Session 06 − **Cardiovascular and Renal Pharmacology**

06.001

Ruthenium red reverts endothelium-dependent relaxations and enhances contractions of arterial rings from pigs, rats and rabbits. Silva JDP[1], Alves Filho FC[2], Ballejo G[3] [1]NPPM-UFPI, [2]UFPI − Pharmacology and Biochemistry, [3]FMRP-USP − Pharmacology

Introduction: In rabbit thoracic aorta ruthenium red (RR) reverses acetylcholine (ACh)-induced relaxations but not those caused by sodium nitroprusside (Alves Filho FC, R06.016, 41º SBFTE Congress, 2009). In this study we continued to characterize this effect of RR in other isolated arterial preparations. **Methods:** Experimental procedures were approved by the local research ethics committee (No. 03/2009). Rings (3-4 mm) of pig right coronary artery, rat thoracic aorta and rabbit arteries (aorta, carotid, iliac, mesenteric and renal) were suspended in an organ bath containing Krebs solution kept at 37°C with diclofenac (10μM), continuously bubbled with carbogenic mixture, for recording of isometric contractions. After the stabilization period (1-2 hours) contractions were induced in the rings by the addition of KCl or U46619 (pig coronary) and phenylephrine (Phe) (other arteries) in the bath; relaxations were elicited by the addition of different agonist [ACh, 4α-PDD and Bradykinin (BK)] once the plateau of contractions was attained. RR was added on the plateau of the relaxation caused by each agonist or on the plateau of the contraction. **Results:** In rat aorta, RR (10μM) significantly enhanced the magnitude of Phe (0.1μM) induced contractions (mean 1.66 ± 0.32 times, n=5), only in endothelium containing rings; RR (10μM) did not affect the basal tone. RR (1-10μM) augmented also the magnitude of U46619 (10-25nM) elicited contractions of pig coronary artery rings with endothelium; RR caused a similar effect in pre-constricted rabbit mesenteric and renal arteries, but not in rabbit iliac, carotid or thoracic aorta. In rabbit aorta, the relaxations elicited by Ach (1μM) were reverted by RR (1-30μM, IC50=4.6μM, n=6). Likewise, RR (10μM) reverted the relaxations caused by BK (0.1 μM) in pig coronary arterial rings contracted with 20 mM K^+. Interestingly, the selective TRPV4 agonist 4α-PDD (1-30μM) caused endothelium dependent relaxations of rat aorta which were also reverted by RR (1-10 μM). In rabbit or rat aorta, other selective TRP agonists such as capsaicin (1-30μM), allylisothiocyanate (1-30μM) and thymol (1-30μM) caused relaxations that were neither endothelium-dependent nor affected by RR (1-30μM). Intriguingly, in rabbit and rat aorta, the ACh-induced relaxations were also reversed by capsazepine (20μM) a well known TRPV1 antagonist which also antagonizes TRPV4 channels. **Discussion:** These findings suggest that among RR pharmacological targets which include TRPV1-4 and TRPA1, TRPV4 channels are most likely involved in the effects being described. Furthermore, they indicate that endothelial cell stimulation to produce relaxing factors (NO, EDHF) by agonists such as ACh and BK appears to share a common signaling pathway: the activation of TRPV4 channels which leads to an increase in intracellular calcium and consequently the synthesis and release of relaxing factors. **Financial support:** FAPESP, UFPI **Acknowledgements**: Dr. Paulo Calvacanti

06.002

Pharmacological and morphological evidences for the presence of TRPV4 channels in endothelial cells from rat vessels. Alves Filho FC[1], Silva JDP[2], Salgado MCO[1], Ballejo G[1] [1]FMRP-USP − Pharmacology, [2]NPPM-UFPI

Introduction: It is well established that endothelial cells require a sustained increase in intracellular calcium, which depends in part on calcium entry from the extracellular medium, for the synthesis and release of relaxing factors (NO, PGs and EDHF). The channels involved in allowing calcium entry in endothelial cells, however, remain to be fully identified; recently it has become apparent that channels belonging to the TRP family, namely TRPC1-6 and TRPV1-4, mediate calcium entry in a variety of cells. The aim of the present study was to determine whether TRPV4 channels are present in endothelial cells of rat vessels and whether their activation leads to the synthesis and release of endothelial derived relaxing factors. **Methods:** Experimental procedures were approved by the local research ethics committee (No. 051/2006). Rings (3-4 mm) of rat thoracic aorta were suspended in an organ bath (Krebs, 37°C, 95% O2/5% CO2, containing diclofenac,10μM), for recording isometric contractions. After the stabilization period (1-2 hours) contractions were induced by the addition of phenylephrine (Phe); relaxations were elicited by the addition of the selective TRPV4 agonist 4a-Phorbol 11, 12 didecanoate (4a-PDD) once the plateau of the contractions was attained. Ruthenium red (RR), vitamin B12a (B12), ODQ and L-NNA were added on the plateau of the relaxation caused by 4a-PDD. Perfusion pressure of isolated mesenteric bed (Krebs, 4ml/min) was monitored continuously with a pressure transducer. Tissue distribution of TRPV4-like immunoreactivity (IR) was determined in cryostatic sections from rat aorta (immunohistochemistry) or from uterine artery (immunofluorescence); TRPV4 like IR proteins were also determined in whole tissue proteins extracted from rat aorta by Western blot. **Results:** In aortic rings preconstricted with Phe (0.1 μM), 4a-PDD (1-30 μM) elicited concentration dependent relaxations which were strictly endothelium dependent and were fully reverted by L-NNA (100 μM), B12a (30 μM) or ODQ (10 μM); RR (3-10 μM) a TRPV/A antagonist, also reverted 4a-PDD induced relaxation. In the isolated and perfused mesenteric bed preconstricted with Phe (3-10 μM) 4a-PDD (1-3 nmoles, bolus injections) decreased perfusion pressure in a dose-dependent manner which was practically abolished by endothelium removal (deoxycholate) or by adding RR (10 μM) to the perfusing solution; interestingly, 4a-PDD decreased also mesenteric perfusion pressure in a dose dependent manner even in the presence of L-NNA/DF. Positive IR was distinctly observed in the endothelial cells of aortic sections (immunohistochemistry staining) as well as in the intima of uterine artery (immunofluorescence). Finally, three bands of IR proteins (95-100 kD molecular weight) were detected in western blot of whole proteins (50 and 100 μg protein) from aortic tissue which were not labeled when the anti-TRPV4 antibody was omitted from the assay. **Discussion:** These findings provide morphological and functional evidences for the presence of TRPV4 channels in the endothelium of rat vessels of different sizes. Furthermore they also show that the pharmacological activation of TRPV4 in the endothelium leads to the synthesis and release of NO and non-NO relaxing factors. **Financial support:** FAPESP, USP, UFPI **Acknowledgements**: The authors wish to thank Osmar Vettore, Eleni T. Gomes and Fabiola L. Mestriner for their excellent technical assistance.

06.003

Reactive oxygen species and PGF_{2alfa} receptor activation modulate SNP relaxation in denuded mice aorta. Kangussu L, Côrtes SF, Bonaventura D UFMG − Farmacologia

Introduction: Sodium nitroprusside (SNP) has been very well characterized as nitric oxide (NO) donor and induces vascular relaxation. Its effect is attributed to a direct action on the vascular smooth muscle cells. The aim of this study was to investigate if the endothelium modulates SNP vasorelaxation by using pharmacological parameters as maximal relaxation effect (Emax) and potency (pD2). **Methods: and results**: All these procedures were in accordance with the guidelines of the Animal Experimentation Ethics Committee, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil. (Protocol number: 164/2009). Vascular reactivity experiments in mice aorta showed that the vasorelaxation induced by SNP was less potent in the absence (pD2: 7.81 ± 0.14, n=06) than in the presence of functional endothelium (pD2: 9.93 ± 0.24, n=10). This effect was not abolished by a non-selective NO-synthase (NOS) inhibitor, L-NAME, (pD2: 8.47 ± 0.20, n=9). On the other hand, in the presence of Tiron, a superoxide anion (O2-) scavenger, the difference observed in SNP vasorelaxation between denuded and intact endothelium aortas was completely abolished (intact endothelium + Tiron: pD2: 9.61 ± 0.16, n=7 and denuded + Tiron: pD2 9.00 ± 0.25, n=5). Considering that there is an exacerbated oxidative stress in mice aortas without endothelium, and reactive oxygen species (ROS) could activate cyclooxigenase (COX), we have also investigated the involvement of vasoconstrictor prostanoids in this negative modulation. Ibuprophen, a non-selective COX inhibitor and AH 6809, a selective prostaglandin F2alfa (PGF2alfa) receptor antagonist, abolished the differences in SNP relaxation between denuded (e-) and intact endothelium (e+) aorta (Ibuprophen pD2 e+: 11.71 ± 0.18, n=8; e-: 11.65 ± 0.11, n=06 and AH 6809 pD2 e+: 11.85 ± 0.03, n= 03; e-: 11.68 ± 0.07, n= 06). **Discussion:** The present findings indicate that lower potency of the SNP-relaxation in aortas without endothelium could be associated with an intense oxidative stress that leads to an enhancement of COX activation and PGF2a receptor activation. **Financial support:** FAPEMIG

