq, Faperj, INBEB.

06.025

Beneficial effects of aldosterone mediated by GPER (G protein-coupled estrogen receptors) activation are decreased in mesenteric arteries of diabetic animals.

Ferreira NS[1], Cau SBA[2], Manzato CP[1], Silva MAB[1], Carneiro FS[1], Tostes RC[1] [1]FMRP-USP – Pharmacology, [2]UFJF – Basic Health Sciences

Introduction: Diabetes is a chronic disease that affects more than 8% of the world population. Vascular dysfunction is related to the major complications of diabetes. Aldosterone (Aldo) has important effects in the vasculature, inducing inflammation, oxidative stress, remodeling and vascular dysfunction in cardiovascular and metabolic diseases, including diabetes. Aldo exerts its effects via activation of mineralocorticoid receptors (MR) and G protein-coupled estrogen receptors (GPER) inducing genomic and non-genomic effects. GPER activation by estrogen or the G1 agonist produces beneficial effects in the vasculature. This study tested the hypothesis that GPER-mediated vascular effects of aldosterone are decreased/abrogated in diabetic animals. **Methods:** The project was approved by the local Ethics Committee (protocol: 012/2013-1). Second-order mesenteric arteries from control (B6BKS-*Lepr^{db}*/ +) and diabetic (db/db) female mice were incubated with 10 nM Aldo, in the presence of either vehicle (veh), the MR antagonist eplerenone (Eple, 10 μM) or the GPER antagonist G15 (1μM), and the effects on acetylcholine (ACh) vascular reactivity were determined. **Results and discussion:** Aldo reduced ACh maximum response (Emax: veh: 99.5 ± 1.4; n=7 vs. Aldo: 85.9 ± 1.7; n=5 $p<0.05$) and potency in arteries from control (pD_2: veh: 7.5 ± 0.04; n=7 vs. Aldo: 7.1 ± 0.06; n=5 $p<0.05$) and db/db (pD_2: veh: 7.4 ± 0.08; n=6 vs. Aldo: 6.8 ± 0.11; n=5 $p<0.05$) mice. Eplerenone abolished responses induced by Aldo in arteries from control (Emax: Aldo: 85.9 ± 1.7; n=5 vs. Eple + Aldo: 95.1 ± 1.7; n=5 $p<0.05$) and db/db (pD_2: Aldo: 6.8 ± 0.11; n=5 *vs.* Eple + Aldo: 7.14 ± 0.11, n=5) mice. G15 further decreased ACh potency in Aldo-treated arteries from the control group (pD_2: Aldo: 7.1 ± 0.06; n=5 *vs.* Aldo + G15: 6.8 ± 0.07; n=4 $p<0.05$), but did not affect ACh responses in db/db arteries (EC_{50}: Aldo: 6.8 ± 0.11; n=5 *vs.* Aldo + G15: 6.8 ± 0.10; n=5. $p<0.05$). These results demonstrate that GPER have protective effects in control arteries, but its effects are decreased in db/db arteries. Increased GPER protein expression [arbitrary units (Au), 192.3 ± 4.5 vs. 100.0 ± 20.5], but not MR expression, (Au, 99.8 ± 6.1 vs. 100.0 ± 12.9, respectively) was detected in arteries from db/db mice **Conclusions**: Confirming our hypothesis, the beneficial vascular effects of aldosterone mediated by GPER activation are decreased in diabetes mellitus. Our results further elucidate the mechanisms by which aldosterone influences vascular function and contributes to vascular dysfunction in diabetes. **Financial support**: CNPq, Capes, Fapesp.

06.026

Chronic treatment with red wine modulates the purinergic neurotransmission and decrease oxidative stress and blood pressure in rats SHR and diabetic-STZ. Musial DC, Bomfim GHS, Miranda-Ferreira R, Rocha KKHR, Jurkiewicz A, Jurkiewicz NH Unifesp – Farmacologia

It is well known that in hypertension and diabetes the sympathetic dysfunction is present. However, the role of purinergic neurotransmission in these diseases is unknown. Therefore we decided to study the alterations of the purinergic neurotransmission and investigate if the treatment with red wine improves the neurotransmission in these diseases. **Methods:** Wistar Kyoto rats-WKY; diabetic streptozotocin-induced-WKY (60 $mg.kg^{-1}$); Spontaneously Hypertensive Rats-SHR; were treated with saline, EtOH (12.5%) or red wine (12.5%) in an identical volume of 3.715 ml/kg/day/v.o during 3 weeks. Just in the end of treatment it was verified the systolic blood pressure-SBP. Isolated atria (left/right) and thoracic aortic (rings) were used in functional experiments and were stimulated using the purinergic agonists (ATP, ADO, β,γ-Me-ATP). The ventricle was used to analyze the lipid peroxide and catalase activity. All experimental procedures were approved by the Ethical committee of Unifesp (protocol number 1169/11). **Results:** In isolated atrium, the P1 receptor response from diabetes and SHRs was decreased about 20% while the P2 receptor response was increased in diabetic (98%) and SHRs (134%). The same was observed in isolated aorta where the P1 response (pIC_{50} to ADO) as decreased about 25% in SHR and increased about 30% in diabetics. Furthermore the P2X function was increased in SHR. All these alterations in purinergic neurotransmission were improved after the treatment with red wine, resulting in a reduction of SPB in diabetic (10.5%) and SHR (8.96%), but not when treated with EtOH. Besides, the treatments with red wine decreased the lipid peroxide in diabetic and SHR, compared with rats treated with saline. However, the lipid peroxide levels were not altered in the control group compared with saline treatment. In relation to catalase activity, there was an increase in diabetic rats treated with red wine compared with diabetes treated with saline, but in others groups it was not altered. **Conclusion:** These findings are a strong indication that purinergic neurotransmission plays a key role in the control of cardiovascular function contributing to the pathogenesis of diabetics and hypertension. Moreover the moderate consumption of red wine is able to provide an improvement in the cardiovascular system influencing the control of blood pressure in part because the control of purinergic neurotransmission and reduction of oxidative stress. **Financial agencies:** Fapesp, Capes and CNPq

06.027

Impaired vascular reactivity in rats fed with moderately high fructose chow involves changes in nitric oxide production and calcium mobilization. Silva RCF[1], de Souza P[1], Da Silva-Santos JE[2] [1]UFPR, [2]UFSC

Introduction: Fructose overconsumption has been involved with the onset of several diseases, including vascular dysfunction. We hypothesized that significantly chronic small amounts of fructose ingested in early life stages is enough to impair the vascular function. The purpose of this study was evaluated how the excessive intake of fructose can modulate the vascular function contributing to the development of cardiovascular diseases. **Methods:** Male Wistar rats were fed with a moderately high fructose diet (6% F group) for six weeks from weaning (the control group received regular chow). The reactivity of isolated aortic rings to vasoactive agents was evaluated. All procedures were approved by the Institutional Ethics Committee of UFPR (protocol number 601). **Results and discussion:** Endothelium-intact (E+) aortic rings from 6% F rats presented reduced responses to phenylephrine (PE, 1 μM: 0.59 ± 0.05 g against 1.26 ± 0.22 g in control), and vasopressin (30 nM: 0.26 ± 0.07 g against 0.61 ± 0.11 g in control). PE-induced constriction was reestablished in endothelium-denuded (E-) rings, as well as after incubation with L-NAME (100 μM) or ODQ (10 μM). In addition, PE-contracted aortic rings (E+) displayed reduced relaxation in response to acetylcholine (ACh, 1 μM), but not to sodium nitroprusside. This study shows that the ingestion of moderately high fructose in early life stages is enough to impair the vascular reactivity of rat aortic rings, a condition fully dependent of the NO/guanylate cyclase pathway. On the other hand, when tested in calcium-free environment, the vessels from animals fed with the fructose diet displayed augmented responses to both caffeine and angiotensin II, suggesting additional changes in intracellular calcium mobilization and/or function. We are currently investigating the contribution of such changes for the development of cardiovascular diseases associated with high fructose intake. **Financial support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Capes, Brazil; Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brazil.

06.028

Pinitol ameliorates pressure natriuresis in diabetic rats. Sousa LGF, Cortez LUAS, Regis SRS, Fonteles MC, Nascimento NRF, Santos CF UECE – Ciências Fisiológicas

Pressure natriuresis (P-N) is a protective phenomenon that counteracts increased renal perfusion pressure by increasing sodium and water excretion. This phenomenon is dependent on increased medullar blood flow, increased interstitial pressure and is positively modulated by nitric oxide and cyclic GMP. This phenomenon is blunted in all forms of hypertension. This is due to increased oxidative stress, impaired pressure transduction along the kidney circulation and endothelial dysfunction. Since endothelial dysfunction is a hallmark of diabetes and endothelial damage and microcirculatory impairment are early pathogenetic events in diabetic nephropathy we hypothesized that pressure natriuresis is blunted in the course of diabetes. All the protocols for this study were approved by the Animal Ethics Committee of the Ceara State University under the document number 12237146-1. Diabetes was induced by i.p. injection of streptozotocin (65 mg/kg) and control rats (euglycemic) received only citrate buffer (pH 4.0). Rats were divided in the following groups: euglycemic (E), diabetic treated with saline (D) and diabetic treated with pinitol (P). The rats were treated after 2 months of diabetes, in order to develop nephropathy, and were treated during the following 2 months with saline (1 ml/kg/12h;v.o) or pinitol (20 mg/kg/12h; v.o). The animals were kept 24h-period in metabolic cages in the time zero, 2-month and 4-month for kidney function studies. After the treatment period the animals were anesthetized with sodium pentobarbital (60 mg/kg; i.p) and cannulas inserted in the jugular for infusion of 3% albumin and 1% inulin diluted in 5% dextrose at 40 µL/min. Cannulation of the right carotid artery using PE-50 provided arterial access for mean arterial pressure (MAP) and heart rate (HR) measurements. Following a midline laparotomy, the right kidney was removed, and the remaining ureter was cannulated (PE-10) to collect urine. We used a variant of the pressure natriuresis model of Roman and Cowley that has been used previously in our laboratory. Renal perfusion pressure (RPP) was increased during the experimental period by tying off the infrarenal aorta and clamping the superior mesenteric and celiac artery. We measured MAP, sodium excretion (UNaV), glomerular filtration rate (inulin clearence), renal blood flow (RBF), fractional excretion of Na+ (FENa) during a 30 minutes control period (normal renal perfusion pressure) and during 30 minutes of high RPP. During pressure natriuresis UNaV increased from 0.08 ± 0.03 to 0.55 ± 0.15 µEq/ml/min/g in the E group but was completely blocked in the D group (0.16 ± 0.06 to 0.09 ± 0.02 µEq/ml/min/g; $p<0.001$). In the group treated with pinitol pressure natriuresis was partially restored (0.1 ± 0.02 to 0.27 ± 0.08 µEq/ml/min/g). During P-N, FENa increased 278% in the E group, 43% in the D group and 118.2% in the P group. The Renal vascular resistance (RVR) was greatly enhanced in the D group when compared to E (20 ± 1.3 mmHg/mL/min vs. 55 ± 12 mmHg/mL/min; $p<0.001$). In the P group RVR was 27.2 ± 2.2 mmHg/mL/min (0,57 ± 0,27µEq/min/g). During P-N, RBF and GFR were not different among groups. Pinitol is able to restore P-N that is impaired in the diabetic group but the mechanisms were not addressed in the present study. This work was supported by CNPq and Funcap.

06.029

Potential vasodilatory and anti-inflammatory effects of a new thienylacylhydrazone derivative LASSBio-788. Motta NAV[1], Fumian MM[1], Brito GB[1], Barreiro EJ[2], Kummerle AE[3], Brito FCF[1] [1]LAFE-UFF, [2]UFRJ – Avaliação e Síntese de Substâncias Bioativas, [3]UFRRJ – Química

Introduction: Atherosclerosis is the major cause of cardiovascular diseases and is characterized by progressive deposition of lipid and fiber in arteries. This disease is closely associated with inflammation and oxidative stress (Ross, RN Engl J Med., 340(2): 115, 1999). The compound LASSBio-788 is a thienylacylhydrazone derivative with antiatherogenic effects in hypercholesterolemic rats (Motta, NAV *et al*., J Pharmacol Sci., 123: 47, 2013). Although LASSBio-788 presents several *in vivo* properties, the mechanisms involved in its effects remain unknown. In this study, we aimed to investigate the mechanism underlying the effects of LASSBio-788. **Methods:** The animal protocols were approved by the Ethics Committee for Experimental Research of the Federal Fluminense University (CEPA/ UFF 00287/12). Adult male Wistar rats (150-200g) were randomly divided into four groups (n= 10, for each group): Control (C) and positive control (C +) group: has received a normal rat chow for 45 days. Hypercholesterolemic (HC) and Hypercholesterolemic + LASSBio-788 (HC + 788) group: have received a hypercholesterolemic diet (HCD) for 45 days. The vehicle (tween, etanol and water, 1:1:8) (0.05 ml/kg/i.p.) was administered to C and HC, whereas LASSBio-1425 (100 µmol/kg/i.p) was administered to the treated groups C + and HC + 788, in the last 15 days treatment. The animals were euthanized by cervical dislocation and decapitated under anesthesia. Blood samples were collected, the thoracic aorta, and liver were excised for ELISA, western blot and histological analysis respectively. Data were analyzed using one-way ANOVA followed by a post-hoc Bonferroni Multiple Comparison Test, $p<0.05$. **Results:** In western blot analysis, we observed a significant reduction of endothelial nitric oxide synthase (eNOS) protein expression (0.43 ± 0.03 vs. 0.64 ± 0.10), an increase in the inducible nitric oxide synthase (iNOS) (3.21 ± 0.24 vs. 1.35 ± 0.05), VCAM-1(1.17 ± 0.01 vs. 0.82 ± 0.01) and nuclear factor kappa B (NF-kB) (2.52 ± 0.11 vs. 1.63 ± 0.08) in the HC group when compared to C group. LASSBio-788 was able to reverse the damage promoted by diet (eNOS: 0.86 ± 0.06; iNOS: 1.59 ± 0.01, VCAM-1: 0.79 ± 0.01, NF-kB: 1.82 ± 0.01) when compared to HC group. We also observed an increase of TNF-α serum levels in HC group (52.2 ± 4.4 vs. 34.7 ± 3.1 pg/ml). On the other hand, LASSBio-788 promoted a decrease of TNF-α serum levels, indicating an important anti-inflammatory effect (33.3 ± 2.6 pg/ml). In histological parameters, the HC group presented a lipid acumulation in the cytoplasm of almost all hepatocytes (microsteatosis). LASSBio-788 protected the liver from injury. Liver morphology also suggests that LASSBio-788 is not hepatotoxic once there were no statistical differences between the C, HC+788 and C+788 groups, suggesting that LASSBio-788 only acts on inflammatory process. **Discussion:** According to the results obtained, the mechanism of action of LASSBio-788 is associated to the inhibition of TNF-α and the decreasing of NF-kB activation. These effects reduce the cytokines synthesis and oxidative stress, reestablishing the endothelial function in vascular bed. **Financial Agencies:** Capes, PROPPI-UFF, Faperj.

06.030

Serotonin and venous hyporeactivity in endotoxemic rats. Carioletti GH[1], Nardi GM[2], Linder AE[1] [1]UFSC – Farmacologia, [2]UNOESC

Introduction: The fall of blood pressure is one of the clinical signs observed in septic shock and is accompanied by hyporeactivity to vasoconstrictor agents. The increase in platelet aggregation is associated with microvascular failure[1]. Platelets are a major deposit of serotonin (5-hydroxytryptamine, 5-HT) at the periphery and, when activated, release 5-HT[2]. Knowing that chronic administration of 5-HT induces a fall in blood pressure [3], we aimed to test the hypothesis that altering 5-HT homeostasis contributes to the changes in vascular tone in experimental sepsis induced by intraperitoneal (ip) administration of lipopolysaccharide (LPS) in rats. **Methods:** Male Wistar rats (250-300 g) were divided into 3 groups: Group 1 received vehicle (1 ml/kg, ip); Group 2 received LPS (10 mg/kg, ip), and Group 3 received ketanserin (5 mg/kg, ip) 5 minutes before LPS (10 mg/kg, ip). After 6 and 24 hours from the treatments, endothelium intact jugular vein rings were isolated and mounted in an isolated organ system for isometric tension measurements. Concentration response curves (CRC) induced by 5-HT (10^{-9}M to 10^{-5}M) were constructed in these rings. The presence of endothelium was assessed by the ability of acetylcholine (1 µM) to relax the jugular vein rings contracted with 5-HT (1 µM). Only the tissues that relaxed more than 50% were considered for this study. Data represent the mean ± standard error of the mean of the results of maximum contraction (MC) expressed in mg or pD_2 values (-log effective concentration of 5-HT which caused 50% of its maximum response) obtained or calculated from CCR from "n" experiments and submitted to the Student's t test. (Experimental protocol approved by the ethics committee: PP00706). **Results and discussion:** The CCR-induced by 5-HT 6 hours after LPS treatment was shifted to the right (pD_2 = 6.4 ± 0.1, N = 4, $p<0.05$) and showed reduced MC (485.4 ± 92.4 mg, $p<0.05$) compared to that obtained in vessels from animals treated with vehicle (pD_2 = 6.8 ± 0.05, MC = 897.7 ± 94.8 mg N = 5). When compared to the group that received only LPS, pretreatment with ketanserin before LPS shifted the CRC induced by 5-HT to the left (pD_2 = 7.1 ± 0.1, N = 5, $p<0.05$) with increased MC (1185.8 ± 191.4, $p<0.05$) in the groups evaluated 6 hours after drug administration. . The contractile response induced by 5-HT was not different between groups 1 and 2 evaluated 24 hours after the treatments. These data suggest that the hyporeactivity to vasoconstrictors in the rat jugular vein observed 6 hours after induction of sepsis can be reversed by treating the rats with ketanserin. This hyporeactivity seems to disappear 24 hours after induction of sepsis. **Conclusion:** The 5-HT released by platelet aggregation contributes to vascular hyporeactivity observed in the experimental model of sepsis induced by LPS. **References:** 1-Mostefai HA *et al.*, 2008. *Am J Respir Crit Care Med*. 1;178(11):1148-55; 2-Mössner R, Lesch KP 1998. *Brain Behavior Immun*. 12: 249; 3-Diaz J *et al.*, 2008. *J Pharmacol Exp Ther* 325(3):1031-8. **Financial Support:** CNPq/FAPESC, PPG-FMC-UFSC.

06.031

Effects of early weaning on nutrition and cardiovascular parameters of rats in different life stages. Barros RBM, Marques EB, Rocha NN, Scaramello CBV UFF – Neurociências

Introduction: The World Health Organization recommends that infants should be exclusively breastfed for the first six months of life (WHO 2003). Interventions in this period may affect offspring in adulthood (Bonomo IT *et al*, J Endocrinol. 198:331,2008). This biological phenomenon is called programming (Lucas A *et al*, *Arch Dis Child* 71:288,1994). Obesity, insulin and leptin resistance in addition to lipid profile worse may be programmed by early weaning in rats (Lima NS *et al*, *Br J Nutr* 105:1405,2011). However, the consequences of those alterations on cardiovascular system remain unknown. **Aim**: Investigate if early weaning modulates nutrition and cardiovascular parameters of rats in different life stages (90 and 150 days-old rats). **Methods:** A day old male Wistar rats were randomly assigned into two groups – Control group (C), which pups had free access to milk during all lactation (21 days-old) or Early Weaning (EW), which pups had free access to milk until 18 days-old. After weaning, pups were placed in cages with water and food *ad libitum*. Offspring body weight and food intake were monitored until 150 days-old allowing feed efficiency ratio determination (Novelli *et al. Lab Anim*, 41:111,2007). Body length, abdominal and thoracic circumferences as well as Body Mass Index were determined to 90 and 150 days-old rats. Echocardiographic studies were performed in these animals and left ventricular mass (LVM), diastolic relative wall thickness (RWT), interventricular septal wall thickness (IVS) and posterior wall thickness (LVPW) were determined. Maximal effortergometer test was also performed (Brooks & White. *J Appl Physiol*, 45:1009, 1978). Additionally, serum samples were analysed in order to measure lipid/glycaemia profile. Data are presented as mean ± SEM, analyzed by Student's t test and considered statistically different if $P<0.05$. Present study was approved by local ethical committee (389/2013). **Results**: At 21 days of life, EW offspring had a higher bodyweight compared to C group (39.1 ± 0.4gX46.5 ± 0.7g), but at 90 days-old EW body weight overcame C group (373.6 ± 5.8gX366.2 ± 4.0 g) and at day 150 no statistical difference was noticed (456.1 ± 6.5gX459.4 ± 7.6 g). Body length, BMI and thoracic/abdominal circumference ratio were similar between C and EW groups in both ages tested. EW group presented a greater Feed efficiency ratio from 21 to 90 days-old (0.22 ± 0.00X0.12 ± 0.00) but no difference was noteworthy from 90 to 150 days-old (0.06 ± 0.01X0.06 ± 0.01). Glycaemia was higher just in 90 days-old EW group than C group at the same age (133.8 ± 3.4 mg/dLX170.7 ± 8.94 mg/dL) but not at day 150 (182.2 ± 2.54 mg/dLX173.6 ± 13.74 mg/dL). No statistical difference was found among groups about lipid profile. Echocardiographic studies showed a greater LVM (1.17 ± 0.03gX1.41 ± 0.04g) as well as diastolic IVS (0.15 ± 0.00cmX0.19 ± 0.00cm), LVPW (0.15 ± 0.00cmX0.19 ± 0.00cm) and RWT (0.42 ± 0.03X0.53 ± 0.03) in 90 days-old EW group. At day 150, EW also showed a higher LVM (1.23 ± 0.04gX1.4 ± 0.07g), IVS (0.14 ± 0.00cmX0.16 ± 0.00) and LVPW (0.28 ± 0.01X0.31 ± 0.01). Both groups had similar performance at maximum effort test. **Discussion**: Indexes of overweight and lipid profile did not point to cardiometabolic risk for EW group, however Echocardiographic studies suggest a diastolic dysfunction where animals evolved from concentric ventricular hypertrophy to eccentric. **Financial Support**: CNPq, Capes, PROPPI/UFF.

06.032

PK 11195 Translocator Protein 18 Kd ligand improves parameters commonly evaluated in experimental sepsis. Quibem LA[1], Ribeiro JT[1], Arruda TB[1], Linder AE[2], da Silva-Santos JE[2], Nardi GM[1] [1]UNOESC – Farmacologia, [2]UFSC – Farmacologia

Introduction: Sepsis is defined as systemic inflammatory response syndrome (SIRS) in which there is an identifiable focus of infection. As a consequence of the overactive SIRS response, the function of various organ and systems may be compromised, resulting in multiple organ dysfunction syndrome and death. SIRS is results from the activation of the innate immune system and it is characterized by intravascular release of pro-inflammatory cytokines and other vasoactive mediators, and the concurrent activation of the innate immune cells. In addition to pro-inflammatory reactions, the host's anti-inflammatory mechanisms are also activated and aimed at counteracting the inflammatory response. Ligands of Translocator Protein 18 Kd (TSPO) plays an important role in the regulation of immune function, as the modulation of the monocyte functions such as cellular chemotaxis, inhibition of inflammatory cytokines production and lymphoid cell proliferation. TSPO, such as PK 11195 (PK), stimulate steroid synthesis in several tissues enhancing the translocation of cholesterol from the outer to inner mitochondrial membranes. This compound exhibits potent anti-inflammatory action in models of acute inflammation. The purpose of the present study is to investigate the role of PK in the experimental model of sepsis. **Methods:** Sepsis was performed by cecal ligation and puncture surgery (CLP) in female Wistar rats (250-300 g) and divided into 4 groups. Group 1 received vehicle (1 ml/kg, ip); Group 2-4 received PK (0.1, 0.3 and 1 mg/kg, ip) 2 h after. After 24 hours from the treatments, blood pressure and vascular reactivity to three consecutive doses of phenylephrine (3, 10 and 30 nmol/kg, i.p.) were evaluated. Additionally, animals were treated with PK (1 mg/kg, ip), 1 h before or 2 h after the CLP procedure and mortality were recorded every 12 h, during 96 h. The results are expressed as mean ± standard error of mean (SEM) or percent inhibition. Statistically significant differences between groups were measured using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical analysis of mortality curve was performed using logrank test. (Experimental protocol approved by the ethics committee: protocol nº 06/2013). **Results and discussion:** CLP reduced the response to phenylephrine ~30 %. Treatment with PK, in doses of 0.3 and 1 mg/kg was effective to restore the vasoconstrictor effect of phenylephrine (30 nmol) in 30 and 62 % in comparison to non-treated animals. Animals submitted to CLP surgery show a substantial hypotension (77.8 ± 6.7 mmHg vs. sham 109.0 ± 6.43 mmHg). PK doses of 0.3 and 1 mg/kg reduced sepsis hypotension in 27 % (98.9 ± 6.5 mmHG) and 25 % (97.5 ± 6.8 mmHG) respectively, in comparison to non-treated animals. Survival of the CLP rats declined sharply in the first 24 h and then progressively up to 96 h. Rats injected with PK, 2 h after the CLP surgery survived significantly longer than the CLP group (80 versus 40%, respectively). These data suggest that treatment with PK might significantly affect survival and improve the cardiovascular parameters in septic rats. **Conclusion:** Thus, stimulation of steroidogenesis by PK 11195 improves parameters commonly evaluated in experimental sepsis. **References:** Torres SR, Fröde TS, Nardi GM, Vita N, Reeb R, Ferrara P, Ribeiro-do-Valle RM, Farges RC. Anti-inflammatory effects of peripheral benzodiazepine receptor ligands in two mouse models of inflammation. *Eur J Pharmacol*. 2000;408:199-211. **Financial Support:** CNPq/FAPESC, Unoesc.

06.033

Serotonergic antagonists in septic shock. Lobo KL[1], Nardi GM[2], Linder AE[1] [1]UFSC – Pharmacology, [2]UNOESC – Biological and Health Sciences

Introduction: In septic shock, it is observed a decrease in mean arterial pressure (MAP) as well as hyporeactivity to vasoconstrictors agents, which contribute to the high mortality rate associated with this pathology [1]. Studies also demonstrate that platelets may be the key players in septic shock, and that these platelets when activated release 5-HT [2]. As 5-HT drop blood pressure in rats[3] the hypothesis is that the use of receptor antagonists of 5-HT may increase vascular reactivity in septic shock, thereby reducing the fall in MAP. **Materials and Methods:** Male Wistar rats received intraperitoneally lipopolysaccharide (LPS, 10 mg/kg) or saline (1 ml / kg). After 6 hours the rats were killed and aortic rings were isolated and mounted on a system for isometric tension measurements. Vascular reactivity was analyzed by a cumulative concentration response curve (CCRC) induced by norepinephrine (NE, 1 nM to 30 mM). In another set of experiments, CCRC induced by NE was also performed on rat aortic rings treated with LPS *in vitro*, but not *in vivo* as above mentioned. The aortic rings were incubated for 12 hours with physiological saline solution (PSS) or LPS (10 mg / ml). Some vessels incubated in the presence of LPS were also treated simultaneously with 5-HT (10 μM) or with the following antagonists of 5-HT receptors: SB269970 (10 μM; 5-HT7 antagonist); GR127935 (10 μM ; 5-HT1B/1D antagonist) ; LY266097 (10 μM, 5-HT2B antagonist) ; Way100135 (10 μM, 5-HT1A antagonist) and ketanserin (10 μM; 5-HT2A/2C antagonist). Data represent the mean + /- standard error of the mean of the results of maximum contraction (MC) expressed in g or of the pD2 values (-log of the effective concentration of NE which caused 50% of its maximum response) obtained or calculated from the CCRC curves of n experiments and submitted to Student's t test (CEUA PP00706). **Results and discussion:** The CCRC induced by NE in aortic rings from animals treated *in vivo* with LPS showed reduced maximum effect (MC = 1.4 ± 0.1, $p<0.05$) and it was shifted to the right (pD2 = 6.9 ± 0.1, n = 8, $P <0.05$) when compared to that obtained in vessels of rats treated with saline (MC = 2.4 ± 0.3, 7.5 ± pD2 = 0.1, n = 6). Incubation for 12 hours *in vitro* with LPS reduced the maximal effect (MC = 0.3 ± 0.05, n = 6, $P <0.05$) induced by NE without displacement of CCRC (pD2 = 6.8 ± 0.4 n = 6) when compared to the results obtained in rings incubated with vehicle (MC = 1.3 ± 0.3, pD2 =6.9 ± 0.4, n = 5). The MC induced by NE was increased by the treatment with the 5-HT receptor antagonists GR127935 (MC = 0.8 ± 0.1, n = 5), ketanserin (MC = 0.6 ± 0.09, n = 6) and SB269970 (MC = 0.09 ± 1.0, n = 5) when compared to the results obtained in rings treated with LPS. Based on these results we can say that the administration of LPS both *in vivo* as well as *in vitro* decrease the MC to vasoconstrictors such as NE. Moreover, some antagonists of 5-HT receptors improved vascular reactivity, suggesting that 5-HT is involved in the vascular changes observed in septic shock. As a next step, we will treat the animals *in vivo* with LPS and with the 5-HT receptor antagonists to verify whether these treatments improve vascular reactivity or even MAP fall in septic shock. **References:** 1) Failure J.F *Rev. Ciênc Farm. Basic Apl*, 29, 119, 2008. 2) Walther, A. *et al.*; *Journal of Surgical Research*, 143, 216, 2007. 3) Diaz, J. *et al.*; The Journal of pharmacology and experimental therapeutics, 325, 1031, 2008. **Financial Support:** CNPq / FAPESC, PPG-FMC-UFSC