06.004

Chronic ethanol consumption decreases the relaxation induced by adrenomedullin and increases its expression in the isolated rat aorta. Hipólito UV[1], Tirapelli DP[2], Jacob Ferreira ALB[3], Batalhão ME[4], Tanus-Santos JE[5], Carnio EC[4], Queiroz RHC[6], Tirapelli CR[7] [1]EERP-USP − Enfermagem Psiquiátrica e Ciências Humanas, [2]FMRP-USP − Cirurgia Anatomia, [3]FCM-UNICAMP − Farmacologia, [4]EERP-USP − Enfermagem Geral e Especializada, [5]FMRP-USP − Farmacologia, [6]FCFRP-USP − Toxicologia, [7]EERP-USP − Farmacologia

Introduction: Epidemiological data suggest that chronic ethanol consumption is a causative factor for cardiovascular diseases. Adrenomedullin (ADM) expression can be increased during cardiovascular disease/stress in vitro and in vivo where it may have a role in several cardiovascular protective actions. This study aimed to investigate the effects elicited by chronic ethanol consumption in the vascular ADM system. **Methods:** The experimental protocols were in accordance with the Ethical Committee from USP (07.1.942.53.5). Male Wistar rats were treated with ethanol (20% vol/vol) for 6 weeks. Vascular reactivity experiments using standard muscle bath procedures were performed on isolated thoracic aorta from Wistar rats. Nitrate and nitrite levels were measured in supernatants from total thoracic aorta homogenates prepared under liquid nitrogen. Metalloproteinase-2 (MMP-2) levels were measured by gelatin zymography. mRNA for pre-pro-ADM, CRLR (calcitonin receptor-like receptor) RAMPS 1,2,3 (receptor-activity-modifying proteins) and endothelial NO synthase (eNOS) was assessed by RQ-PCR. **Results:** Blood ethanol levels in the ethanol-treated rats averaged (1.87 ± 0.15 mg/ml n=11). Body weight of the rats before beginning the treatment averaged (276.7 ± 3.4g) in control group and (281 ± 4.4g) in ethanol group. The treatment for 6 weeks reduced the body weight of the rats from ethanol group (496 ± 8.5g) when compared to control group (591.8 ± 9.1g) ($P<0.05$, Student's t test). Chronic ethanol consumption significantly decreased ADM-induced relaxation (29.3 ± 3.4%, n=7) in endothelium intact rings when compared to control group (43.4 ± 2.2%, n=4) ($P<0.05$, Student's t test). However, in endothelium denuded rings no differences were observed in control and ethanol treated rats. Calcitonin gene-related peptide (CGRP), acetylcholine and sodium nitroprusside-induced relaxation were not affected by ethanol consumption. No difference on the basal nitrate and nitrite generation was found after treatment with ethanol. Similarly, chronic ethanol consumption did not alter aortic (MMP-2) levels. Ethanol consumption increased the mRNA levels for pre-pro-ADM and RAMP1. However, ethanol intake did not alter mRNA levels for CRLR, RAMP 2, 3 and eNOS. **Discussion:** Chronic ethanol consumption reduces ADM-induced relaxation and this response is endothelium dependent. Finally, ethanol increases the expression of pre-pro-ADM, a peptide that has been described to exert protective effects in the cardiovascular system. Supported by: FAPESP.

06.005

Effect of quercetin on diabetic nephropathy in hypercholesterolemic mice. Gomes IBS[1], Santos, MCLFS[2], Ricardo KFS[3], Meyrelles SS[4], Vasquez EC[5] [1]UFES − Pharmaceutical Sciences, [2]UFES − Pathology, [3]FAESA, [4]UFES − Physiological Sciences, [5]UFES-EMESCAM − Physiological Sciences

Introduction: Dyslipidemia contributes to the increased risk of death and morbidity in patients with diabetes by accelerating macro and microvascular disease. Furthermore, clinical observations have suggested that hyperlipidemia is a contributory factor to the progression of diabetic renal disease. Quercetin has been reported to have many beneficial effects on human health, including cardiovascular protection and anti-inflammatory effects. Considering the deleterious effects of diabetes the aim of this study was to evaluate the effect of quercetin on diabetic nephropathy in C57BL/6 and apoE deficient mice (apoE(-/-)) with experimental diabetes. **Methods:** Male mice C57 and apoE(-/-) 8-week-old were separated in groups: control (n=6), diabetic vehicle (n=6) and diabetic treated with quercetin (n=6). Diabetes was induced by injection of streptozotocin (STZ,100mg/kg/day, i.p.) diluted in citrate buffer, for 3 days, the vehicle group received only citrate buffer. The STZ-treated animals with glycemia lower than 250mg/dL were excluded from the study. Six weeks after diabetes induction, the animals were divided in vehicle and quercetin groups, which received vehicle (soy oil), or a suspension of quercetin (10 mg/kg, daily) orally during 4 weeks. In the 10th week mice were euthanized, blood was collected for dosage of glucose, triglycerides, creatinine, urea; urine was collected for dosage of creatinine and determination of proteinuria. All procedures were approved by the Animal Ethics Committee for Use of Animals of EMESCAM College of Health Sciences (013/2007). Data are expressed as mean ± SEM. Statistical analysis was performed using ANOVA followed by Fisher´s post hoc test. P< 0.05 was considered significant. **Results:** Diabetic mice showed significant increase in blood glucose (C57: 134,3 ± 5,3 vs. 388,0 ± 41,07* mg/dL; apoE: 192,9 ± 10,4 vs. 476,4 ± 45,4#mg/dL), polyuria (C57: 2,1 ± 0,2 vs. 24,2 ± 1,6**mL/24h; apoE: 1,9 ± 0,4 vs. 17,4 ± 3,3##mL/24h) and proteinuria (C57: 4,1 ± 0,6 vs. 10,9 ± 3,2*mg/24h; apoE: 4,0 ± 0,6 vs. 14,9 ± 2,1##mg/24h) compared with control group. Diabetic groups also exhibited renal dysfunction, as evidenced by increased plasma creatinine (C57: 0,2 ± 0,02 vs. 0,4 ± 0,03**mg/dL; apoE: 0,2 ± 0,01 vs. 0,4 ± 0,01##mg/dL), plasma urea (C57: 69,8 ± 2,9 vs. 115,3 ± 6,5**mg/dL; apoE: 83,6 ± 4,5 vs. 110,3 ± 8,5#mg/dL) and proteinuria. The treatment with quercetin decreased the plasma glucose level of both diabetic groups (C57: 388,0 ± 41,07 vs. 261,6 ± 30,2**mg/dL; apoE: 476,4 ± 45,4 vs. 358,3 ± 38,7##mg/dL), as well as reduced high plasma concentrations of triglycerides (C57: 109,6 ± 11,1 vs. 34,0 ± 5,5**mg/dL; apoE: 158,2 ± 24,6 vs. 69,8 ± 13,8#mg/dL) and creatinine (C57: 0,4 ± 0,03 vs. 0,2 ± 0,03**mg/dL; apoE: 0,4 ± 0,01 vs. 0,2 ± 0,03## mg/dL). The evaluation of renal function improved the creatinine clearance (C57: 150,7 ± 25,6 vs. 203,0 ± 22,7 µL/min; apoE: 159,1 ± 11,8 vs. 223,8 ± 36,0 µL/min) and decreased proteinuria (C57: 10,9 ± 3,2 vs. 4,4 ± 0,5*mg/24h; apoE: 14,9 ± 2,1 vs. 13,6 ± 1,1 mg/24h). **Discussion:** Reactive oxygen species play a key role in the pathophysiological processes of renal diseases. Thus, antioxidants, as quercetin, are expected to decrease the vulnerability of the kidney to oxidative challenges. Quercetin demonstrated a beneficial antidiabetic effect and ameliorated diabetic renal damage. **Financial support:** CAPES, CNPq, FAPES-PPSUS, Lab. Bioclínico