06.034

Endothelium-dependent dilatory effect of estrone, an equine estrogen, in rat thoracic aorta. Oliveira TS, Oliveira LM, Peixoto LF, Filgueira FP, Ghedini PC UFG – Farmacologia Bioquímica e Molecular

Introduction: The hormone replacement therapy (HRT) with conjugated estrogens presents cardioprotective effects; however, recent clinical trials do not have supported these benefits. Knowing that the conjugated equine estrogens (CEEs) is a pharmaceutical preparation commonly used in the HRT and considering the controversy about its health benefits, the present study was designed to investigate the vascular effects of the estrone (E1), that is the more abundant estrogen in the CEE (48% of its content). **Methods:** The vasorelaxant effect of E1 was evaluated in preparations of thoracic aorta rings of male Wistar rats (200-300g) using organ chambers. Concentration-response curves to E1 (0.1μM – 100μM) were constructed in phenylephrine pre-contracted aortic rings with either intact or denuded endothelium. Relaxation responses to E1 in intact endothelium rings were performed in the absence or presence of the estrogen receptor antagonist ICI 182,780 (10μM), the G-protein-coupled receptor (GPER) antagonist G15 (30μM), the calcium-calmodulin ($Ca2^{+}$-CaM) complex inhibitor Calmidazolium (10μM), the PI3K inhibitor Wortmannin (1μM), the p38 MAPK inhibitor SB 22580 (10μM), the nitric oxide synthase inhibitor L-NAME (100μM), the soluble guanylate cyclase inhibitor ODQ (10μM), the cyclooxygenase inhibitor (Indomethacin (10μM) or the non-selective K+ channel blocker TEA (1mM). All experiments were conducted in accordance with the Brazilian Society of Laboratory Animal Science (SBCAL) and were approved by the local Ethics in Research Committee (Protocol CEUA/UFG 20/2013). Data are presented as mean ± SEM of 4-7 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 and discussion:** E1 induced concentration-dependent relaxation in endothelium-intact aortic rings (E_{max} = 41.04 ± 1.89%), whereas in endothelium-denuded vessels it did not have any effect. The incubation of aorta rings with ICI 182,780, Calmidazolium, Wortmannin, L-NAME or ODQ significantly reduced E1-induced relaxation (E_{max} = 13,39 ± 3.06%, 30.34 ± 1.32%, 12.37 ± 1.25%, 7.97 ± 2.63% and 7.12 ± 1.36%, respectively). On the other hand, the incubation with G15, SB 203580, Indomethacin or TEA did not inhibit the relaxation induced by E1 (E_{max} = 46.57 ± 4.36%, 37.68 ± 3.22%, 45.53 ± 4.34% and 46.76 ± 9.61%, respectively). These results suggest that the vasorelaxant effect of E1 is endothelium-dependent and mediated by activation of estrogen receptors which in turn results in activation of NO/cGMP pathway by PI3K signaling and a $Ca2^{+}$/CaM complex -activation dependent manner. **Financial Support:** Capes, FAPEG, CNPq

06.035

Morphometric and biochemistry evaluation in protective effect on myocardial of the hydroalcoholic extract of red propolis in infarcted rats with isoproterenol. Clementino-Neto J, Tenório EP, Ferreira AKB, Beiriz RV, Moura MTD, Oliveira JMS, Nascimento TG, Ribeiro EAN ESENFAR-UFAL

Introduction: Propolis is a resin that bees collect from different plant sources. This resin has a complex chemical composition. The existence of a red coloured propolis has been reported in the northeastern coast of Brazil (states of Alagoas, Bahia, Paraiba, Sergipe and Pernambuco). Red propolis is biologically active, having antimicrobial and antioxidant activity, among others. The present study was designed to evaluate the cardioprotective effects of hydroalcoholic extract of red propolis from Alagoas (HAERP) against isoproterenol-induced myocardial infarction in rats by studying cardiac markers and morphometric changes. **Methods:** Male Wistar rats (200-300g) were divided into 5 groups (n = 5), (G1 = 0.3 mL Saline P.O for 30 days), (G2 = infarction by isoproterenol 85 mg/kg s.c on 2 consecutive days), (G3, G4 and G5 treaties with HAERP 50, 75 and 150 mg/kg, respectively, orally for 30 days and underwent infarction with isoproterenol 85 mg/kg s.c in 29 and 30 days of treatment). On the 31th day the animals were euthanized, serum was collected and incubated in biochemical kits for the evaluation of CK-NAC, CK-MB, LDH markers of tissue damage. For morphometric analysis, the hearts were measured the thickness ventricular of left and right, and reason cardiac weight by body weight (TLV, TRV and WC/WB). The results were expressed as mean ± SEM, and analyzed statistically by ANOVA one-way followed by Newman-keuls. Considered significant when *p<0,05, **p<0,01, ***p<0,001 vs. G1; + p<0,05,++p<0,01,+++ p<0,001 vs. G2; ·p<0,05, ··p<0,01, ···p<0,001 vs. G3; #p<0,05, ##p<0,01, ###p<0,001 vs. G4; °p<0,05, °°p<0,01, °°°p<0,001 vs. G5. All protocols were approved by the Ethics Committee of UFAL under number 12A/2014. **Results and discussion:** WC (G1 = 1.1 ± 0.1; G2 = 1.4 ± 0.1**; G3= 1,3 ± 0,1*; G4= 1,2 ± 0,1^{+}; G5=1,2 ± 0,1^{+} g, respectively), TLV (3.8 ± 0.4; 4,9 ± 0.1***; 4,6 ± 0,2***; 3,8 ± 0,2$^{\cdots/+++}$; 3,8 ± 0,2$^{\cdots/+++}$ mm), TRV (3,1 ± 0.2; 4.0 ± 0.1***; 3,6 ± 0,2^{+++} ; 3,1 ± 0.1^{+++} ; 3,0 ± 0.1^{+++} mm), WC/WB (4,20 ± 0,12; 5,43 ± 0,10***; 4,71 ± 0,20^{+++} ; 4,77 ± 0,20^{+++} ; 4,78 ± 0,20$^{+++)}$, CK-NAC (471,2 ± 31,2; 1035 ± 134,7***; 697,7 ± 47,6^{++}; 578,7 ± 57,14^{+++} ; 397,9 ± 34,58^{+++} $^{/\cdot}$ U/L), CK-MB (414,4 ± 18,5; 740,8 ± 77,3***; 505,6 ± 43,5^{+++} ; 496,0 ± 32,9^{++}; 376,8 ± 6,3^{+++} U/L), LDH (131,9 ± 13,1; 233,2 ± 31,4*; 131,1 ± 14,9^{+}; 195,1 ± 29,1; 158,7 ± 13,63 U/L). The results indicate that the treatment with HAERP was able to prevent hypertrophy concentric cardiac, it is observed that there is a decrease in TLV and WC/WB. In the biochemical analysis observed a reduction in counting enzyme CK-NAC and CK-MB fraction after treatment with HAERP in three doses. There was no significant change in LDH counting, in three doses, when compared to saline control. The results indicate that treatment with HAERP was able to promote protective activity in myocardial infarction. **Financial support:** CNPq and FAPEAL.

06.036

Ethanol withdrawal induces oxidative stress: Role of AT1 receptors. Gonzaga NA[1], Tirapelli CR[2] [1]FMRP-USP – Farmacologia, [2]EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas

Introduction: Cardiovascular, physiological and behavioral changes developed after partial or total interruption of chronic ethanol consumption are known as ethanol withdrawal syndrome (EWS), which is characterized by tremors, anxiety, agitation, seizures, *delirium tremens* and hypertension. Activation of the renin-angiotensin system with consequent increase in systemic levels of angiotensin II (ANG II) is also described during EWS. The aim of our study was to evaluate the role of AT_1 receptors on oxidative stress induced by ethanol withdrawal. With this purpose, we induced a pharmacological blockade of the AT_1 receptor with losartan. **Methods:** Male *Wistar* rats (250g) were divided into 6 groups: Control/Control: animals received water *ad libitum* for 23 days and vehicle by gavage (water); Control/Losartan: animals received water *ad libitum* for 23 days and losartan (gavage, 10 mg/kg/day); Ethanol/Control: chronic ethanol treatment was started with an ethanol solution 3% (vol/vol) and gradually increased every three days to 6% (day 4) and 9% (day 7 onwards), being this concentration maintained until day 21, and daily gavage of water; Ethanol/Losartan: the group was treated according to the description of the Ethanol/Control group and with losartan (gavage, 10 mg/kg/day); Withdrawal/Control: animals were treated in the same way of the ethanol groups until day 20. Then, ethanol solution 9% was removed and returned the next day (day 21) for 2h. After the end of this period the animals received water until day 23 and daily gavage of water; Withdrawal/Losartan: the group was treated according to the description of the Withdrawal/Control group and with losartan (gavage, 10 mg/kg/day) during the period of ethanol abstinence. Plasma thiobarbituric acid reactive substances (TBARS-nmol/mL) were measured colorimetrically. Aortic O_2^- generation (RLU/ mg protein) was measured by lucigenin chemiluminescence. Data are presented as means ± standard error of the mean. Groups were compared using one-way ANOVA. All protocols were approved by the Ethics Committee (Protocol: 11.1.1432.53.5). **Results:** Ethanol withdrawal increased plasma TBARS (20.1 ± 2.2; n=9) when compared to control groups (Control: 10.7 ± 1.9; n=7; Control + Losartan: 12.4 ± 1.9; n=7) or ethanol groups (Ethanol: 13.2 ± 1.5; n=9; Ethanol + Losartan: 14.5 ± 1.6; n=7). Losartan prevented the increase in plasma TBARS induced by ethanol withdrawal (9.8 ± 1.9; n=7). Ethanol withdrawal induced a significant increase in O_2^- generation (655.7 ± 51.2; n=7) when compared to control groups (Control: 248.7 ± 19.8; n=8; Control + Losartan: 214.9 ± 18.8; n=8) or ethanol groups (Ethanol: 228.5 ± 18.7; n=8; Ethanol + Losartan: 214.0 ± 21.0; n=7). Losartan prevented the increase in O_2^- generation induced by ethanol withdrawal (199.0 ± 24.3; n=8). **Conclusion:** Our data show that ethanol withdrawal induces systemic and vascular oxidative stress by a mechanism that involves AT_1 receptor activation. **Financial support:** CNPq and Fapesp.

06.037

Evaluation of the antiacoagulant agent curcumin in bleeding models by transection of the tail in mice. Macêdo AJR, Neta MJCN, Campêlo JAC, Rosa LD, Feitosa ML INTA – Farmácia

Introduction The *Curcuma longa* or safflower, as it is also popularly known is considered a precious spice. The *Curcuma longa*, besides extensively used in popular medicine to treat various diseases, it is also often cited in the literature for the variety of activities it presents. The main compounds responsible for the activities of the plant are curcumin and its derivatives. Curcumin is apolyphenoic compound responsible for yellow color characteristic of the rhizome of *Curcuma longa* main component. It has anticoagulant actions identified through pharmacological studies and medicinal activities. Hemostasis is a complex instrument, which the organism coordinates and acclimate the blood fluids within blood vessels to prevent thrombosis or bleeding. The bleeding time (BT) is one of the most requested laboratory tests in the preoperative routine to identify the risk of bleeding. Thus, the bleeding time test despite simple, evaluates a large number of complex interrelated events. The present work aims to carry out an assessment of the anticoagulant action of Curcumin by testing *in vivo* bleeding time "tail transection" in mice. **Methodology** The research was of the experimental type with a quantitative and qualitative approach. Adult micés of the Swiss species were used (25-30 g). In the research to develop heparin 100 IU (intraperitoneally) was used as a positive standard. Curcumin was used at a concentration of 100 and 200 mg/kg orally. Curcumin was dissolved in 6% DMSO and then diluted in sodium phosphate buffer. The mice were randomized into different groups subjected to the following treatments: Curcumin at concentrations 100 and 200 mg/kg and a positive control with 100 IU heparin. Control animals received vehicle (distilled water 10 ml / kg). The Curcumin 100 and 200 mg/kg groups and the control group were treated orally in an acute form 3 h before the experiment. The heparin group was treated intraperitoneally in an acute form 1 h before the experiment. Bleeding was induced by tail end section of 3 mm from the tip with the help of a scalpel. The statistical analysis was performed using Graph Pad Prism version 5.0 for Windows (Graph Pad Software, San Diego California USA) software. The results, which followed a parametric distribution were analyzed by ANOVA followed by Student Newman Keuls (post hoc) test, and the values represented by the mean ± SEM. The criterion of significance was $p<0.05$. The project was approved by the ethics committee of colleges INTA with the protocol number 201307005. **Results** Curcumin increased the bleeding time in a dose-dependent way, in the same way, the positive control group (heparin) showed the same effect. In the control group the mean bleeding time in minutes was 14:33 ± 2.65 in the Curcumin group at a dose of 100 mg/kg was 27.86 ± 2:44 increasing approximately 94.4% over the control and 200 mg/kg was 35.54 ± 5:13 with an increase of 148% compared to control and in the group of heparin at a dose of 100 IU was> 60. **Discussion:** It is known that heparin is the main prescribed anticoagulant in nowadays and which has extensive clinical experience. However, this substance has disadvantages, such as side effects of systemic anticoagulation induced by unfractionated heparin (UFH) thrombocytopenia. Curcumin has shown such anticoagulant action. However, there is an imminent desire to identify new resources for getting new drugs, which have greater pharmacological activity, lower toxicity and lower rates of drug interactions. **Acknowledgments:** We are thankful for INTA for financial support.

06.038

Apocynin prevents oxidative stress induced by chronic ethanol consumption in the rat mesenteric bed. Hipolito UV[1], Leite LN[2], Tirapelli CR[1] [1]EPCH-USP, [2]USP – Farmacologia

Introduction: The chronic ethanol consumption may be associated with many cardiovascular diseases such as hypertension. However, little is known about the molecular mediators associated with cardiovascular effects induced by chronic ethanol intake. Studies suggested that endothelial dysfunction is a potential mechanism responsible for the increase in blood pressure associated with ethanol consumption and the formation of ROS derived from NAD(P)H oxidase lead to vascular endothelial dysfunction. Therefore, the aim of this study is to evaluate the involvement of NAD(P)H oxidase in the effects induced by chronic ethanol consumption in the rat mesenteric bed through its inhibition by apocynin (APO). **Methods:** The experimental protocols were approved by the Ethical Committee from USP (13.1.472.53.5). Male Wistar rats (200-250g) were divided into 4 groups: Control group (C): received drinking water by oral gavage; Control + APO group (AC): APO (30 mg/kg/day, oral gavage); Ethanol group (E): ethanol 20% (v/v) for 6 weeks and drinking water by oral gavage; Ethanol + APO group (AE): ethanol 20% and APO (30 mg/kg/day, oral gavage. Systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and heart rate (HR) were measured by tail-cuff plethysmography. Superoxide anion levels in mesenteric bed homogenates was evaluated by lucigenin-derived chemiluminescence assay. Nitrate levels were measured in plasma and supernatants from mesenteric bed homogenates. **Results**: Chronic ethanol consumption increase the systolic arterial pressure (SAP) ($p<0.05$, ANOVA).On the other hand chronic ethanol did not affect diastolic arterial pressure (DAP), heart rate (HR) and mean arterial pressure (MAP). Superoxide anion levels (RLU/ mg protein) were higher in mesenteric bed from ethanol-treated rats (42.8 ± 6.9, n=5) when compared with control (14.5 ± 3.1, n=8). APO prevented the increase in superoxide anion levels induced by ethanol (AC: 18.0 ± 5.4 n=7 AE: 25.8 ± 2.5 n=6) ($p<0.05$, ANOVA). Ethanol decreased nitrate levels in mesenteric bed (16.5 ± 4.4 n=5) when compared with control group (45.2 ± 6.4 n=). APO prevented this effect (36.5 ± 3.0 n=6) ($p<0.05$, ANOVA). **Conclusion:** Chronic ethanol consumption induced oxidative stress and reduced nitrate levels in mesenteric bed, being these responses prevented by APO. **Financial Support:** Fapesp.

06.039

Cardiovascular effects induced by tetrahydrofurfuryl nitrate (NTHF), a new nitric oxide donor, in spontaneously hypertensive rats. Silva TAF[1], Furtado FF[2], Machado NT[1], Queiroz TM[3], Alustau MC[1], Assis VL[3], Vasconcelos WP[1], Oliveira-Filho AA[1], Veras RC[1], Santos AF[3], Athayde-Filho PF[3], Medeiros IA[1] [1]DCF-CCS-UFPB, [2]ETSC-CFP-UFCG, [3]CCS-UFPB

Introduction: The organic nitrates are the most commonly nitric oxide (NO) donors used in the treatment of cardiovascular diseases, mainly due the pronounced vasodilator effect. Previous studies have shown that the tetrahydrofurfuryl nitrate (NTHF), an organic nitrate obtained through of synthetic route from sugarcane, induces vasorelaxation in mesenteric artery with involvement of the NO-cGMP-PKG pathway, in addition, caused hypotension and bradycardia in normotensive conscious rats. The current research aimed to investigate the effects of NTHF on cardiovascular parameters in spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats. **Methods**: All protocols were approved by CEUA/UFPB (0310/09 and 0207/12). For *in vivo* experiments, SHR and WKY rats were anesthetized. Abdominal aorta and inferior vena cava were cannulated for pressure recordings and administration of drugs, respectively. For *in vitro* experiments, isolated rat superior mesenteric rings were suspended by cotton threads for isometric tension recordings in a Tyrode's solution at 37 ºC, gassed with 95% O_2 and 5% CO_2; and primary cultures of rat thoracic aortic smooth muscle cells were obtained by the explant Method: **Results and discussion:** NTHF increased NO levels in rat aortic smooth muscle cells, detected by NO-sensitive probe DAF-2T by flow cytometry. The intravenous injection of NTHF (10, 20, 30, 40 e 50 mg/kg, randomly) produced a dose-dependent hypotension in SHR (8.4 ± 1.6 ; 21.6 ± 4; 28.8 ± 6.2; 55.6 ± 5; 59.10 ± 5%) and WKY (7.5 ± 2; 14.2 ± 3.0; 32.1 ± 14.8; 58.2 ± 4.6; 74.8 ± 5%) and bradycardia in SHR (6.2 ± 1.5; 9.17 ± 4.3; 39.9 ± 10; 85.8 ± 3.5; 90 ± 2%) and WKY (3.8 ± 1.4; 5 ± 1.7; 28.3 ± 14; 90 ± 2.7; 90 ± 2.3%). In mesenteric artery rings from SHR and WKY rats pre-contracted with phenylephrine (1 µM), NTHF (10^{-18}-10^{-6} M) induced a concentration-dependent vasorelaxation in presence (SHR: MR=90 ± 7%, pD_2 = 11.9 ± 0.3; WKY: MR = 99.1 ± 6.4%, pD_2 = 11.7 ± 0.3) or absence (SHR: MR=105 ± 8%, pD_2 = 11.4 ± 0.2; WKY: MR= 105 ± 6.4%; pD_2 = 11.8 ± 0.3) of endothelium. Furthermore, in the presence of PTIO (300 µM), a free radical form of NO ($NO^{\bullet}$) scavenger, the vasorelaxation induced by NTHF was decrease in SHR (MR= 67.8 ± 8%) and WKY (MR= 57 ± 5.5%). However, in the presence of L-cysteine (3 mM), a reduced form of NO (NO^-) scavenger, the NTHF effect was not changed in SHR (pD_2= 11.9 ± 0.29, MR=96.7%) and WKY (pD_2= 12.6 ± 0.5, MR=93.9%). The NTHF effect was attenuated in the presence of cyanamide (1 mM), a mitochondrial aldehyde dehydrogenase inhibitor (mtALDH) in SHR (MR= 52.2 ± 9.8%) and WKY (MR=56.98 ± 9.5%). The vasodilation was also reduced in the presence of ODQ (10 µM), a soluble guanylyl cyclase inhibitor (sGC) in SHR (MR= 33 ± 7%) and WKY (MR=20.48 ± 4%); and TEA (3 mM), a non-selective K+ channel blocker in SHR (MR=66.4 ± 8.4%) and WKY (MR=46.96 ± 10%). These results together suggest that NTHF induces hypotension and bradycardia and promotes vasorelaxation due to $NO^{\bullet}$ release by a mechanism dependent of mtALDH metabolism and consequent activation of sGC and K channels in SHR and WKY rats. **Financial support**: CNPq and Capes

06.040

Functional and molecular analyses of heart in different experimental models of sepsis in rats. Gonçalves RPM, Assreuy J, da Silva-Santos JE UFSC – Farmacologia

Introduction: Bacterial lipopolysaccharide (LPS) injection and the polymicrobial sepsis induced by the cecal ligature model (CLP) have been widely used to address the cardiovascular dysfunction in sepsis. However, the existence of model-specific differences between these approaches has been scarcely investigated. This study was designed to compare the profile of changes in the *in vivo* cardiac function of rats subjected to LPS and CLP models. **Methods:** Male Wistar rats received a single injection of LPS (10 mg/kg, i.p; LPS group), or were subjected to CLP (50% of cecal ligature, 4 holes using a 18G needle; CLP group). The cardiac function was analyzed with a pressure-volume catheter (SPR-901, Millar Instruments Inc.) inserted in the left ventricle (LV) using the closed-chest method, in rats previously treated with 2 mg/kg xylazine (i.p.) and under anesthesia (isofluorane, 1-3%). The analyses were performed at the peak of the hypotensive state (mean arterial pressure-MAP ≤ 60 mmHg), which was observed at 6 h and 48 h in LPS and CLP groups, respectively. The effects of a single injection of 30 nmol/kg dobutamine (i.v.) were evaluated to better explore the cardiac function. Control values were recorded in naïve rats (C). The heart was collected and used for Western blot analyses. All procedures were approved by the Animal Care and Use Committee of UFSC (protocol PP00566). **Results:** LPS and CLP groups presented MAP of 56 ± 3 and 59 ± 1 mmHg (C: 73 ± 1 mmHg). The ejection fraction (C: 67 ± 4%) was similarly reduced in both LPS (42 ± 4%) and CLP (47 ± 3 %) groups. Although both LV developed pressure (LVDP) and dP/dt_{max} (C: 90 ± 2 mmHg and 5205 ± 318 mmHg/s, respectively) have also been reduced, these parameters were significantly ($p<.05$) more depressed in LPS than in CLP rats (LVDP: 71 ± 2 and 80 ± 3 mmHg; dP/dt_{max}: 2787 ± 165 and 3665 ± 369 mmHg/s, respectively). In addition, only LPS group presented reduced stroke work and stroke volume, while the end-systolic volume was significantly increased in the CLP but not in the LPS group. The heart rate increased only also in LPS group (C: 281 ± 17 vs 324 ± 14 bpm, $p<.05$). In the time evaluated, there were no changes in cardiac output or end-diastolic volume, neither in LPS nor in CLP groups. Dobutamine administration improved the ejection fraction, LVDP and dP/dt_{max} systolic parameters similarly in both LPS and CLP groups. The end-systolic volume, however, presented a significant reduction only in LPS group after dobutamine (C: 50 ± 13 vs 8 ± 2 µL for LPS group). Interestingly, the expression levels of both the ryanodine receptor and the RhoA small GTPase were increased in the LV of CLP rats, but were found reduced or unchanged in the LPS group. **Discussion:** According to our results, both CLP and LPS models present the classical septic myocardium depression evidenced by reduction of the systolic parameters studied. The basal and after dobutamine cardiovascular analyses, however, showed a different cardiodynamic profile on the experimental models used. We are currently investigating how the changes in intracellular calcium mobilization, evidenced by the increased expression of ryanodine and RhoA contribute for the results observed. **Research support**: CNPq and FAPESC (TO 1366/2010-8, TR2012000367 and TR201200078)

06.041

β-citronellol evokes vago-vagal bradycardiac and hypotensive reflex in normotensive rats. Veras HRF, Silva CMS, De Siqueira RJB, Lahlou S, Magalhães PJC UFC Physiology and Pharmacology

Introduction: β-Citronellol (βC) is a monoterpene found in the essential oil of plants from the *Cymbopogon* genus, some used in folk medicine to treat hypertension such as *C. citratus* (DC) Stapf. βC possesses hypotensive and vasorelaxant properties, but the mode by which it induces such effects remains unclear. Our major aim was to characterize how βC induces its cardiovascular effects. **Methods**: Under anesthesia, male Wistar rats (270-300 g) were subjected to cannulation of the left femoral artery and vein for mean arterial pressure (MAP) monitoring and drug injection, respectively (CEPA Nº: 37/2014). Subcutaneous electrodes allowed heart rate (HR) recordings. MAP and HR changes elicited by intravenous (i.v.) βC (1, 5, 10 and 20 mg/kg) were determined in awake rats pretreated or not with methylatropine (MA; 2 mg/kg, i.v.). Other group of rats was tracheostomized under anesthesia (urethane, 0.8 g/kg; i.p.) for simultaneous MAP, HR and respiratory rate (RR) responses to intravenous injection of bC (10 mg/kg) before and after bilateral vagotomy (BV) or perineural treatment (PT) of both cervical vagus nerves with capsaicin (250 µg/ml). **Results:** Administered i.v. to awake rats (n=7), βC elicited hypotensive and bradycardiac effects characterized by a rapid (P1; maxima effect (Emax) of -67.4 ± 3.2% of MAP and -89.2 ± 1.9% of HR at 20 mg/kg βC) followed by a delayed phase (P2; Emax of -72.6 ± 1.0% of MAP and -85.3 ± 4.7% of HR at 20 mg/kg βC), but only P2 followed a dose-dependent behavior ($p<0.001$, ANOVA). At 10 mg/kg, βC elicited submaximal effects of 51.5 ± 8.2% of MAP and a 56.7 ± 4.7% of HR in P1, which peaked at 1.9 ± 0.2 and 1.5 ± 0.3 s after βC injection, respectively. In addition, P2 was characterized by a hypotension corresponding to -51.5 ± 8.2% (peak at 90.0 ± 0.3 s) and bradycardia of -56.7 ± 4.7% (peak at 36.5 ± 3.5 s). To animals subjected to βC (10 mg/kg), treatment with MA abolished P1 of hypotension and bradycardia, while it was inert to change the hypotensive effect in P2 (-48.0 ± 2.6% of MAP; n = 4; $P > 0.05$, Student's t-test). In contrast, bradycardia in P2 was significantly reduced by MA (-7.1 ± 1.5%; $p<0.001$; t-test; n = 4). In anaesthetized animals subjected to βC (10 mg/kg), apnea was also observed during P1 and both BV and PT abolished P1 of hypotension and bradycardia, but not apnea. Hypotension was not altered ($P > 0.05$, Student's t-test) in P2 by BV (-61.8 ± 8.5%; n=5) or PT (-51.8 ± 4.3%; n=5), but the bradycardia in P2 was significantly lower after BV (-18.8 ± 3.2%; $p<0.05$; t-test; n = 4) or PT (-14.0 ± 2.0%; $p<0.001$; t-test; n=4). **Discussion:** βC induces biphasic hypotension and bradycardia, being P1 of a vago-vagal reflex origin because it was abolished by the interruption of the vagus neural traffic by BV, or by the afferent desensitization after PT. Such phenomena induced by βC could not be of central origin. The vagal efferent component of this reflex recruits muscarinic receptors because the peripheral antimuscarinic MA abolished it. In P1, the bradycardia is the primary event that leads to hypotension since it peaked in a time shorter than the peak of hypotension. It seems that P2 is caused by a direct vasorelaxant action of βC. In P2, the residual significant bradycardia may be due to direct action of βC on cardiac muscle. **Financial Support:** CNPq.

06.042

Ethyl acetate fraction from the inflorescences of *Mimosa caesalpiniifolia* reduces intracellular calcium in smooth muscle. Santos MEP[1], Silva-Filho JC[1], Moura LHP[1], Arcanjo DDR[1], Citó AMGL[2], Paulo M[3], Restini CBA[4], Bendhack LM[3], Oliveira AP[1] [1]UFPI – Plantas Medicinais, [2]UFPI – Química, [3]FCFRP-USP, [4]FMRP-USP

Introduction: The *Mimosa caesalpiniifolia* Benth. (Mimosaceae) is a woody plant that occurs naturally in the Brazilian northeastern "cerrado region". The medicinal use of this species is based on the flowers tea for the treatment of hypertension (Albuquerque UP, *J. Ethnopharmacol*., 114, 325, 2007). Previous studies have been reported the endothelium-independent vasorelaxant effect induced by the *M. cesalpiniifolia* ethanol extract or its ethyl acetate fraction (Mc-AcOEt) in rat mesenteric artery. **Material and Methods:** Male Wistar rats (270 ± 30 g) were used for all experiments (Animal Research Ethics Committee/UFPI -008/12). Rat mesenteric artery rings were obtained, and the vasorelaxant effect of Mc-AcOEt was evaluated on phenylephrine (10^{-5} M) -, KCl (80 mM) or S-(-)-BayK-8644 (10^{-5} M)-induced pre-contractions, and the isometric tension was recorded by a force transducer coupled to a data acquisition system (AECAD 1604, AQCAD 2.8.0., AVS Projects, SP, Brazil) (Oliveira AP, *Vascul Pharmacol*, 44, 338, 2006). The fluorescence intensity of the Ca2+ ion was measured by confocal microscopy (Oliveira APS, *Vascul Pharmacol*, 50, 153, 2009). The cytotoxicity of Mc-AcOEt was evaluated by VSMC and HUVEC cells viability using MTT test. All values were expressed as mean ± S.E.M. Student's t-test, and results were considered significant when $p<0.05$. (GraphPad™ Prism 5.0). **Results and discussion:** The Mc-AcOEt promoted endothelium-independent vasorelaxant effect. A similar effect was observed on KCl-induced pre-contractions (pD_2=2.62 ± 0.05). Mc-AcOEt induced vasorelaxation in mesenteric rings pre-contracted by S-(-)-BayK-8644 (pD_2 = 2.35 ± 0.08), an activator of the Ca_V.L channels. Likewise, Mc-AcOEt decreased intracellular calcium concentration in approximately 30%(70.5 ± 9.525%) observed by confocal microscopy. The relaxation caused by Mc-AcOEt did not was altered in preparations with tetraethylammonium (TEA) 3mM. The Mc-AcOEt did not reduce cell viability of both HUVECs (viability control: 100.0 ± 1.5%, 105 µg/mL: 99.60 ± 2.20%, 500 µg/mL, 107.60 ± 3.30%, n=3) and VSMCs (viability control: 100.0 ± 1.55%, 105 µg/mL: 107.13 ± 2.10%, 500 µg/mL: 98.30 ± 3.30%, n=3). Thus, Mc-AcOEt probably reduced intracellular calcium concentration in smooth muscle cells leading to vasorelaxation. The fraction did not affect cell viability in VSMC and HUVEC cells. **Financial Support:** This work was supported by FAPEPI, Capes, and CNPq.