06.006

Characterization of the hypertensive mechanism of ethanolic extract of *L. ericoides*. de Paula DCC[1], Souza ACM[1], Guzzo LS[1], Guimarães HN[2], Saúde-Guimarães DA[1], Grabe-Guimarães, A[1] [1]DEFAR-UFOP, [2]UFMG − Engenharia Elétrica

Introduction: Several species of Lychnophora are used in Brazil to treat bruises, wounds, as antinociceptive and anti-inflammatory. These species are widely used by oral route, requiring the evaluation of their general activity providing basis for the rational use by the population. In this context, the cardiovascular activity of natural products poorly understood. Guzzo (2006) showed that the ethanolic extract of L. ericoides presents hypertensive effect at the highest dose evaluated. **Methods:** Aerial parts of Lychnophora ericoides Mart., were dried at 400C, reduced to powder and extracted with ethanol, at room temperature. The solvent was then removed. The extracts solutions were dissolved in DMSO (dimethylsulfoxide), Tween 80 and distilled water (1:1:8). All the procedures were approved by the UFOP Ethical Committee under number 2009/11. Male Wistar rats (260 ± 40 g) were divided into 4 groups that received an orally single dose of L. ericoides ethanolic extract (1,5 g/kg - 100 mg/ml) dissolved in capryol:water (6.5:3.5) (n=18): 1) only extract, 2) extract+atenolol (5 mg/kg), 3) extract+captopril (10 mg/kg) 4) extract+prazosin (1 mg/kg). The animals were anesthetized with thiopental (60 mg/kg), 30 min after administration of the extract, and had catheters implanted into the femoral artery and vein, in order to obtain arterial pressure (AP) signal and for drugs administration, respectively. Electrodes were inserted subcutaneously for electrocardiogram (ECG) recording (DII). The drugs were administered 105 min after oral administration of the extract. The signals were obtained for more 20 min. **Results:** Compared to the group that received only the extract, atenolol did not reduce the systolic AP (SAP) and diastolic AP (DAP) and reduced significantly the heart rate (HR) (maximum of 30 %). Captopril was able to reduce 23 % of SAP and 28 % of DAP reduction. Prazosin significantly reduced 30 % of SAP after 125 min and 36 % of DAP after 115 min. **Discussion:** Considering the hypertensive activity already demonstrated for the L. ericoides extract (GUZZO, 2006), in the present work were used antagonists that interfere with the cardiovascular activity and can indicate its action mechanism. Prazosin.was able to induce the greater reduction of AP and normal values were reached soon after its IV administration. The results demonstrated that the likely mechanism of hypertensive action induced by the L. ericoides extract is a sympathetic nervous system hyperactivity mainly at vascular level. Supported by: UFOP, FAPEMIG. Citation: GUZZO, L. S., 08.068 SBFTE, 2006.

06.007

Vasodilatatory activity and antihypertensive profile of a new N-acylhydrazone derivative: LASSBio-1027. Leal CM[1], Kummerle AE[2], Leal DM[3], Barreiro EJ[2], Fraga CAM[2], Sudo RT[5], Zapata-Sudo G[5] [1]UFRJ − Farmacologia Básica e Clínica, [2]FF-UFRJ – LASSBio, [3]UFRJ − Farmacologia, [4]UFRJ − LASSBio, UFRJ, [5]UFRJ

Introduction: LASSBio-1027 was synthesized using the prototype LASSBio-294, a potent vasodilator, as lead compound. The goal of the present work is to investigate the mechanisms of action involved in the vasodilatory activity of LASSBio-1027 in aortic rings and evaluate its action on the blood pressure of normotensive (Wistar Kyoto, WKY) and spontaneously hypertensive rats (SHR). **Methods:** This experimental protocol was approved by research ethics under license DFBCICB 013.Thoracic aorta was dissected from male Wistar rats (240–280 g) and prepared for isometric tension recording. Aorta was cut in rings of 2–3 mm and placed in vertical chambers filled with modified Tyrode solution. Preparations were stabilized under 1 g resting tension for 2 h and then, the contractile response to phenylephrine (10 μM) was measured before and after exposure to increasing concentrations of derivatives. A phenylephrine-induced contracture was followed by exposure to acetylcholine (10 μM) to test the integrity of the endothelium. To evaluate possible mechanisms involved in the vasodilation induced by the derivatives, aortic rings were pretreated with the following blockers: L-NAME, inhibitor of nitric oxide synthase (100 μM), glibenclamide, inhibitor of potassium channels sensitive to ATP (5 μM), ZM 241385 selective antagonist of adenosine A2A receptor (100 nM). Male WKY and SHR (13 weeks old) were intraperitoneously injected daily with a single dose of 10 mg/kg LASSBio-1027 for 14 days. Systolic (SP), diastolic (DP) and mean arterial pressure (MAP) were measured using a noninvasive method through the pressure meter (LE 5001, PanLab). For the histological analysis, aorta, heart, brain, liver, kidney, lung and skeletal muscle were excised, fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin. **Results:** LASSBio-1027 promoted vascular relaxation in a concentration-dependent manner, in which the concentration necessary to induce 50% of relaxation (IC50) was 6.9 ± 1.4 μM (n= 6). Removal of endothelium shifted the concentration-response curve to the right with a significant increase of IC50 to 154.7 ± 17.4 μM (n= 6; $P<0,05$). Pretreatment of aorta with preserved endothelium with L-NAME increased the IC50 to 179.0 ± 9.1 μM (n= 4). Vascular relaxation induced by LASSBio-1027 was reduced after exposure to glibenclamide, glibenclamide + L-NAME or ZM241385. Daily treatment of SHR with LASSBio-1027 significantly reduced SP, DP and MAP from 219.4 ± 5.7 to 118.0 ± 1.4 (n= 6; $P<0,05$); from 171.7 ± 4.7 to 95.5 ± 4.1(n= 6; $P<0,05$) and from 187.2 ± 4.8 to 103.3 ± 3.1 mmHg (n= 6; $P<0,05$), respectively. No significant alterations were observed in SP, DP and MAP in WKY. No morphological modifications were observed after prolonged treatment with LASSBio-1027. **Discussion:** The vasodilation induced by LASSBio-1027 is mediated by nitric oxide production, opening of ATP-sensitive K+ channels and involves the activation of adenosine receptors. Reduction of blood pressure was observed during daily treatment with LASSBio-1027 in SHR indicating its potential as a new antihypertensive agent. **Acknowledgements**: CAPES, FAPERJ, CNPq, FUJB, INCT.

06.008

Analysis of the mechanisms underlying the vasorelaxant action of THE Kaurane acid 16-metoxicauran-19-oic in the isolated rat aorta. Palazzin NB[1], Bonaventura D[2], Ambrósio SR[3], Hipólito UV[4], Tirapelli CR[5] − [1]EPCH-EERP-USP, [2]UFMG, [3]UNIFRAN − Bioprospecção e Biotransformação, [4]FMRP-USP − Farmacologia, [5]EERP-USP − Farmacologia

Introduction: The present work aimed to investigate the mechanisms underlying the vasorelaxant effect of the diterpene 16-Metoxicauran-19-oic (KAOCH3). **Methods:** Vascular reactivity experiments using standard muscle bath procedures were performed on isolated thoracic aorta from Wistar rats. The experimental protocols were in accordance with the Ethical Committee from USP (09.1.706.53.1). Data was analyzed by ANOVA followed by Bonferroni's multiple comparison test ($P<0.05$ was considered statistically significant). **Results:** Pre-incubation of aortic rings for 30 and 60 min with the diterpene (10, 50 and 100 mmol/L) showed that the period required by KAOCH3 to achieve its maximal inhibitory activity is 30 min. KAOCH3 (10, 50 and 100 mmol/L) concentration-dependently inhibited phenylephrine-induced contraction in either endothelium-intact or denuded rat aortic rings. On the other hand, KAOCH3 did not interfere with Ca2+ release from intracellular stores mediated by either phenylephrine (1 µmol/L) or caffeine (30 mmol/L). KAOCH3 (1-450 mmol/L) concentration dependently relaxed phenylephrine-pre-contracted rings with intact (80.6 ± 2.8%, n=10) or denuded endothelium (80.8 ± 6.4%, n=6). The diterpene also relaxed KCl-pre-contracted rings with intact (85.4 ± 3.9%, n=6) or denuded endothelium (85.1 ± 8.0%, n=6). **Discussion:** Collectively, our results provide functional evidence that the vascular effects elicited by KAOCH3 are endothelium-independent and probably involve extracellular Ca2+ influx blockage. Supported by: FAPESP

06.009

Effects of chronic ethanol consumption on the reactivity and adrenomedullin mRNA levels of components of this system in the rat mesenteric bed. [1]Rocha JT, [3]Hipólito UV, [2]Tirapelli DP, [2]Jacob-Ferreira AL, [1]Batalhão ME, [2]Tanus-Santos JE, [1]Carnio EC, [1]Tirapelli CR, [1]EERP-USP, [2]FMRP-USP, [3]EERP-USP / FMRP-USP