06.043

Impaired vascular function in sepsis-surviving rats: evidence for endothelial dysfunction mediated by angiotensin II, increased ROS/RNS Generation and augmented activity of RHO-kinase. de Souza P[1], Scheschowitsch K[2], da Silva LM[1], Guarido KL[2], Werner MF[1], Assreuy J[2], da Silva-Santos JE[2] [1]UFPR – Pharmacology, [2]UFSC – Pharmacology

Introduction: Several epidemiological studies reveal that the mortality rate among those who survive sepsis is strikingly higher when compared with age-matched people. We hypothesized that impairment of vascular function contribute to this higher mortality rate. **Methods:** Male Wistar rats were subjected to cecal ligation and puncture to induce sepsis (mortality rate ~ 30%). The isometric responses of aortic rings from survived animals were tested in organ baths at 30 or 60 days after (S30 and S60 groups, respectively). Functional and molecular changes were explored by Western blot and fluorescence techniques. The results obtained were compared with data from control (CT) rats; authorization from CEUA/UFPR: 527. **Results:** The effects of KCl, phenylephrine, angiotensin I, $CaCl_2$, acetylcholine and sodium nitroprusside were unchanged in aortic rings from S30 and S60 groups, compared with the CT. In contrast, angiotensin II (AII)-induced vasoconstriction was increased by 70% in aortic rings from S60 group. Interestingly, in opposite to vessels taken from CT rats, endothelium removal, incubation of L-NAME (100 µM) or losartan (1 mM), was unable to alter the contractile effects of AII in aortic rings from the S60 group. On the other hand, the NADPH oxidase inhibitor apocynin (1 mM), the O_2^- scavenger tempol (0.3 mM), and superoxide dismutase (SOD; 300 U/mL) fully avoided the enhanced responses to AII in the S60 group. When compared with the CT group, the activity of SOD in aortic homogenates of the S60 group was decreased by 45 ± 3%, while the generation of hydroperoxides lipids (LOOH) and glutathione (GSH) were increased by 41 ± 12% and 98 ± 3.9%, respectively. An increased ROS generation (up to 30%) was found in vessels from S60 rats after AII-stimulation, as detected by the fluorescent probe DHI. Importantly, immunofluorescence approaches revealed increased levels of tyrosine nitration (up to 70%), when compared to CT vessels, indicating exacerbated peroxynitrite production in vessels from the S60 rats. In addition, aortic rings from the S60 group presented a rightward shift of the concentration-response relaxation curve induced by the Rho-kinase (ROCK) inhibitor Y-27632 – EC50 of 0.43 (0.33-0.55) versus 1.03 (0.76 – 1.40) µM, in CT and S60 groups, respectively. Moreover, incubation with 0.3 µM Y-27632 reduced by 60% the effects of AII in aortic rings from CT, but was completely ineffective against AII-induced contraction in vessels from the S60 group. Western blot analysis revealed that when compared with the CT, vessels from the S60 group presented increased levels of RhoA, ROCK II and the phosphorylated MYPT-1, the main target of ROCK, without changes in the expression of ROCK I and total MYPT-1. **Discussion:** Our study discloses that under stimulation by AII, aortic rings from sepsis-surviving rats display endothelial dysfunction mediated by increased production of O_2^-, which in turn reduces the bioavailability of NO and increases the formation of $ONOO^-$. This increased oxidative and/or nitrosative stress may enhance the calcium sensitization mediated by the RhoA-ROCK pathway, leading to increased contractile responses to AII. In conclusion, this is the first study demonstrating long-lasting changes in the vascular and endothelial function of sepsis-surviving rats. **Research support:** Capes and FAPESC (2012000367 and 201200078).

06.044

LASSBio-1425 – antiatherogenic and anti-inflammatory activity of a new phtalimide derivate. Fumian MM[1], Motta NAV[1], Leite TRS[1], Maia RC, Barreiro EJ[2], Brito FCF[1] [1]UFF – Fisiologia e Farmacologia, [2]UFRJ – Síntese e Avaliação de Substâncias Bioativas

Introduction: Atherosclerosis was considered a consequence of dyslipidemia, but recent investigations have revealed that chronic inflammatory processes associated with dyslipidemia and endothelial dysfunction are also important contributors to its development (ZHANG *et al.*,Trends Pharmacol., 28: 286, 2008). LASSBio-1425, a phtalimide derivative, showed relevant anti-inflammatory activities (Dissertação de mestrado Milla Fumian, 2010, UFRJ). Previous studies have demonstrated hypolipidemic activity of phtalimides derivatives (CHAPMAN *et al.*, J. Med. Chem., 26: 237, 1983; WYRICK *et al.*, J. Med. Chem., 28: 286, 1984). Our hypothesis is that LASSBio-1425 plays a protective role in cardiovascular diseases. **Methods**: The animal protocols were approved by the Ethics Committee for Experimental Research of the UFF (CEPA 00287/12). Adult male Wistar rats were randomly divided into three groups: Control group has received a normal rat chow for 45 days. Placebo and LASSBio-1425 group have received a hypercholesterolemic diet for 45 days. The vehicle (tween-ethanol-water: 1:1:8) (0.05 ml/kg) was administered to Control and Placebo groups, whereas LASSBio-1425 (100 µmol/kg) was administered to the LASSBio-1425 group. The treatments were orally given, once a day, at the last 15 treatment days. The animals were euthanized by cervical dislocation under anesthesia. Blood samples were collected and the thoracic aortas were excised. Data were analyzed using one way analysis of variance with the Bonferroni's test ($p<0.05$). **Results**: Evaluating the effect of LASSBio-1425, this treatment was able to reduce serum levels of total cholesterol (1619.0 ± 123.6 x 1061.0 ± 130.6 mg/dL), triglycerides (192.5 ± 46.0 x 107.3 ± 7.8 mg/dL), LDL (873.7 ± 64.0 x 665.0 ± 67.0 mg/dL), and increase serum HDL levels (25.4 ± 3.8 x 96.7 ± 17.0 mg/dL) when compared with the placebo group. The treatment with LASSBio-1425 was able to reduce platelet aggregation induced by collagen (5 µg/mL) in 25%. The treatment with LASSBio-1425 produced a significant potency reduction of phenylephrine induced contractions (CE_{50}= $8.3x10^{-8}$M x CE_{50} = 7.4 x 10^{-7}M), and on the other hand promoted an increase in acetylcholine induced vasodilation effect (CE_{50}= $2.2x10^{-7}$M x CE_{50}= 1.5 x 10^{-7}M) when compared to placebo group. Treatment with LASSBio-1425 was also able to inhibit the production of TNF-α and IL-6 (52.1 ± 4.4 x 31.8 ± 2.0 e 110.7 ± 5.4 x 49.4 ± 1.2 pg/mL, respectively, comparing to the placebo group). The treatment with LASSBio-1425, reduced the expression of NF-kB (2.5 ± 0.1 x 1.6 ± 0.1 arbitrary units NF-kB/β actin), iNOS (3.2 ± 0.2 x 1.5 ± 0.1 arbitrary units iNOS/α-tubulina) and increased eNOS expression (0.4 ± 0.1 x 0.84 ± 0.03 arbitrary units eNOS/β actin) in aortic tissues, when compared to the placebo group. The treatment with LASSBio-1425 produced a significant decrease in the thickening of the aorta wall (142.0 ± 1.5 µm x 106.3 ± 5.5 µm) when compared to placebo group. **Conclusion**: The data indicate the compound LASSBio-1425 as a promising candidate for the treatment of atherosclerosis, highlighting its potential antiatherogenic effect when administered *in vivo*. **Financial Agencies**: Capes, PROPPI-UFF, Faperj.

06.045

***In vitro* tolerance induced by sodium nitrite in mice aorta**. Araújo NF, Bonaventura D
UFMG – Farmacologia

Introduction: Organic nitrates are NO donors used in cardiovascular disease treatment. The clinical limitation of organic nitrates is the loss of hemodynamic effects during chronic treatment, a phenomenon that characterize tolerance. The occurrence of tolerance associated with sodium nitrite, another NO donor, is unknown. Based in these facts the aim of this study is to verify whether sodium nitrite induces tolerance and, if it occurs, which factors are involved. This study was approved by Ethics Committee of UFMG (n° 038/2010). **Methods:** Vascular reactivity study was performed in thoracic aortas isolated from Balb/C mice. We analyzed the maximal effect (Emax) and potency (pD_2) for sodium nitrite. Cumulative concentration effect curves were performed in aorta, with or without endothelium, pre-contracted with phenylephrine (Phe 100 nM). *In vitro* tolerance was induced by incubation with EC_{50} and EC_{100} of sodium nitrite for 15 minutes. After incubation, aortas were washed for 15 minutes and then pre-contracted with Phe to performed sodium nitrite relaxation. Once characterized the tolerance phenomenon, protocols were performed to verify which factors could mediate nitrite-induced tolerance. In this way, during induction of tolerance, L-NAME (0.1 mM), a nitric oxide synthase (NOS) inhibitor or L-arginine, a substrate for NOS, or Tiron, a superoxide scavenger or Ibuprofen, a nonselective COX inhibitor, were added. **Results:** The nitrite pD_2 values are similar in intact (pD_2: 3.92 ± 0.06) and denuded (pD_2: 4.02 ± 0.11) aortas and the EC_{50} and EC_{100} of sodium nitrite are respectively, 0.01 µM and 0.1 mM. In tolerance analysis, the results showed that previous exposition to EC_{50} for 15 did not alter the nitrite relaxation in intact (pD_2: 4.00 ± 0.06) and denuded (pD_2: 4.24 ± 0.14) aortas. However, in aortas treated with EC_{100} for 15 minutes there is a significant reduction of pD_2 values of nitrite in intact (pD_2: 3.52 ± 0.08) and denuded (pD_2: 3.62 ± 0.10) aortas, suggesting the occurrence of tolerance in both preparations. During evaluation of the factors that may be related with nitrite tolerance, the results showed that incubation with L-NAME during tolerance induction prevented tolerance for sodium nitrite (pD_2: 3.98 ± 0.08) in intact aortas, suggesting the involvement of NOS in this phenomenon. Similarly, the incubation with L-arginine was also able to reverse nitrite induced tolerance (pD_2: 3.74 ± 0.04) in intact aorta. Incubation of denuded aortas with Tiron, prevented the tolerance phenomenon, but, in intact aortas this effect was not observed. On the other hand, Ibuprofen was able to prevent nitrite tolerance in intact and denuded aortas. **Conclusion:** In conclusion, our results demonstrate that, *in vitro*, nitrite EC_{100} is able to induce tolerance and this phenomenon seems to involve NOS uncoupling caused by decreased bioavailability of L-arginine and increased production of superoxide anion associated to COX participation. **Financial Support:** CNPq and FAPEMIG

06.046

Bufalin induced vasoconstriction at pharmacological concentrations involves adrenergic pathway in guinea pig vascular segments. Amorim LS[1], Oliveira IMB[1], Freire MSS[1], Siqueira RJB[2], Santos CF[1], Nascimento NRF[1], Fonteles MC[1] [1]UECE – Fisiofarmacologia Cardiovascular e Renal, [2]UFC

Bufalin is a cardiotonic steroid (CS) originally isolated from amphibians, but recently detected in tissues and body fluids of mammals. CS are classically known as Na+ -K+ -ATPase (NKA) inhibitors, and may contribute to the genesis of certain forms of hypertension. However, several aspects about the pathophysiological significance of these compounds remains unclear, such as whether they promote vascular effects at physiological levels and what mechanisms are involved in these responses. We used the guinea-pig as an animal model since they are sensitive to NKA inhibitors. We choose to evaluate the *in vitro* effects of bufalin (BUF; 0.1 – 100μM) in aortic and 2nd branch of guinea pig mesenteric artery rings. The concentration-response curves to BUF were evaluated in the presence or absence of guanethidine (GUA – 10μM) or 1μM prazosin, in order to check the role of adrenergic nerves. Tissues were mounted horizontally, in 5ml organ baths, for isometric contractions recordings, and bufalin was added cumulatively at intervals of 3 minutes. All the responses were expressed as percentage of the maximal contraction promoted by phenylephrine (a complete concentration curve was done in each experiment). For the branches of mesenteric artery, the wire myograph-Mulvany apparatus was used. The protocols were approved by the Ethics Committee on Animal Research – UECE under the protocol #12781206-7. BUF promoted a slow onset contractile response in the aortic rings that reached a maximal response of 101.3 ± 19.87 %-PE, but this was achieved only at 100 μM concentration. In the 2nd branch of the mesenteric artery, BUF promoted a lower contractile response 46 ± 9.6 %-PE again only at the dose of 100 μM. The 1h treatment of tissues with guanethidine (GUA + BUF) attenuated BUF vasoconstriction in aortic rings (39.7 ± 8.4%, $p<0.05$). The contractions were also completely blocked by 1 μM prazosin. The contractions elicited by BUF in both conductance and resistance arteries is achieved only in high concentrations and seems to be dependent on the activation of the local adrenergic system. **Funding**: CNPq, Capes, FUNCAP.