Introduction: Chronic ethanol consumption is a causative factor for cardiovascular diseases such as hypertension. The expression of the peptide adrenomedullin (ADM) is increased during cardiovascular disease/stress where it displays cardiovascular protective actions. Our study aimed to investigate the effects elicited by chronic ethanol consumption in the vascular ADM system in the mesenteric bed (MB). **Methods**: The experimental protocols were in accordance with the Ethical Committee from USP (06.1.1094.53.7). Male Wistar rats were treated with ethanol (20% vol/vol) for 6 weeks. Vascular reactivity experiments were performed on isolated MB from Wistar rats. Nitrate and nitrite levels were measured in supernatants from total MB homogenates prepared under liquid nitrogen. Metalloproteinase-2 (MMP-2) levels were measured by gelatin zymography. mRNA for pre-pro-ADM, CRLR (calcitonin receptor-like receptor) RAMPS 1,2,3 (receptor-activity-modifying proteins) and endothelial NO synthase (eNOS) was assessed by RQ-PCR. **Results:** Vascular reactivity experiments showed that basal perfusion pressure (mmHg) was not different between the groups (Control: 17.9 ± 0.8, n=10; Ethanol: 19.5 ± 0.9, n=9). Chronic ethanol consumption did not alter the endothelium-dependent relaxation induced by ADM as well as the endothelium-independent relaxation induced by sodium nitroprusside. Conversely, ethanol consumption significantly decreased acetylcholine-induced relaxation (29.9 ± 3.5%, n=7) in endothelium intact rings when compared to control group (55.5 ± 1.6%, n=9) ($P<0.05$, Sudent's t test). Phenylephrine-induced contraction was increased in endothelium-intact MB from ethanol-treated rats (137.6 ± 1.7 mmHg, n=7) when compared to control (106.3 ± 3.3 mmHg, n=9). ($P<0.05$, Sudent's t test). No difference on the basal nitrate and nitrite generation was found after treatment with ethanol. Similarly, chronic ethanol consumption did not alter pro- or active-MMP-2 levels in rat MB. Ethanol consumption increased mRNA levels for pre-pro-ADM. However, ethanol intake did not alter mRNA levels for CRLR, RAMP 1, 2, 3 and eNOS. **Discussion:** Chronic ethanol consumption did not alter ADM-induced relaxation by significantly reduced the endothelium-dependent relaxation induced by acetylcholine. Finally, ethanol increases the expression of pre-pro-ADM, a peptide that has been described to exert protective effects in the cardiovascular system. Supported by: FAPESP.

06.010

Characterization of L-arginine-NO-cGMP pathway in spontaneously hypertensive rat platelets: the effects of pregnancy. Ognibene DT, Bello PHP, Moss MB, Soares de Moura R, Brunini T, Mendes Ribeiro AC, Resende AC UERJ − Farmacologia e Psicobiologia

Introduction: Nitric oxide (NO) is a short-lived intercellular messenger that provides an efficient vascular regulatory mechanism to support homeostasis and prevent thrombosis. Endothelial dysfunction and reduced NO bioavailability play a central role in hypertension associated with pregnancy. The purpose of this study was to investigate the impact of pregnancy on the L-arginine-NO-cGMP pathway in platelets and its correlation to platelet function and blood pressure in normotensive and spontaneously hypertensive rats (SHR). **Methods:** The experiments were approved by the Ethics Committee of the UERJ (CEP/HUPE – CAAE:0018.0.228.000-07). Platelets were obtained from blood in the 20th day of pregnancy from female SHR (SHR-P) and normotensive controls (-P) or age-matched non-pregnant rats (SHR-NP and –NP). Systolic blood pressure (SBP, mm Hg) was measured by the tail-cuff method after mating and at 7, 14 and 20 days of pregnancy and in control non-pregnant rats. Total L-[3H]-arginine transport (100 μM) and basal nitric oxide synthase (NOS) activity measured by the conversion of L-[3H]-arginine (1 μM) to L-[3H]-citruline were evaluated. The endothelial NOS (eNOS) and inducible NOS expression (iNOS), as well as the phosphodiasterase 5 (PDE5) and soluble guanilate cyclase (sGC) were evaluated by Western Blotting. The intraplatelet cGMP measurement was performed by ELISA and the platelet aggregation was induced by ADP (12 μM). **Results:** The SBP was increased in SHR-NP compared to –NP rats (-NP: 137 ± 1.9/ SHR-NP: 182 ± 4.9) and it was reduced at the end of pregnancy (-P at day 1: 131 ± 2.3/ -P at day 20: 110 ± 2.7), as well, as in SHR-P rats (SHR-P at day 1: 183 ± 5.4/ SHR-P at day 20: 128 ± 3.4). Intraplatelet NOS activity was reduced in –P compared to –NP (-NP: 0.27 ± 0.02/ -P: 0.15 ± 0.01), despite unchanged L-arginine influx (-NP: 0.55 ± 0.06/ -P: 0.60 ± 0.01). The expression of eNOS and iNOS were decreased during pregnancy in normotensive rats. Paradoxically, cGMP levels were similar between –NP and –P (-NP: 0.16 ± 0.02/ -P: 0.21 ± 0.01), as well as PDE5 expression (-NP: 6.25 ± 0.16/ -P: 4.35 ± 0.59) and platelet aggregation induced by ADP (-NP: 46.28 3.65/ - P: 43.66 ± 4.67). In SHR, L-arginine influx was reduced in SHR-P compared to SHR-NP (SHR-NP: 0.68 ± 0.07/ SHR-P: 0.43 ± 0.05). SHR-P had impaired NOS activity (SHR-NP: 0.28 ± 0.03/ SHR-P: 0.18 ± 0.03) and reduced iNOS expression compared with SHR-NP. sGC and PDE5 expressions were lower in SHR-P compared to SHR-NP (SHR-NP:17.3 ± 1/SHR-P:14 ± 0.5; SHR-NP:10.2 ± 1/SHR-P:6.5 ± 0.3, respectively) while no differences were noted in cGMP levels between groups (SHR-NP: 0.26 ± 0.03/ SHR-P: 0.35 ± 0.05). However, increased levels of cGMP levels were observed in SHR-P compared to normotensive groups and platelet aggregability remained unaltered (SHR-NP: 42.12 ± 4.19/ SHR-P: 38.62 ± 3.27). **Discussion:** This study reveals evidence for an active L-arginine-NO-cGMP pathway in rat platelets. Moreover, pregnancy decreases L-arginine transport, NOS activity and iNOS expression in platelets from hypertensive rats compared to non-pregnant hypertensive rats. Despite reduced platelet NO bioavailability in pregnant hypertensive rats, platelet aggregability remains unaltered, which may be related to increased levels of cGMP and reduced expression of PDE5. **Financial support:** CNPq and FAPERJ.

06.011

Effects of intermittent hypoxia on biochemical parameters of rats fed with different diets. Simões RR[1], Dutra AL[1], França RT[2], Lopes STA[2], Portela LOC[3], Zanchet EM[1] [1]UFSM − Fisiologia e Farmacologia, [2]UFSM − Clínica de Pequenos Animais, [3]UFSM − Educação Física e Desportos