06.047

Pravastatin and rosuvastatin reduce fibrinogen receptor activation and platelet adhesion to fibrinogen of high fat-fed rats. Goulart G[1], Araujo HN[2], Lopes Pires ME[1], Monteiro PF[1], Antunes E[1], Delbin MA[3], Marcondes S[1] [1]Unicamp – Pharmacology, [2]Unesp-Rio Claro – Physical Education, [3]Unicamp – Structural Biology and Functional

Introduction: Platelets are key cells in the thrombus formation. They are activated by different agonists that lead to increase of intracellular $Ca^{++}$and fibrinogen receptor (integrin αIIbβ3) activation, which is important to stabilize the platelet aggregate. Dyslepidemia is a risk factor for atherosclerosis and thrombus formation. Studies have demonstrated that statins, despite of your actions of lipid-lowering, modulate platelet activity. Statins reduce platelet aggregation as well as the thromboxane A2 synthesis and p-selectin expression. However, the studies about the effect of statins on platelet adhesion are scarce. Therefore, in the present work we decided to investigate the effects of pravastatin and rosuvastatin on platelet adhesion of high fat-fed rats. We also decided to investigate the effect of these statins on fibrinogen receptor activation. **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – Unicamp, protocol number 3202-1). Rats received high fat diet or normal chow diet for 16 weeks and after that the animals were euthanized and blood was collected. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 200 g for 15 min and the platelets were washed using citrated buffer (pH 6.0). Washed platelet adhesion was evaluated using fibrinogen-coated 96-well microtiter plates. Platelets, in absence or presence of pravastatin or rosuvastatin, 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. Western blotting assays were carried out for phosphorylation of beta3 on the Tyr773 and Tyr785 residues to investigate fibrinogen receptor activation. **Results:** High fat diet increased body weight (16%), epididymal weight (62%) and triglycerides (48%) of the animals compared to normal chow diet. High fat diet significantly increased non-stimulated platelet adhesion (4 ± 04% to 6.5 ± 0,7% of adhesion for normal chow and high fat diet, respectively). However, thrombin (50 mU/ml) or ADP (10 μM)-stimulated platelet adhesion were similar in both diet. Pravastatin and rosuvastatin inhibited 30% non-stimulated-platelet adhesion, but nearly abolished ADP- or thrombin-stimulated platelet adhesion. The effect of these statins on stimulated-platelet adhesion was accompanied by significant reduction of $beta3^{tyr785}$ chain phosphorylation but not at Tyr773 residue. Reduction of beta3 phosphorylation was not observed on non-stimulated-platelet. **Conclusion**: Therefore, the present results show that inhibition of stimulated-platelet adhesion to fibrinogen of high fat-fed rats is mediated by reduction of Tyr785 residue phosphorylation of beta3 chain of alphaIIbbeta3 integrin. **Supported by:** CNPq

06.048

Vasorelaxant effect of the beta-phenylethylamine on rat isolated aortic rings. Nóbrega FC, Brito TS, Lima FJB, Magalhães PJC UFC – Physiology and Pharmacology

Introduction: Beta-phenylethylamine (β-PEA) is considered as an endogenous sympathomimetic amine, known as trace amine, since it is found in very low concentrations in mammalian brain. Recent studies described the existence of receptors for trace amines and low or high plasma concentrations of β-PEA may be associated with specific psychological disorders such as attention deficit hyperactivity disorder, depression and schizophrenia (Hossain *et al*., *Neurochem Int*, 73:27,2013).This compound is also found in the diet in foods such as chocolate. However the physiological functions of β-PEA are still not fully understood. Considering that β-PEA has a chemical similarity with 1-nitro-2-phenylethane, a volatile compound with myorelaxant properties (Brito *et al*., *Biochem Pharmacol* 85:780, 2013), we aimed to investigate whether β-PEA could promote vasorelaxant effects on rat isolated aorta. **Methods:** Aortic rings were obtained after euthanasia of Wistar rats (200 – 250 g) under ethical guidelines (Institutional Animal Ethics Committee number 22/2014). Rings were maintained in Krebs solution (pH 7.4, 37 ˚C, continuously aerated with carbogen mixture, resting tension = 1 g). Contractions were recorded isometrically through force transducers connected to a data acquisition system. **Results:** β-PEA (1- 300 μM) relaxed the phenylephrine-induced contractions (PHE, 1 μM, n = 15) in a concentration-dependent manner ($p<0.001$, Holm-Sidak test) with EC_{50} values of 85.61 [70.81– 101.5] μM, an effect that became significant at the concentration of 30 μM. Pretreatment of endothelium-intact aortic rings with propranolol (a non-selective beta-adrenoceptor antagonist, 5 μM, n = 6) or glybenclamide (inhibitor of the K+ channels activated by adenosine triphosphate [ATP]; 10 μM, n = 9) did not affect the vasorelaxant effect induced by β-PEA (EC_{50} values of 90.75 [68.42 – 120.4] μM and 79.5 [48.23 – 131.07] μM, respectively). In contrast, the vasorelaxant effect of β-PEA was significantly ($P<0.05$, Holm-Sidak test) decreased by vascular endothelium removal (n = 8) or by pretreatment with L-NAME (a nitric oxide synthase inhibitor; 100 μM, n = 6), ODQ (inhibitor of the guanylate cyclase; 10 μM, n = 6), tetraethylammonium (TEA, a nonspecific blocker of K+ channels; 5 mM, n = 6) or 4-aminopyridine (4-AP, a selective blocker of voltage-operated K+ channels; 1 mM, n = 8), since the contractile response to PHE (1μM) in the maximum concentration used of β-PEA (300 μM) was reduced to 59.4 ± 5.03 %, 69.2 ± 5.85 %, 59.95 ± 3.2 %, 72.34 ± 4.3 % and to 49.74 ± 4.15 %, respectively. **Discussion:** In the concentration range of 1 – 300 μM, β-PEA relaxed rat aortic rings with recruitment of the NO-cGMP pathway. Part of its pharmacological effects could be explained by the opening of voltage-operated K+ channels to produce vasorelaxation. Moreover, neither β-adrenoceptors nor K^+ channels activated by ATP appears involved in the vasorelaxant effects of β-PEA. **Financial Support:** Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq

06.049

Chronic treatment with apocynin improves the resistance vessel relaxations to acetylcholine and increases the nitric oxide concentration in endothelial cells of SHR.

Potje SR, Graton ME, Troiano JA, Perassa LA, Antoniali C Unesp – Basic Sciences

Introduction: The endothelial dysfunction in spontaneously hypertensive rat (SHR) has been associated to an increased expression of subunits of the enzyme NADPH-oxidase (NOX), increased concentration of reactive oxygen species (ROS) and decreased bioavailability of nitric oxide (NO). We hypothesized that apocynin (APO), a NOX inhibitor, reverses the impaired relaxation to acetylcholine (ACh) in resistance vessels of SHR. **Methods:** SHR was treated from the 4th to 10th week of life with APO (30 mg/kg / day, in drinking water). Then, we realized concentration-effect curves to ACh on the 2nd branch of mesenteric artery (MA) in DMT-myograph system (PowerLab 8/35). The fluorescence intensity (FI) to DAF-2DA (NO probe) and DHE (ROS probe) was analyzed by flow cytometry (Applied Biosystems Attune – Focusing Cytometer), in aortic endothelial cells (EC) at basal conditions or after ACh stimulation. The results were expressed as mean ± SEM and were compared between normotensive Wistar rats (WST, n=5), SHR-C (Control, untreated, n=5) and SHR-T (treated, n=5) (ANOVA, $p<0.05$). Results: The vasorelaxation to ACh was impared in MA of SHR (Emax: 74.3 ± 3.3 % of relaxation to Phe contraction) when compared to MA of WST (Emax: 93.8 ± 1.8 %). In MA of SHR-T, ACh reactivity was increased (Emax: 94.9 ± 1.1%), and it was not diferent from that observed in MA of WST. The basal concentration of NO ([NO]) in EC of SHR was lower (2057.0 ± 0.081 U) than that observed in the EC of WST (3180.0 ± 0.220). There was no difference in the [NO] between EC of WST and EC of SHR-T (3368.0 ± 0.27). ACh (1 μM) increased the [NO] in the EC of WST (4526.0 ± 0.263 U), but not in untreated SHR (2259.0 ± 0.108 U). However, ACh induced about a 2-fold higher increase in the [NO] in the EC of SHR-T (6320.0 ± 0,345 U) than in the EC of untreated SHR. In EC of SHR-C, the baseline [ROS] was higher than in the EC of Wistar rats (2880.0 ± 0.155 U and 1902.0 ± 0.127 U, respectively). However, in EC of SHR-T, the [ROS] (2090.0 ± 0.174 U) was lower than in the cells of SHR-C and similar to that observed in WST. ACh did not alter the $[ROS]_c$ in the EC of the groups. Conclusion: Apocynin improves the ACh relaxation in MA of SHR by increasing [NO] and decreasing [ROS] in EC of SHR. Our results suggest that apocynin reverses endothelial dysfunction in SHR. **Financial support**: Fapesp 2011/20998-0; CNPq: 141323/2013-2; IC-Fapesp (2012/01733-9). Animal Research Ethics Committee at the School of Dentistry of Araçatuba – Unesp (CEEA-FOA/Unesp, protocol. 01561-2011).

06.050

Uroguanylin stimulates distal potassium secretion via PKG pathway. Gonzaga JLD, Godinho AN, Fonteles MC, Lessa LMA UECE – Fisiofarmacologia Cardiorenal

Uroguanylin (UGN) is the member of guanylin family of peptides with the most pronounced renal effects. It has been postulated that this peptide participates of an axis connecting the kidneys to intestine, regulating salt and water homeostasis. Besides promoting natriuresis and diuresis, our group has demonstrated that UGN also stimulates kaliuresis, probably via activation of Maxi-K channels. The present study was designed to investigate the signaling pathways involved in the kaliuretic effect of UGN in rat renal distal tubules. After the approval by the Ethics Committee on Animal Experimentation, under protocol number 11518128-8/67, the *in vivo* stationary microperfusion experiments were performed. By this technique a segment of proximal tubule was punctured by a micropipette contenting control or experimental perfusion solution. Then, a segment of distal tubule was impaled by a potassium selective double barreled microelectrode. This method allows the measurement of the K+ secretion in distal tubules. When compared to the control group, the perfusion with UGN (1μM) stimulated significantly the potassium secretion (JK-nmol.cm-2.s-1: 0.24 ± 0.05control (n=14) x 0.85 ± 0.06UGN (n=11)*), which was followed by decreased half-time of K+ secretion (t1/2, s: 15.33 ± 1.42 control (n =14) x 10.60 ± 1.00 UGN (n = 11)*) and increased level of stationary potassium ([Kmax], mM: 10.36 ± 1.89 control (n = 14) x 22. ± 1.11 UGN (n = 11)*) (*p<0.05 vs. control). The cGMP analogue, 8br-cGMP, mimicked UGN effect on distal potassium secretion at the same concentration (JK, nmol.cm -2.s-1: 0.51 ± 0.04; t1/2, s: 9.02 ± 1.43; [Kmax], mM – 12.68 ± 1.93). In order to investigate the involvement of guanylate cyclase / cGMP / PKG pathway in the stimulatory effect of UGN on distal potassium secretion, distal tubules were perfused with the combination of 1μM UGN and 1μM KT5823 (PKG inhibitor). The PKG inhibitor not even prevented the stimulatory effect of UGN, but also promoted a inhibition of distal potassium secretion (JK, nmol.cm-2.s-1: 0.14 ± 0.02, n = 9) which was followed by an increase in half-time of potassium secretion (t1/2, s: 18.89 ± 2.40 (n = 9) and reduced stationary potassium ([Kmax], mM: 10,096 ± 1.33 (n = 9) (p<0.05 vs control and vs UGN). Taken together, these findings suggest the involvement of GC/cGMP/PKG pathway activation in the UGN effect on distal potassium transport. Furthermore, our data also suggests an inhibition of potassium secretion induced by PKG inhibitor, since in the presence of this compound we observed a inhibition of potassium secretion, indicating a constitutive modulatory action of PKG on the distal potassium secretion. **Financial support**: FUNCAP, Capes, and CNPq.

06.051

Alpha-1 antagonism of molecules that contains pirimidone rings (6-oxo-2,4-diaryl-1,6-dihydro-pyrimidine-5-carbonitriles). Costa CP[1], Wanderley AG[1], Anjos JV[2], Araújo AV[3]
[1]UFPE – Physiology and Pharmacology, [2]UFPE – Fundamental Chemistry, [3]CAV-UFPE

Introduction: Arterial Hypertension is one of the main causes of cardiovascular diseases, which represent one third of the deaths in Brazil and in the world. However, despite the advances in the anti-hypertensive therapies, an important number of patients do not have their pressure levels appropriately controlled. Therefore, the development of new anti-hypertensive therapies, with greater efficacy and less side effects, is very important. Alpha 1–adrenergic antagonists are one of the classes used for the treatment of Arterial Hypertension and includes prazosin and doxazosin, that have a pyrimidine ring in their structures. It prompted us to develop pryrimidine drugs with vasorelaxant activity. **Aim:** To evaluate the alpha 1-antagonism of four new molecules that contains pirimidinone ring (sodium salts derivatives of thiomethyl pyrimidines) in aortic rings from Wistar rats. **Methods:** Cumulative concentration-effect curves for phenylephrine were constructed in the absence or in the presence of the molecules (0.1 mmol/L) in denuded aortic ring from male Wistar rats. Maximum effect and potency were analyzed. All protocols were approved by the Animal Experimentation Ethics Committee of the UFPE – UFPE (license no. 021446/2013-12). **Results:** The maximum effect of phenylephrine (ME: 2.72 ± 0.19 g, n=7) was diminished in the presence of 2-methylsulfanyl-6-oxo-4-phenyl-1,6-dihydro-pyrimidine-5-carbonitrile sodium salt (ME: 1.87 ± 0.31 g, n=5, inhibition of 31.12%), 2-methylsulfanyl-6-oxo-4-(*m*-nitrophenyl)-1,6-dihydro-pyrimidine-5-carbonitrile sodium salt (ME: 1.45 ± 0.03, n=4, inhibition of 47.02%), 2-methylsulfanyl-6-oxo-4-(*p*-nitrophenyl)-1,6-dihydro-pyrimidine-5 – carbonitrile sodium salt (ME: 2.33 ± 0.24, n=4, inhibition of 15.95%) or 2-methylsulfanyl-6-oxo-4-(*p*-methoxyphenyl)-1,6-dihydro-pyrimidine-5-carbonitrile sodium salt (ME: 2.34 ± 0.34 g, n=4, inhibition of 15.67%). **Conclusion:** The data suggest that the new molecules may be alpha1-antagonists, although more studies are necessary to confirm this action. **Financial support:** CNPq.

06.052

Increased nitric oxide generation after low level laser therapy (LLLT) in rat myocardial infarct (MI) model. Manchini M[1], Santana E[1], Serra A[1], Antonio E[2], Craijonas R[3], Girardi A[3], Tucci P[2], Silva JA Jr[1] [1]Uninove – Ciências Médicas, [2]Unifesp – Fisiologia Cardiovascular, [3]FMUSP-HC-InCor

Introduction: The purpose of this study, therefore, was to determine the mechanisms by which low level laser therapy (LLLT) may exert some of its angiogenic and cardioprotective effects and induce neovascularization, a necessary event of cardiac remodeling after myocardial infarction (MI). **Methods:** Female Wistar rats (200-250g) were anesthetized and after thoractomy, a single laser irradiation at 660 nm for 60s (power 15 mW, laser beam spot size 0.785 cm^2, energy density 22.5 J/cm^2, energy delivered 1.1 J) was applied in the region of the descending left coronary artery occlusion. The effects of LLLT on nitric oxide (NO) secretion, eNOS and iNOS mRNA and VEGF expression in the MI remote area were determined. **Results:** Laser irradiation enhanced endothelial nitric oxidase synthase (eNOS) gene expression by 30% when compared to control rats ($p<0.05$). MI increased inducible NOS mRNA expression in 6-fold change, however, LLLT performed after MI diminished iNOS mRNA content in 4-fold change ($p<0.05$). In addition, an increased plasmatic NO was found in rats with MI treated with LLLT (36.1+/- 2.1 uM) in comparison to MI (26.6+/- 1.7 uM) and control (18.9+/-0.6 uM) rats ($p<0.05$). However, at the dosage used in this study, LLLT was unable to increase VEGF protein expression in the myocardium ($p>0.05$). LLLT failed to increase neovascularization assessed by capillaries density quantification ($p>0.05$). **Conclusion:** The results of this study suggest that LLLT after MI increased NO generation and secretion by modulating NO synthase gene expression. An increased NO might help the vasodilation in the MI remote area, and may contribute to cardiomyocytes survival after MI. **Summary:** Institutional Research Ethics Committee of the Nove de Julho University (Number 0015/2012), São Paulo, Brazil. **Financial support** by Fundação de Amparo à Pesquisa do Estado de São Paulo (2009/54225-8), Conselho Nacional de Desenvolvimento Científico e Tecnológico (477458/2009-2) and Uninove.

06.053

Uroguanylin inhibits H-ATPase activity and surface expression in renal distal tubules by a PKG dependent pathway. Godinho AN[1], Lima VS[1], Crajoinas RO, Carraro-Lacroix LR[2], Dias JLG[1], Dariolli R[3], Girardi ACC[3], Fonteles MC[1], Malnic G[2], Lessa LMA[1] [1]ISCB-UFC, [2]ICB-USP – Physiology and Biophysics, [3]FMUSP-HC-InCor

Uroguanylin (UGN) is a member of the guanylin family that connects the intestine to the kidneys, modulating salt and water homeostasis. We reported previously that uroguanylin (UGN) inhibits hydrogen secretion in renal distal tubule. In the present study, we investigated the functional and molecular effects of UGN on H^+-ATPase activity in rat renal distal tubules and in MDCK-C11 cells, after approval by the Ethics Committee on Animal Experimentation, under protocol number 11518128-8/67. By *in vivo* stationary microperfusion experiments we were able to show that UGN inhibits H^+-ATPase activity by a PKG and not PKA dependent pathway, since KT5823 (PKG inhibitor) abolished UGN effect on distal bicarbonate reabsorption and H89 (PKA inhibitor) was unable to prevent it, as can be seen through the respective values found for bicarbonate reabsorption (JHCO3-,nmol.cm-2.s-1: control – 0.86 ± 0.12 (n=6), UGN – 0.51 ± 0.07 (n=6)*, KT5823 – 0.77 ± 0.05 (n=16), KT5823 + UGN 0.91 ± 0.08 (n=9), H89 0.86 ± 0.09 (n=8), H89 + UGN 0.43 ± 0.05 (n=7)* (*p<0,05 versus control). The *in vivo* results were confirmed by the *in vitro* experiments, where we used fluorescence microscopy to measure intracellular pH (pHi) recorvery after an acid pulse with NH_4Cl. By this technique our data showed that UGN and 8br-cGMP inhibited H^+ -ATPase-dependent pHi recovery, and that UGN inhibitory effect was abolished in the presence of PKG inhibitor. Our study also demonstrated by RT-PCR technique that MDCK-C11 cells express guanylate cyclase-C. Besides, UGN were able to stimulate increase of both cGMP content and PKG activity, but was unable to stimulate increase of cAMP content and PKA activity. Furthermore our data showed by biotinylation and western blot methods that UGN incubation promotes a reduction of surface abundance of H^+-ATPase B1 subunit in MDCK-C11 cells, and that this effect was also abolished by PKG inhibitor. Thus, our study indicates that UGN inhibits H^+-ATPase activity and surface expression in renal distal tubule by a cGMP/PKG dependent pathway. Financial Support: CNPq e FUNCAP.

06.054

Cardiac intracellular Ca^{2+} handling and β-adrenergic activity in rats submitted to neonatal leptin treatment. Marques EB[1], Silva RM[1], Pinto LMO[2], Nascimento JHM[2], Scaramello CBV[1] [1]UFF – Farmacologia Experimental, [2]IBCCF-UFRJ

Introduction: Previous data of our group showed cardiovascular risk and cardiac leptin receptor upregulation programmed in rats by neonatal leptin treatment (Marques *et al*., FeSBE2011, panel 24.033; SBFTE2013, panel 06.010). This work aimed to evaluate β-adrenergic activity and Ca^{2+} -regulatory proteins expression in rats submitted to neonatal hyperleptinaemia. **Methods**: Pups were divided into two groups: during lactation (first 10 days), injections of leptin (8µg/100g sc) (L group) or saline (C group) were performed. Rat hearts (1, 3 and 5 months-old) were removed for analysis. In perfused hearts, Left Ventricular Developed Pressure (LVDP) and ventricular performance index (± dp/dt) were measured, before and after the addition of Isoprenaline (1-100nM) (Vassalo *et al*., *Pharmacol Res*, 29; 251, 1994). SERCA, phospholambam (PLB), PMCA, Na+ /K+ ATPase, FKBP12 and β1-adrenergic receptor (β1-AR) protein expression were determined by Western blot assays (Laemmli *Nature*, 227:680, 1970). Data are presented as mean ± SEM, analyzed by Student's *t* test and considered statistically different if $P<0.05$(*). The use of animals was approved by Ethics Committee (CEPA/UFF389-13). **Results**: Langendorff assays showed that heart spontaneous activity is decreased in rats submitted to neonatal hyperleptinaemia. One month-old rats presented impairment of lusitropism (-dp/dt: C=-454.1 ± 23.9 vs L=-536.3 ± 38.1*) while 5 months-old rats presented decrease of inotropism (LVDP: C=93.4 ± 4.2 vs L=74.8 ± 3.6*/ + dp/dt: C=297.3 ± 0.9 vs L=262.3 ± 6.1*). Isoprenaline, a β-agonist, determined a greater inotropic and lusitropic effects at 1 month-old hearts (LVDP: C=70.9 ± 6.5 vs L=129.6 ± 22.0*/ + dp/dt: C=874.6 ± 21.5 vs L=1034.4 ± 24.9*/- dp/dt: C=-1054.1 ± 37.1 vs L=-1330.3 ± 27.1*). At 5 months-old only its inotropic effect was improved (+ dp/dt: C=360.9 ± 44.7 vs L=556.5 ± 47.0*). Western blot analysis showed a significant increase of SERCA protein expression at 1 (C=1.5 ± 0.2 vs L=2.5 ± 0.4*) and 5 month-old rats (C=1.5 ± 0.2 vs L=2.6 ± 0.3*) due to neonatal leptin treatment. However Na^+/K^+ ATPase expression was decreased at 5 months-old (C=4.3 ± 0.6 vs L=1.8 ± 0.3*). Leptin treatment does not affect PLB, PMCA, FKBP12 and β1-AR protein expression. **Discussion**: Langendorff assays suggest diastolic dysfunction at 1 month-old rats due neonatal leptin treatment accompanied by systolic dysfunction at 5 months-old rats. This is corroborated by our previous Echocardiography data (Marques *et al*., FeSBE2011, panel 24.033). However, Isoprenaline (100nM) addition increased ventricular performance at 1 and 5 months-old hearts in this group. This is not related to an increase of β1-AR expression being probably associated to this receptor efficacy improvement. SERCA expression raise, Na^+/K^+ ATPase expression decrease and PMCA/PLB expression conservation explain our previous observation about these P-type ATPases activities in this experimental model (Marques *et al*., SBFTE2013, panel 06.010). FKBP12 expression also remained unchanged, minimizing Ca^{2+} leak from sarcoplasmic reticulum. These data suggest that neonatal leptin treatment programmed an increase of intracellular Ca^{2+} stock that favours Isoprenaline inotropic and lusitropic cardiac effect being a compensatory mechanism related to cardiac injury. **Financial Support**: Faperj, CNPq, Capes, PROPPI/UFF.

06.055

Dipeptidyl-peptidase IV activity in Bothrops alternatus snake venom and renal tissue of rats. Fernandes PCL, Torres-Huaco FD, Hyslop S Unicamp – Farmacologia

Introduction: Bothrops snake venoms contain peptides capable of inhibiting proteases, such as metalloproteinases, and peptidases, as angiotensin-converting enzyme. These venoms also contain peptidases, including neutral peptidases, aminopeptidases and dipeptidyl-peptidases (principally DPP-IV). In addition to its presence in venoms, DPP-IV is an important endogenous enzyme in vertebrates where it plays a role in immunological responses and in the control of blood glucose levels. In this work, we examined the influence of the low molecular mass (peptide-containing) fraction of *Bothrops alternatus* venom on venom and rat renal DPP-IV activity. **Methods and Results**: The experimental protocols involving animals were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 3044-1). Male Wistar (250-300g) were anesthetized with 2% isoflurane, perfused with 0.9% NaCl via the aorta, and the kidneys then removed and stored at -80 oC for later use as a source of DPP-IV. When required, kidneys were homogenized in 0.1 M Tris-sucrose, pH 7.4, at 4 ˚C, centrifuged (3.000 g, 4 ˚C, 10 min) and the precipitate discarded. The supernatant was centrifuged again (20.000 g, 4˚C 25 min) and the precipitate then washed twice with buffer and resuspended in assay buffer. DPP IV activity was assayed using the fluorogenic substrate Gly-Pro-β- naphtylamine in an assay mixture containing 40 µL of buffer (50mM Tris-HCl, pH 8,0), 20 µL of substrate (final concentration: 100 µM) and 100 µL of sample, in a final volume of 200 µL. Venom (~70 mg) was dissolved in 1 ml of Ambic buffer (see below), centrifuged (13,000g, 10 min, 4 ^{o}C) was fractionated by gel filtration on a column (1.6 x 60 cm) of Superdex 75 equilibrated with 50 mM ammonium bicarbonate (Ambic), pH 8.0. Proteins were eluted at a flow rate of 0.5 mL/min and the elution profile was monitored at 280 nm; fractions of 1.5 mL were collected. Quantitative results were expressed as the mean ± SEM and were analyzed by ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. Venom DPP-IV activity was 12019 ± 3513 fluorescence/mg (n=3). Gel filtration of venom resulted in six major peaks (Pl-PVI) with DPP-IV activity occurring in peak II. In agreement with this finding, the venom (final concentration:1,25-10,0 mg/mL) had a concentration-dependent stimulatory effect on the DPP-IV activity of rat renal DPP-IV, with a 150-fold stimulation being observed at the highest concentration (similar to that observed in other Bothrops snake venom). Finally, when venom was ultrafiltrated (by centrifugation) through Amicon 3-5 kDa cut-off filters there was a marked decrease in venom DPP-IV activity that was restored by mixing the filtrate (low molecular mass fraction) with the retenate **Conclusion:** Together, these findings indicate that *B. alternatus* venom contains a low-molecular mass component(s) capable of stimulating DPP-IV activity. The identity of this component remains to be determined. **Financial Support:** CAPES, CNPq, FAPESP.

06.056

Electrolyte disorder in the acute renal failure induced by gentamicin in rats. Bona MD, Rodrigues FAP, Coelho YP, Prata MMG, Oliveira DMN, Pereira JM, Costa PHS, Silva PLB, Monteiro HSA, Havt A UFC – Fisiologia e Farmacologia

Introduction: Gentamicin (GM) is a aminoglycoside antibiotic with ample action spectrum against infections caused by gram-negative bacteria (Quiros *et al*, **Toxicol sci**, v. 119, p. 245-256, 2011). However, its use is associated with the development of acute renal failure by acute tubular necrosis and this is found in 30% of the patients (Derakhshanfar, **Iran Vet Research**, v. 8, p. 231-238, 2005). GM inhibits membrane transporters of the renal proximal cells, collaborating to the reduction of ATP levels and the conduction of necrosis and death (Quiros *et al*, **Toxicol sci**, **v. 119**, p. 245-256, 2011). This study evaluated the electrolyte disorder pattern and the expressions of Aquaporin-2 (AQP-2) in the kidneys of animals treated with aminoglycoside. **Material and methods:** We used male rats Wistar (n=7), weight 240-280g. The study was accredited by UFC Ethics Comission CEUA-UFC (protocol 68/12). The animals were divided, randomly, in two groups: control group (CT) and gentamicin group (GM). The GM group received, daily, 0.6mL of GM, intraperitoneally (i.p), (100 mg/kg for 7 days) and the CT group received the same volume of NaCl at 0,9% i.p. The groups were maintained in metabolic cages. At the end of the experimental period, the urine, blood and kidney were collected. We measured urinary and plasmatic electrolytes (Na+ and K+) by the electrolyte analyzer 9180 (Roche, Brazil). Then, we could calculate the fraction of sodium and potassium excretion (FENa+, FEK+). The renal tissue was conducted to mRNA isolation to evaluate AQP-2 transcription level using Real Time PCR Method: **Results and discussion:** The treatment with Gentamicin had a trend to increase the urinary flow (0,011 ± 0,004 ml/min) when compared to CT (0,007 ± 0,002 ml/min). The data also showed that GM caused an increase of FENa+ (3,2% ± 1,4%), significantly different from CT group (1,2 ± 0,2%), and an increase of FEK+ (72,05 ± 12,4%) compared to CT (32,1 ± 17,2%) (*P<0,05*). The relative transcription of the AQP-2 was decreased when compared to CT (GM: 0.75 ± 0,3 *vs* CT: 1.3 ± 0.4, *P<0,05*). **Conclusion:** The model of renal injury by Gentamicin caused changes in the ionic renal function, due to the increase of FENa+ and FEK+, and the decrease of the relative expression of the AQP-2. **Financial support**: Capes.

06.057

Effect of chronic salt intake in cardiovascular and metabolic parameters in Dahl rats.