Introduction: and aims: Intermittent hypoxia uses breathing with a low oxygen concentration with intervening periods of normal breathing. It is employed for pre-acclimatation to high altitude and in the treatment and prophylaxy of several diseases (Serebrovskaya, T.V. High Alt Med Biol, 3(2):205, 2002). This research aimed at evaluating the effect of intermittent hypoxia on biochemical parameters of rats fed with normo fat (NF) or high fat (HF) diets. **Methods:** Two groups of Wistar rats (200-250 g) were fed with NF or HF for 17 weeks. On the 12 week , the rats were redivided and continued receiving the same diets, but were subjected to intermittent hypoxia (IH) or normoxia (N) sessions. The groups NF/IH, NF/N, HF/IH and HF/N were formed. IH program was administered for 34 days. The program consisted of 15 minutes of hypoxic exposure with 5 minutes reoxygenation and a total duration of 2 hours per day. The normoxia group was subjected to the same conditions, but was exposed to normal O2 concentrations. After this period, the rats were anesthetized with halothane and blood was collected by cardiac punction for biochemical parameters analysis. **Results and Discussion:** When data were compared through two-way ANOVA, followed by Duncan's test, there was no significant difference in total cholesterol, glycemia and LDL levels among the different diets and O2 conditions (IH or N). Triglyceride levels were significantly lower in NF/IH group compared to NF/N group. Although there was no statistical difference, triglyceride value was lower in HF/IH group than in HF/N group. HDL values were different only in the hypoxic conditions (NF/IH; HF/IH) between the different diets. Diet/O2 Condition NF/ N NF/IH HF/N HF /IH Triglycerides (mg/dL) 83,1 ± 25,27* 58,21 ± 0,6*# 91,33 ± 26,06# 72,44 ± 12,5 Total Cholesterol (mg/dL) 87,8 ± 16,15 82,4 ± 13,1 86,17 ± 17,63 84,44 ± 13,63 HDL (mg/dL) 35,9 ± 4,3 37 ± 2,78 *# 33,3 ± 2,4 31,8 ± 3,5*# LDL (mg/dL) 35,2 ± 12,85 33,6 ± 12,38 34,7 ± 15,68 38,22 ± 13,19 Glycemia (mg/dL) 90 ± 7,16 98 ± 12,51 99,83 ± 9,41 94,8 ± 9,62 * Difference between IH or N on diets NF or HF; # difference between diets; $p < 0,05$. Our findings indicate that IH significantly reduced triglycerides in HF group. Likewise, triglyceride values were lower in HF/IH group than in NF/N group. The present data may provide new insights into alternative treatments of dislipidemic disorders. No financial support. Approved by the Ethical Committee on Animal Experimentation of UFSM. Process 230681.009917/2009-69.

06.012

Effects of intermittent hypoxia on oxidative parameters of rats fed with different diets.

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Introduction: and aims: Intermittent hypoxia (IH) refers to the method that uses cyclic exposure to moderate hypoxia with intervening periods of normoxia. IH is employed for pre-acclimation to high altitude, sports and in the treatment of several diseases (Serebrovskaya, T.V. High Alt Med Biol, 3(2):205, 2002). The aim of this study was to evaluate whether intermittent hypoxia alters oxidative parameters in cardiac tissue of rats fed with different diets. **Methods:** Two groups of Wistar rats (200-250 g) were fed with normal fat (NF) or high fat (HF) diets for 17 weeks. On the 12 week, the rats were redivided and continued receiving the same diets, but were subjected to intermittent hypoxia (IH) or normoxia (N) sessions. The groups NF/IH, NF/N, HF/IH and HF/N were formed. The IH program was administered during 34 days. The program consisted of 15 minutes of hypoxic exposure with 5 minutes reoxygenation and a total duration of 2 hours per day. The normoxia groups were subjected to the same conditions, except that O2 concentrations were normal. In the end of the 34 sessions, the rats were euthanized with halothane and the hearts collected, homogenized in phosphate buffer (30mM, pH 7.4) and centrifuged at 3500xg for 10 minutes. Supernatants were used to determine lipoperoxidation through the measurement of thiobarbituric acid reactive substances (TBARS) and the superoxide dismutase enzyme (SOD). **Results and Discussion:** The comparison between groups NF/IH (0,59 ± 0,14 nmol/mg protein) and NF/N (0,57 ± 0,14 nmol/mg protein) did not evince significant changes in TBARS levels. The same was verified in groups HF/N (0,13 ± 0,02 nmol/mg protein) and HF/IH (0,18 ± 0,02 nmol/mg protein). SOD values reduced significantly ($p < 0,05$) in group NF/IH (10,71 ± 2,42 USOD/mg protein) when compared to group NF/N (15,20 ± 4,31 USOD/mg protein). IH significantly altered SOD levels in heart of rats fed with NF diet, suggesting a possible protective role of IH in the formation of reactive oxygen species. Nonetheless, more studies are needed to confirm these findings. No financial support. Approved by the Ethics Committee on Animal Experimentation of UFSM. Process 230681.009917/2009-69.

06.013

Role of renin-angiotensin system and oxidative status on the maternal cardiovascular regulation in spontaneously hypertensive rats. Bello PHP, Ognibene DT, Carvalho LCRM, Costa CA, Soares de Moura R, Resende AC UERJ − Farmacologia e Psicobiologia

Introduction: During pregnancy the systemic maternal circulation adapts to favor uteroplacental perfusion, through increases in plasma volume and cardiac output. Despite an expressive increase in renin-angiotensin system (RAS) activity in the normal pregnancy, blood pressure does not increase. The purpose of this study was to investigate the contribution of RAS and oxidative status on the maternal cardiovascular regulation at the end of pregnancy in normotensive and spontaneously hypertensive rats (SHR). **Methods:** The experiments were approved by the Ethics Committee of the UERJ (protocol–CAAE:0018.0.228.000-07). Mesenteric arterial bed (MAB) was isolated from female SHR (SHR-P) and normotensive controls (-P) in the 20th day of pregnancy or age-matched non-pregnant rats (SHR-NP and –NP). Systolic blood pressure (SBP, mm Hg) was measured by the tail-cuff method. Vasodilator effects of angiotensin II (Ang II) and angiotensin 1-7 (Ang 1-7) were evaluated in preconstricted MAB. MAB endothelial nitric oxide synthase (eNOS), AT1 and AT2 receptors and angiotensin-converting enzyme (ACE) and ACE2 expressions were evaluated by Western Blotting. MAB homogenates were used to determine oxidative damage by formation of thiobarbituric acid reaction substances (TBARS nmol/mg protein) and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities (U/mg protein) by spectrophotometry. **Results:** The SBP in –P rats was reduced at the end of pregnancy (-P at day 1:131 ± 2/-P at day 20:110 ± 3) as well as in SHR-P (SHR-P at day 1:183 ± 5/SHR-P at day 20:128 ± 3). The vasodilator effects of Ang II (300nmol; -NP:32 ± 2/-P:38 ± 1;SHR-NP:41 ± 1/SHR-P:49 ± 2) and Ang 1-7 (300nmol;-NP:28 ± 2/-P:32 ± 2;SHR-NP:26 ± 1/SHR-P:43 ± 2) were higher in MAB from SHR-P than in other groups. MAB eNOS expression was increased in –P and SHR-P compared to their non-pregnant counterparts (-NP:129 ± 5/-P:184.5 ± 8; SHR-NP:141 ± 15.3/SHR-P:195.2 ± 12.4). AT1 receptor expression was increased in MAB from SHR-NP compared to normotensive groups and pregnancy reduced AT1 expression in SHR (-NP:15.27 ± 0.9/-P:19.9 ± 1;SHR-NP:36 ± 4/SHR-P:22 ± 2). No difference was observed in AT2 receptor expression among the groups. ACE expression was increased in SHR-NP compared to normotensive groups and pregnancy reduced ACE expression in SHR (-NP:90 ± 2/-P:95 ± 4;SHR-NP:215 ± 22/SHR-P:152 ± 18). ACE2 expression was higher in MAB from hypertensive than normotensive groups (-NP:155 ± 16/-P:156 ± 16;SHR-NP:242 ± 9/SHR-P:250 ± 5). TBARS levels were reduced in the pregnant groups compared to their non-pregnant counterparts (-NP:0.85 ± 0.03/-P:0.44 ± 0.06;SHR-NP:0.79 ± 0.02/SHR-P:0.34 ± 0.02). SOD activity was reduced in SHR-P compared to non-pregnant group (-NP:66.3 ± 7.3/-P:55.1 ± 5.5;SHR-NP:68.2 ± 6.7/SHR-P:36.8 ± 4.8). Pregnancy increased CAT activity in normotensive rats (-NP:0.021 ± 0.004/-P:0.066 ± 0.01;SHR-NP:0.042 ± 0.006/SHR-P:0.04 ± 0.01), and increased GPx activity in SHR (-NP:0.024 ± 0.001/-P:0.026 ± 0.001;SHR-NP:0.028 ± 0.002/SHR-P:0.045 ± 0.001). **Discussion:** the results suggest that the reduction of blood pressure to normal values at the end of pregnancy in SHR may be related to an increased NO production and vasorelaxation to Ang II and Ang 1-7 associated with decreased expression of vascular ACE and AT1 receptors and oxidative status. **Financial support:** CNPq and FAPERJ.