Farah V[1], Arnold A, Araujo IC, Pereira J, Silva I, Brandão M, Sacchi J, Oliveira R, Fiorino P, Fonteles MC Mackenzie University

Introduction: It is well known that salt plays an important role in the development of hypertension and cardiovascular disease. The aim of this study was to evaluate the effect of 1% of salt intake on the metabolic and cardiovascular parameters of Dahl salt-sensitive rat. **Methods**: Animals were divided in Male Dahl salt-sensitive (MDS, n=8); Male DAHL salt-resistant (MDR, n=4); Female Dahl salt-sensitive (FDS, n=12) and Female DAHL salt-resistant (FDR, n=8) and received 1% of salt in drinking water during 5 weeks. Body weight, food and water intake were evaluated in all groups. At the end of the protocol blood pressure was evaluated by tail-cuff plethysmography (Amplifier Model 229, IITC Life Science, USA). Systolic blood pressure (SBP) was measured five times, and the average value was calculated. (Animal Ethics Committees: 11516758-7/61). **Results:** At the end of the protocol the male rats showed an increase in body weight gain when compared to female: MDS=63 ± 12 and MDR=57 ± 4g vs FDS=27 ± 4 and FDR=25 ± 5g. There were no differences in water and food intake between the groups. However, the MDS showed an increase (19%) in SBP when compared to MDR as well as the FDS when compared to FDR (14% of increase): MDS=183 ± 8 mmHg; MDR=154 ± 4 mmHg; FDS=179 ± 3mmHg and FDR=157 ± 2 mmHg. **Discussion:** These data indicate that 1% salt in the drinking water for 5 weeks increased the blood pressure in both males and females Dahl salt-sensitive rats. Moreover, the male rats showed an increase in body weight when compared to female rats. In conclusion, our data showed that there was no difference in blood pressure responses induced by salt between males and females Dahl salt-sensitive rats. **Financial *support***: *CNPq-Fapesp-MackPesquisa*

06.058

The A1 and P2Y1 receptor are the receptor involved in the cardiac arrest produced by ATP? Rodrigues JQD, Silva Junior ED, Camara H, Godinho RO, Jurkiewicz A Unifesp – Farmacologia

Background: The A1, P2Y1 and P2Y6 receptors are expressed in cardiac tissue, which are activated by purines (ATP – adenosine triphosphate) released from sympathetic neurons, endothelial cells and other tissues such as cardiomyocytes, modulating the cardiac inotropism and chronotropism. Therefore, this study aimed to investigate the role of purinergic receptors on cardiac arrest induced by ATP and its signaling pathway in isolated right atria (RA) of Wistar rats (WR). **Methods**: RA from WR (4-6 months) were isolated and mounted in isolated organ bath. The RA presented spontaneous beatings and frequency between 180 and 280 bpm was considered an inclusion criterion. We studied the effect of ATP (0.001 mM to 1 mM) on frequency of RA in the absence or presence of DPCPX– a selective antagonist of the adenosine A1 receptor (1- 100 nM / pre-incubated for 40 min), MRS 2179 – a selective antagonist of P2Y1 receptor (1- 100 nM / pre-incubated for 30 min) and MRS 2578 – a selective antagonist of P2Y6 receptor (1- 100 nM / pre-incubated for 30 min). We use ATPγS to verify if the effect of cardiac arrest produced by ATP was due to ATP or its degradation in Adenosine.The results were analyzed by unpaired t test and one-way ANOVA. All experiments procedures were approved by the Ethics Committee of Unifesp (n° 0778/11). **Results**: ATP (1 mM to 30 μM) produced an initial negative chronotropic effect (NCE) which lasted 60-90 s and a negative inotropic effect (NIE). After that, the chronotropism gradually increased, showing a positive chronotropic effect (PCE) that lasted 400 s and a positive inotropic effect (PIE). ATP at 300 μM induced only a NCE. At 1mM ATP abolished the atrial contraction. DPCPX (30 nM) and MRS 2179 (100 nM) prevented the cardiac arrest produced by ATP (1 mM) in Wistar rats. However, MRS 2578 was unable to inhibit this effect. The ATPγS, non-hydrolyzable, produced the phenomenon of cardiac arrest. **Conclusion**: The results suggest that the cardiac arrest induced by ATP was due to the activation of A1 and P2Y1 receptors from Wistar rats.We observed that the effect of cardiac arrest caused by ATP is due to its direct action on purinergic receptors and not its degradation to adenosine.

06.059

Uroguanylin inhibits proximal bicarbonate reabsorption in Dahl salt rats. Lessa LMA, Martins ICMT, Godinho AN, Nascimento NRF, Fonteles MC ISCB-UFC

Uroguanylin (UGN) is a member of guanylin family of peptides that participate in an endocrine axis linking the intestine to the kidney, regulating salt and water excretion in dependence on dietary intake. Thus, it has been proposed an important role for UGN in the regulation of salt and water homeostasis and consequently fluid volume and blood pressure. Our group has previously demonstrated the inhibitory effect of UGN on NHE3 activity in renal proximal tubules of Wistar rats. NHE3 is the main route for sodium bicarbonate reabsorption in renal proximal tubules. The present study was designed to evaluate the UGN effects on proximal bicarbonate reabsorption in Dahl salt sensitive rats (DS), by the stationary renal microperfusion technique. By this method we are able to measure hydrogen secretion in renal proximal tubules by means of hydrogen selective microelectrodes. Similarly to our previous findings with Wistar rats (WR), the net of bicarbonate reabsorption after perfusion of proximal segments with 1µM UGN in DS (before development of salt sensitive hypertension) was significantly reduced when compared to the perfusion of control solution ($JHCO_3^-$,nmol.cm^{-2}.s^{-1}– WR: $2,4 \pm 0,26_{control}$ x $1,56 \pm 0,21_{UGN}$ $P<0,05$; DS: $2,09 \pm 0,46_{control}$ x $1,03 \pm 0,09_{UGN}$ $P<0,05$). This UGN effect was followed by increased half-time of acidification (T1/2) and stationary bicarbonate ($[HCO_3^-]$) in DS when compared to the control (t1/2,s – $5,11 \pm 1,00_{control}$ x $7,89 \pm 0,39_{UGN}$; $[HCO_3^-]$, mM – $3,39 \pm 0,82_{control}$ x $7,48 \pm 1,41_{UGN}$ $P<0,05$). Taken together, these data indicate that UGN inhibits proximal bicarbonate reabsorption in DS. Considering the major importance of NHE3 for proximal bicarbonate reabsorption, our findings also suggest an inhibitory effect of UGN on NHE3 in DS rats in the pre-hypertensive phase. However further studies are needed to elucidate the implications and mechanisms involved in salt sensitive hypertension in DS. So, the effects of UGN in Dahl rats in the pre-hypertensive phase will be compared with its effects in hypertensive phase in order to check whether the sensitivity to UGN is impaired.

06.060

Reversibility of oxidative and glomerular damage in an experimental model of renal injury by gentamicin. Coelho YP, Rodrigues FAP, Prata MMG, Alves NTQ, Oliveira DMN, Pereira JM, Silva PLB, Costa PHS, Monteiro HSA, Bindá AH UFC – Physiology and Pharmacology

Introduction: Gentamicin (GM) is a typical antibiotic, inexpensive and widely used for its broad spectrum of action against gram-negative microorganisms. This antibiotic triggers acute kidney injury (AKI) by acute tubular necrosis (ATN) in about 10 to 20% of patients (Safa *et al*., *Iran J Kidney Dis*, v. 4, p. 285, 2010) characterized by changes in renal function and the production of reactive oxygen species (ROS) (Kang *et al*., *Toxicol Res*, v. 9, p.61-67, 2013). The present study investigated the spontaneous reversibility of renal function in a model of AKI by GM after 3 days of its last administration. **Methods:** Wistar rats weighing 240-280g(n = 6) were used. The study was accredited by UFCEthics Comission CEUA-UFC (protocol 68/12).Experimental groups were classified as: 1)nephrotoxic injury group (GM) -their induction wasintraperitoneally (ip) with 100 mg/kg of GM; 2) Control group (CT) – received 0.9% NaClfor 7 days (ip). 3) Late nephrotoxic injury(GML): induced with GM 100 mg/kg (ip)or 4) 0.9% NaCl (ip) (CTL) for 7 days and only sacrifice in the tenth day after the last administration. Immediately after the last administration, the animals were kept into metabolic cages for a 24h urine collection and, at the end of this period, blood and renal tissue were collected. Dosages of urinary protein (PU) and urinary creatinine were performed by commercial kits (Labtest ®, Fortaleza, CE), and the calculation of UP:C was done to evaluate proteinuria. Additionally, we measured urinary malondialdehyde (MDA) (Walker & Shah, 1990) and renal nitrite by Griess Method: Data was evaluated by ANOVA and Bonferroniand $p<0.05$ was considered significant. The study was accredited by UFCEthics Comission CEUA-UFC (protocol 68/12). **Results and discussion:** The GM group after 7 days of administration showed increased values of PU (82.5 ± 6.8 mg/dl), UP:C (4.1 ± 0.6), MDA (101.9 ± 9.7 nM/g of creatinine) and nitrite (10.4 ± 2.6 nM/g of protein) (*P<0,05*) compared to control group PU (32.9 ± 6.4 mg/dl), UP:C (0.53 ± 1.8), MDA (46.9 ± 11.0 nM/g of creatinine) and nitrite (3.8 ± 0.7 nM/g of protein). Values remained elevated in late nephrotoxic injury group (10 days): PU (86.1 ± 6.518 mg/dl), UP:C (3.7 ± 1.0), MDA (79.2 ± 9.8 nM/g of creatinine) and nitrite (10.1 ± 1.5nM/g of protein) (*P<0,05*) when compared to control groups: PU (40.5 ± 4.4 mg/dl), UP:C (0.30 ± 1.4), MDA (38.75 ± 6,7 nM/g of creatinine) and nitrite (3.4 ± 0.7 nM/g of protein).No statistical difference was observed between the GM and GML groups as well as between CT and CTL, characterizing renal dysfunction as irreversible considering 10 days of evaluation. **Conclusion:** Treatment with GM causes kidney damage which was not reversed in ten days. **Financial support**: Capes.

06.061

Apocynin modulates phenylephrine and acetylcholine reactivity in aortic rings of spontaneously hypertensive rats (SHR). Graton ME[1], Potje SR[1], Troiano JA[1], Perassa LA[1], Antoniali C[1] [1]FOA-Unesp – Basic Sciences

Introduction: NOX/NAPDH oxidase activity has been associated with hypertension. Vascular responses to vasoconstrictor drugs can be increased by reactives oxygen species (ROS). Our hypothesis is that the SHR treatment with apocynin (NOX inhibitor) could alter the vascular reactivity of aorta rings to phenylephrine (Phe) and to acetylcholine (ACh). **Methods**: SHR were treated (SHR-T), or not (SHR), from the 4th to the 10th week of life with apocynin (30 mg/kg/day/p.o.). Wistar (WST) rats were used as controls. Intact aorta rings (Krebs solution, pH 7.4, 37ºC, 95%O_2 and 5%CO_2, 2.0g basal tension) were stimulated with Phe (0.1 nmol/L – 10 µmol/L) or ACh (0.1 nmol/L – 10 µmol/L), after Phe contraction (0.1 µmol/L). The potency (pD2) and efficacy (Emax) of drugs were evaluated. In another set of experiments, thoracic aortic rings were loaded with fluorescent probe DHE (dihydroethidium) to analyze the reactives oxygen species (ROS). The fluorescence intensity (FI, in arbitrary units) was evaluated. The results were expressed as mean ± SEM and compared between the groups (ANOVA, $p<0.05$). **Results**: In SHR intact aortas, Phe reactivity was higher than in WST aortas. However, in SHR-T aortas, it was observed a reduced reactivity to Phe when compared to SHR aortas. The vasodilator response to ACh was impaired in SHR intact aortas when compared to WST aortas. In SHR-T aortas, the potency of ACh was higher than in SHR.There was no difference between ACh potency between SHR-T and WST aortas. SHR aorta rings showed higher FI to DHE (0.21 ± 0,04 U) if compared to aorta rings of WST (0,06 ± 0,02 U). There was no difference on FI between SHR and SHR-T (0,21 ± 0,02 U) aorta rings. Conclusion: Our results demonstrated an increased vascular endothelium modulation of the Phe contraction and an increased ACh relaxation in SHR-T aortas. The results suggest that the chronic treatment of SHR with apocynin reverses the vascular endothelium dysfunction. **Financial support**: Fapesp 2011/20998-0; CNPq: 141323/2013-2; IC-Fapesp (2012/01733-9). Animal Research Ethics Committee at the School of Dentistry of Araçatuba – Unesp (CEEA-FOA/Unesp, protocol. 01561-2011).

06.062

Chronic candesartan treatment prevents erectile dysfunction in streptozotocin induced diabetic rats. Neves NCV[1,2,3], Damasceno EC[1], Felipe-Batista K[1], Guimarães HN[4], Grabe-Guimarães A[1], Leite R[1] [1]UFOP – Farmácia, [2]ICB-UFMG, [3]FASAR – Bioquímica e Farmacologia, [4]UFMG – Engenharia

Aim: Diabetes mellitus is a leading health problem and world is witnessing a pandemic. Erectile dysfunction is a common complication of diabetes, with prevalence varying from 35–90% in different studies. The purpose of this study was to evaluate the beneficial effects of increasing levels of circulating angiotensin-(1-7) [Ang-(1-7)] observed in chronic treatment with angiotensin type 1 receptor blocker (ARB), such as candesartan, on the erectile dysfunction (ED) that has been described in streptozotocin (STZ)-induced diabetic male rats. **Methods and Results:** Diabetic stage was induced by 3 consecutive injections of 40 mg/kg of STZ in male Wistar rats (190-220g). Where considered diabetic the rats presenting glucose level superior to 350 mg/dL 5 days after the last injection. Penile erection was evaluated in control (normoglycemic), diabetic, and diabetic rats treated with candesartan (2 mg/kg) for 60 days after the injection of vehicle or STZ. For that, the animals were anesthetized by ketamine/xylazine (100/14 mg/100g), had the major pelvic ganglion isolated and electrically stimulated. Intracavernosal pressure (ICP) and mean arterial pressure (MAP) were measured and presented as an index of erection (ICP/MAP). Frequency-response curves (1-12 Hz, 4V, 5ms pulse and 30 seconds for each frequency) were obtained from rats of all groups. Erectile function was severely reduced in hyperglycemic compared to control normoglycemic rats. Candesartan treatment did not affect the ganglionic induced erectile response in normoglycemic rats, but significantly improved, almost to a normal level, the impaired responses observed in diabetic hyperglycemic rats. **Conclusion:** Our data indicate that chronic treatment with ARB such as candesartan, that has been known to increase the circulating levels of Ang-(1-7) and improve erectile function, can also reverse the erectile dysfunction observed in STZ-induced diabetic rats. We speculate that the high levels of Ang-(1-7) could be in part responsible for the improvement of the erectile response in diabetic rats. These results can be very useful if applied to diabetic man who presented ED. **Financial support:** FAPEMIG, CNPq, UFOP.