06.014

Molecular mechanisms involved in the dual blockade of the renin angiotensin system (RAS) on the left ventricular remodeling in renal hypertensive rats (2K-1C). Corrêa JWN[1], Callera GE[2], Yogi A.[2], He Y[2], Araújo AV[1], Vercesi JA[3], Riul ME[4], Prado CM[4], Rossi MA[4], Touyz RM[2], Bendhack LM[3] [1]FMRP-USP − Farmacologia, [2]University of Ottawa − Kidney Research, [3]FCFRP-USP − Física e Química, [4]FMRP-USP − Patologia

Introduction: Whether the dual blockade of the RAS would lead to better blood-pressure control and potentially cardioprotective effects remains under discussion. Moreover, the molecular mechanisms of these beneficial effects have yet to be identified. Objective: To evaluate molecular effects of dual blockade of the RAS on renal hypertension (2K-1C rats) and cardiac remodeling and to assess whether the hypertrophic NFAT (cytosol-nuclear translocation) signaling pathway is influenced by dual RAS blockade. **Methods:** Renal hypertension was induced in 2K-1C male Wistar rats (180-200g). Control rats were sham-operated (2K). After six weeks, rats were treated with losartan (L, 30mg/Kg/d), enalapril (E, 20mg/Kg/d), losartan+enalapril (L+E, 20 and 30mg/Kg/d, respectively) or saline by gavage for 14 days. Systolic blood pressure (SBP) was measured by tail cuff technique every 3 days. Rats were euthanized and the hearts removed, weighed and collected for western blot analysis and histology. Experimental procedures are in accordance to Ethics Committee (CEUA-USP 07.1.607.53.1). **Results:** SBP was higher in 2K-1C than in 2K control rats (200 ± 23 vs 98 ± 4 mmHg P<0.0001). All the treatments had no effect in 2K, but progressively reduced SBP in 2K-1C with an additional hypotensive effect in the group treated with L+E. The increased cardiac hypertrophy index (heart/body weight) and collagen content in 2K-1C, that is indicative of hypertrophy and fibrosis, were similarly reduced by dual and mono therapy. In the left ventricle, ACE1 expression was similar in 2K and 2K-1C, without effect of the treatments. AT1R expression was higher while AT2R, MASR and ACE2 were lower in 2K-1C than in 2K. Activation of NFAT, a hypertrophic transcription factor, was increased and expression of calmodulin (CaM) was decreased in 2K-1C vs 2K. Calcineurin and total NFAT expression were similar in 2K and 2K-1C. Expression of CAMKII and Phospho-JNK, enzymes, that negatively regulates NFAT signaling, were reduced in 2K-1C. Treatment of 2K-1C with E or L+E corrected all changes associated with hypertension. The only additional effect induced by L+E was MASR upregulation in 2K-1C. Treatment with L did not normalize the expression of AT1R, MASR, CAMKII and P-JNK and only partially inhibited NFAT activation. Conclusions: Molecular mechanisms associated with cardiac hypertrophy in 2K-1C hypertensive rats involve upregulation of the calcineurin-NFAT pathway, increased expression of AT1R and reduced expression of MASR and AT2R. Beneficial effects of dual blockade are associated with normalization of Ang II and calcineurin-NFAT signaling. Monotherapy with L or E had variable actions on molecular processes, despite having similar effects on blood pressure and cardiac hypertrophy. These findings suggest that E and L act in distinct ways to produce similar pharmacological inhibitory effects upon cardiac remodeling. Supported by FAPESP, CAPES, CNPq, CIHR and Heart and Stroke Foundation of Canada.

06.015

Renovascular hypertension alters the contribution of alternative pathway to ace in the renal vascular response in isolated kidney. Sivieri-Jr DO[1], Pereira HJV[2], Oliveira EB[2], Salgado MCO[3] [1]UFVJM − Farmácia, [2]FMRP-USP − Bioquímica e Imunologia, [3]FMRP-USP − Farmacologia

Introduction: We evaluated the renal vascular response to Ang I and its metabolism in the renal perfusate (RP) and renal filtrate (RF) from isolated kidney of false-operated (FO, n=4-5) and two-kidney, one-clip hypertensive rats (2K1C, n=3-6). **Methods:** The renal artery was cannulated with a polyethylene tube and the kidney was removed and perfused with Tyrode solution (4 mL/min) in a water-jacketed organ bath maintained at 37°C. Dose-response curves to Ang I (1-500pmol) were obtained during a control period and in the presence of lisinopril (LIS; 10 μM). The RP and RF were collected by perfusing kidney for 2 h at the same conditions described above. The RP, 260 mL, was concentrated 185-fold, and the RF, 1.4 mL, was collected through a cannula implanted in the ureter. Processing of Ang I was evaluated by incubating 20 nmol of peptide for 40 min at 37ºC with samples of RP or RF. The reactions were carried out in the absence or presence of the protease inhibitors phosphoramidon (PHO; 10 μM), bestatin (BES; 10 μM), chymostatin (CHY; 100 μM) or ortophenantroline (ORT; 1 mM) and the products generated were analyzed by HPLC. All experimental protocols used were reviewed and approved by the Animal Care and Use Committee of the Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (Protocol nº 052/2008). **Results:** Dose-response curves to Ang I were similar in both groups and LIS blunted these responses in the controls kidneys. The degradation of Ang I by proteolytic activity present in the RF was greater in the 2K1C than FO. On the other hand, the RP-catalyzed reactions of FO and 2K1C were similar. The major products formed in the reactions carried out with RP and RF were Ang 3-10 and Ang 1-7. PHO decreased Ang 1-7 formation in the RP-catalyzed reactions and abolished it in the reactions carried out with RF of both groups. PHO increased the Ang 3-10 generation only in reactions carried out with RF of 2K1C. ORT decreased the generation of both fragments in all reactions. BES increased the generation of Ang 1-7 only in RP-catalyzed reactions of FO. CHY did not affect the generation of fragments. **Discussion:** The effect of LIS suggests that the hypertensive state increase the contribution of alternative Ang II-forming pathway in the renovascular tissue. The processing of Ang I effected by soluble peptidases was similar concerning the total angiotensinolytic and the proteolytic specificities of the effector enzymes present in RP of both groups. Differently, the hypertensive state increases the degradation of Ang I in the reactions carried out with RF. It is noteworthy that the major proteolitic activities observed in both fluids belong to the metallopeptidase family of proteases. The neutral endopeptidase 24.11 seems to be the major proteolitic activity involved in the RF-catalyzed cleavage of Ang I. Although the angiotensin-converting enzyme is important in the renal vascular tissue it is not secreted to the perfusate. Supported by: FAPESP.

06.016

The disruption of intracellular Ca^{+2} homeostasis is associated with a change of heart function in rats chronically malnourished. Silva DB[1], Mendes LVP[1], Nascimento JHM[2], Einicker-Lamas M[2], Vieyra A[2], Cunha VMN[1], Lara Morcillo LS[1] [1]UFRJ − Farmacologia Celular e Molecular, [2]IBCCF-UFRJ

Introduction: It has been shown that chronic malnutrition promoted by regional basic diet (RBD) alters the homeostasis of intracellular Ca2+, by affecting the activity of the Ca2+ pumps that are important for ensuring the contractile function of cardiac muscle. Previously, we observed changes in vesicular ATP-dependent Ca2+ accumulation, as SERCA and PMCA ATPase activities were increased and decreased, respectively. Furthermore, it was seen a decrease in expression of PMCA while SERCA expression remained in control levels. We suggested that these changes could cause heart damage as well as heart pathologies. The aim of the present work was to evaluate possible histological and cardiovascular parameters alterations related to imbalance Ca2+ homeostasis promoted by malnutrition. **Methods:** After weaning, rats from healthy mothers were submitted to the RBD diet (RBD-CR, n=6) for 10 weeks, while the control group was fed with a conventional diet (Cont, n=6). After this period, the animals were subjected to electrocardiography and echocardiography for heart functional evaluation. After sacrifice (CEUA DFBCICB 007), ventricles and lungs were removed for histological analysis (with hematoxylin-eosin (HE) and picrosirius staining) and weight measure, respectively. **Results:** Electrocardiographic analysis showed a significant decrease of several hemodynamic parameters: cardiac output (0.0925L/min vs 0.044L/min), ejection fraction (85.8% vs 24.75%) and, sistolic volume (0.352 mL vs 0.180mL), as well as in left diastolic ventricular diameter (0.715cm vs 0.558cm) between control and RDB-CR groups, respectively ($p<0.05$, n=5). Conventional histological assays showed no change in the tissue sections stained with HE or picrosirius between control and RDC-CR rats ($p>0.05$, n=9), but a computational quantitative analysis showed an increase of collagen in the cardiac tissue of malnourished group ($p<0.05$, n=9). Furthermore, the weight of the lungs of malnourished was lower than for control rats (1.09g vs 1.79g, respectively), and the lung weight / body weight ratio increased (0.7 mg/g vs 0.4 mg/g) ($p<0.05$, n=7). Conclusions: During the period of malnutrition, cardiac molecular mechanisms (as showed before) are modified to disrupt the homeostasis of intracellular Ca2+. Chronic malnutrition promotes an increase of SERCA ATPase activity, trying to maintain the appropriate Ca2+ content of in heart sarcoplasmic reticulum. This molecular modification leads to an impact on the heart hemodynamic parameters compromising the physiology of the cardiac muscle and all of these alterations are linked to the presence of fibrosis and accumulation of fluids in the lungs. It is concluded that chronic malnutrition can be responsible for the establishment of the initial phase of left heart failure process in young adult rats. **Financial support:** Projeto Casadinho-CNPq; PROCAD-CAPES; FAPERJ Primeiros Projetos, Programa ALV.

06.017

Endothelial oxidative stress induced by diabetes mellitus I increases maximum contraction evoked by angiotensin II in rat carotid artery. Pernomian L[1], Gomes MS[2], Oliveira AM[2]
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Introduction: Diabetes Mellitus I is characterized by endothelial dysfunction involving an increase in contraction induced by some agonists (Yousif MHM et al., Cell Biochem Funct 24, 13, 2006) and in vascular reactive oxygen species (ROS) production (Lund DD et al., Pharmacol Physiol 29, 305, 2002), including superoxide anion (O2-). The main source of vascular O2-, i.e. NADPH oxidase, is activated by AT1 receptors occupied by angiotensin II (Ang II), during vasoconstriction (de Gasparo M et al., Pharmacol Rev 52, 415, 2000). Based on these, we tested the hypothesis that Diabetes Mellitus I augments Ang II maximum contraction in rat carotid artery, involving the participation of O2-. **Methods:** This study was approved by Ethical Commission from School of Medicine from Ribeirão Preto / University of São Paulo (nº 007/2009). Diabetes Mellitus was induced in male Wistar rats (60 days) by streptozotocin (50mg/kg) and experiments were performed 6 weeks days after induction. Concentration-response curves for Ang II (10-11 to 10-6 mol/L) were obtained in endothelium-intact or -denuded carotid rings from normoglycemic and diabetic rats, in absence or presence of the non-selective NOS inhibitor L-NAME (10-4 mol/L, 30 min), the NO scavenger hydroxycobalamin (10-4 mol/L, 30 min) or the O2- scavenger tempol (10-3 mol/L, 20 min). Endothelial ROS quantification was performed by flow cytometry in endothelial cells from carotid artery of normoglycemic and diabetic rats, with the ROS fluorescent dye dihydroethidine (DHE, 2.5 x 10-6mol/L, 20 min), in absence or presence of the selective O2- scavenger tiron (10-3 mol/L, 30 min). Oxidative stress was assessed by nitrotyrosine immunohistochemical analysis in carotid sections from normoglycemic and diabetic rats, stained with rabbit monoclonal anti-nitrotyrosine (1:300). **Results:** Ang II maximum contraction is significantly augmented in endothelium-intact carotid rings from diabetic rats (Emax = 1.65 + 0.084 g/mg, n=7) in relation to normoglycemic rats (Emax = 0.56 + 0.035 g/mg, n=7). Endothelium removal reduced Ang II maximum contraction in carotid rings from diabetic rats (Emax = 0.71 + 0.029 g/mg, n=7). Ang II maximum contraction in endothelium-intact carotid rings from diabetic rats was not altered by L-NAME (Emax = 1.61 + 0.036 g/mg, n=7) or hydroxycobalamin (Emax = 1.67 + 0.068 g/mg, n=7). Tempol reduced Ang II maximum contraction in endothelium-intact carotid rings from diabetic rats (Emax = 0.82 + 0.024 g/mg, n=7). DHE fluorescence intensity (FI) was significantly augmented in endothelial cells from diabetic carotid arteries (FI = 28,181.6 + 1,555.7 U, n=5) when compared to normoglycemic samples (FI = 11,047.0 + 771.1 U, n=5). Tiron normalized DHE fluorescence in endothelial cells from diabetic carotid arteries (FI = 8,097.2 + 507.7 U, n=5). Staining for nitrotyrosine was augmented in whole extension of carotid sections from diabetic rats, especially in endothelium. **Discussion:** Diabetes Mellitus I increases Ang II maximum contraction in rat carotid artery involving the inactivation of NO derived from NOS by endothelial O2-, which production is augmented. **Financial support:** CNPq and FAPESP.

06.018

Hypercholesterolemia and aging: deleterious effects on renal function. Balarini CM[1], Gava AL[1], Pereira TMC[1], Vasquez EC[2], Meyrelles SS[1] [1]UFES − Ciências Fisiológicas, [2]EMESCAM-UFES − Ciências Fisiológicas

Introduction: Aging is a physiological process with deleterious consequences for renal function, which could be exacerbated when concurrent with pathological situations, such as dyslipidemia. The aim of this study was to determine whether aging and hypercholesterolemia could affect the renal function in mice. **Methods:** Male hypercholesterolemic apolipoprotein E-deficient mice (ApoE, n=13) and age-matched C57BL/6 control mice (C57, n=15) were studied at 2- and 8-month-old. At each time point, animals were placed in metabolic cages for 24 hours to urine volume and urinary creatinine determination. Blood samples were collected for serum cholesterol, urea and creatinine measurements. Glomerular filtration rate (GFR) was estimated by the creatinine clearance. Mesangial expansion was evaluated by Periodic Acid Schiff staining and neuronal nitric oxide synthase (nNOS) expression in the kidney was performed by Western Blotting. All procedures were approved by the Animal Ethics Committee for Use of Animals of EMESCAM College of Health Sciences (Protocol #003/2009). Data are presented as mean ± SEM. For statistical analysis, 2-way ANOVA was used, followed by Fisher's post hoc test. The significance level was set at $p<0.05$. **Results:** Total plasma cholesterol was increased about 5-fold in ApoE mice at both time points compared with C57 animals. At 2-month-old, GFR was already markedly reduced in ApoE compared with C57 mice (187 ± 28 vs 358 ± 92 µL/min, $p<0.05$). Aging caused a significant reduction of GFR in C57 mice (-77%) and this parameter was worsen in ApoE mice (-50%). In addition, serum urea was significantly increased in ApoE animals already at 2-month-old compared to C57 mice (11 ± 1.3 vs 7 ± 0.9 mmol/L, $p<0.01$). A significant mesangial expansion was also observed at 2-month-old in ApoE mice compared with C57 mice (35 ± 0.6 vs 30 ± 0.9%, $p<0.05$). Aging aggravated this condition in ApoE (40 ± 3%) and induced the mesangial expansion in C57 (35 ± 3%) 8-month old mice. Hypercholesterolemia of young ApoE mice did not affect the renal nNOS expression compared with age-matched C57 animals (0.24 ± 0.02 and 0.24 ± 0.02 o.d.). On the other hand, aging reduced this parameter in about 50% in both strains (0.12 ± 0.02 and 0.14 ± 0.03, o.d. $p<0.05$, respectively). **Discussion:** These data show that both aging and hypercholesterolemia contribute to the loss of renal function, even in the absence of atherosclerosis. Reduced nNOS expression and mesangial expansion could be considered two mechanisms involved in the progression of renal disease in these strains. **Financial support:** Capes, CNPq, Fapes, Facitec

06.019

Impact of kidney ischemia-reperfusion on primary active Na^+ transporters and its modulation by lysophosphatidic acid. Gonsalez SR[1], Verdoorn KS[2], Beiral HJV[2], Vieyra A[2], Einicker-Lamas M[2], Lara Morcillo LS[1] [1]ICB-UFRJ − Farmacologia Celular e Molecular, [2]IBCCF-UFRJ

Introduction: Kidney damage after injury processes such as ischemia-reperfusion (I/R) is sufficient to cause cell death by apoptosis followed by necrosis. The administration of lysophosphatidic acid (LPA) shows controversial effects on tissue damage depending on the experimental model. The aim of this work is to study the effect of I/R on the renal active Na+ transporters and the role of LPA in this process. **Methods:** Left renal artery from male Wistar rats (200g) was clamped for 30 minutes followed by 24 h reperfusion. The contralateral kidney was used as control (n=8). After sacrifice according to institutional ethical procedures (CEUA IBCCS087), kidneys were dissected for histological analysis using Hematoxylin and Eosin staining and Picrosirius red for assessing collagen deposition and fibrosis. ATPase activity in the homogenized fractions from cortex and medulla was determined according to the method described by Grubmeyer & Penefsky (1981). (Na++K+)ATPase and Na+-ATPase activities were determined by the inorganic phosphate released by ATP hydrolysis in the presence and absence of specific inhibitors: ouabain and furosemide, respectively. The expression of the (Na++K+)-ATPase and LPA receptors (LPA1 and LPA3) were quantified by Western blotting. **Results:** Histopathological analysis of the kidney after I/R shows loss of brush border, infiltration of inflammatory cells and increase in collagen deposition related to fibrotic areas. The biochemical analysis showed that I/R differentially changed the (Na++K+)ATPase activity, which is increased in the renal cortex (from 139 ± 37 to 436 ± 117 nmolPi.mg-1.min-1, $p< 0.05$) and reduced in renal medulla (from 535 ± 20 to 302 ± 14 nmolPi.mg-1.min-1, $p< 0.05$). The α1 subunit of (Na++K+)ATPase is also reduced in the renal medulla of the clamped kidney when compared to the contralateral control, but not altered in the cortex. Na+-ATPase activity is lowered in 40% only in the cortical ischemic kidney ($p< 0.05$). The LPA1 receptor expression is decreased in the cortex and increased in medulla while the LPA3 was not altered. **Discussion:** I/R alters kidney structure, decrease the medullary demand of ATP leading to the inhibition of the (Na++K+)ATPase activity and expression. The increase in the (Na++K+)ATPase activity and decrease in Na+-ATPase activity in the cortex suggest a possible compensation for Na+ renal reabsorption. The differential LPA1 expression in kidney cortex and medulla could explain its controversial effect and its regulatory effect on the Na+ transporters. **Financial support:** CNPq; FAPERJ

06.020

Antihypertensive profile of a novel *N*-acylhydrazone derivative (LASSBio-1289) in spontaneously hypertensive rats. Pereira SL[1], Oliveira LGT[1], Kummerle AE[2], Fraga CAM[2], Barreiro EJ[2], Sudo RT[1], Zapata-Sudo G[1] [1]UFRJ − Desenvolvimento de Fármacos, [2]FF-UFRJ − LASSBio

Introduction: The new N-acylhydrazone compound, LASSBio-1289, was synthesized from structural modification in the lead compound 3,4-methylenedioxybenzoyl-2-thienylhydrazone (LASSBio-294) in order to improve its vasodilatory activity. The aim of this work was to evaluate the blood pressure during acute and prolonged treatment with LASSBio-1289 in normotensive (Wistar-Kyoto, WKY) and spontaneously hypertensive rats (SHR). **Methods:** In the protocol of acute treatment, male or female WKY and SHR (20-25 weeks old) were anesthetized with ether for dissection and cannulation of the right carotid artery to measure the systolic (SP) and diastolic arterial pressure (DP). Electrodes were attached to the thorax of the animals for electrocardiogram (ECG) recording. The external jugular vein was used for bolus injection of LASSBio-1289 (3 mg/kg). The prolonged treatment with LASSBio-1289 was evaluated during daily i.p. injection (10 mg/kg) for 14 days in male WKY and SHR (20-25 weeks old). SP and DP were measured before and after 1, 3, 7, 11 and 14 days of treatment through a non-invasive measurement (Plethysmograph, LE 5001). **Results and Discussion:** Intravenous injection of LASSBio-1289 reduced significantly DP, SP and mean arterial pressure (MAP) from 142.2 ± 13.7 to 73.6 ± 9.5 mmHg ($P<0.05$, n=7), from 209.4 ± 15.7 to 171.8 ± 12.8 mmHg ($P>0.05$, n=7) and from 164.6 ± 14.0 to 106.3 ± 10.0 mmHg ($P<0.05$, n=7), respectively. In contrast, no significant alterations were observed on SP, DP and MAP in WKY group. Those parameters were 131.3 ± 2.9; 83.4 ± 3.2 and 99.4 ± 3.1 mmHg before intravenous administration of LASSBio-1289 and 129.1 ± 3.1; 83.7 ± 3.9 and 98.8 ± 3.6 mmHg after treatment with LASSBio-1289 ($P>0.05$, n=5). In the protocol of prolonged treatment in SHR, LASSBio-1289 reduced SP from 216.6 ± 1.4 (day 0) to 174.2 ± 6.9 mmHg (day 14) ($P<0.05$, n=6), DP from 194.0 ± 1.8 (day 0) to 150.4 ± 4.7 mmHg (day14) ($P<0.05$, n=6) and the MAP from 201.2 ± 1.5 (day 0) to 157.9 ± 4.9 mmHg (day 14) ($P<0.05$, n=6). There was no significant change in SP, DP and MAP compared to vehicle (DMSO) when WKY were treated with LASSBio-1289 daily. LASSBio-1289 induced antihypertensive effect in SHR during acute and prolonged treatment, probably consequent to its in vitro vasodilatory activity. Animal ethics committee number: DFBCICB 013 **Research grant:** CNPq, FAPERJ, INCT.

06.021

Increased circulating cell-free DNA levels in preeclampsia and gestational hypertension. Amaral LM[1], Palei ACT[2], Sandrim VC[3], Cavalli RC[4], Tanus-Santos JE[1] [1]FMRP-USP − Farmacologia, [2]FCM-UNICAMP − Farmacologia, [3]Santa Casa de Belo Horizonte, [4]FMRP-USP − Ginecologia e Obstetrícia

Background: Preeclampsia is an important hypertensive disorder of pregnancy and its pathophysiology remains unclear. A major focus of research has been the identification of biomarkers that could predict the development of this disorder. Several studies have reported increased levels of fetal cells, cell-free maternal and fetal DNA in pregnancies complicated by preeclampsia. However, there is no information regarding cell-free DNA concentrations in gestational hypertension. Objectives: To compare the circulating cell-free DNA levels in preeclampsia and gestational hypertension with those found in normotensive pregnancies. Design and **Methods:** Approval for the use of human subjects was obtained from the institutional review board at the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo (n°4682/2006), and subjects gave informed consent. The procedures followed were in accordance with institutional guidelines. We studied 270 pregnant women (93 healthy pregnant women with uncomplicated pregnancies, 90 with gestational hypertension and 87 with preeclampsia). DNA was extracted from plasma samples using QIAamp DNA Blood Mini Kit and quantified using Quant-iT™ PicoGreen® dsDNA detection kit. **Results:** We found significantly higher circulating cell-free DNA levels in preeclampsia and in gestational hypertension (195.0 ± 43.2 ng/mL and 179.3 ± 54.0 ng/mL, respectively), both $P < 0.001$ compared with those found in healthy pregnant women (132.6 ± 27.7 ng/mL). We found no correlation between the circulating cell-free DNA levels with clinical characteristics including body mass index. Conclusions: Increased circulating cell-free DNA levels were found in both hypertensive disorders of pregnancy, thus suggesting that this biomarker may not be useful to help in the differential diagnosis of both conditions. However, these results suggest that both conditions may share common pathophysiological alterations. **Acknowledgments** This study was supported by Fundação de Amparo à Pesquisa do Estado de Sao Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).]

06.022

Alveolar bone healing process of upper incisor in spontaneously hypertensive rats (SHR) subjected to treatment with β-adrenergic antagonist. a histomorphometric and immunohistochemistry study. Cursino NM[1], Pereira CCS[2], Garcia LMG[3], Micaroni S[4], Okamoto R[2], Carvalho AAF[5], Perri SHV[6], Luvizuto EL[2], Antoniali C[4] [1]FOA-UNESP − Odontologia Infantil e Social, [2]FOA-UNESP − Cirurgia e Clínica Integrada, [3]FORP-USP − Materiais Dentários e Prótese, [4]FOA-UNESP − Ciências Básicas, [5]FOA-UNESP − Patologia e Propedêutica Clínica, [6]FOA-UNESP − Apoio, Produção e Saúde Animal

The alveolar healing (AH) after tooth extraction may be altered by various systemic diseases and medications. Recent studies have shown that treatment of patients or animals with b-adrenergic antagonists, was directly involved in bone mass gain, increased density and bone formation. The aim of this study was to evaluate, by histomorphometric and immunohistochemistry analysis, the effect of atenolol on AH in SHR. Wistar rats and SHR treated or not with atenolol (100 mg/kg/day, p.o), underwent surgery for extraction of right upper incisor. Treatment with atenolol started a week before surgery and was kept until the sacrifice of animals (7, 14, 21, 28 and 42 days after surgery). The right maxillas were removed, cleaned, decalcified, cleared, embedded in paraffin. Sections were stained with hematoxylin and eosin or subjected to immunolabeling reaction for receptor activator of NF-kB ligand (RANKL) and osteoprotegerin (OPG). The middle third of dental socket (DS) was evaluated (light microscopy, 20x objective) in each histological section. The images were obtained with a digital camera attached to the microscope and analyzed by Leica Qwin Color/ RGB software. Bone percentage (BP) and bone trabeculae thickness (BTT) (μm) were evaluated and compared in different times and between groups (ANOVA). In Wistar DS, increase in the BP and the BTT was observed at 14, 21, 28 and 42 compared to 7 days. At 42 day, the BTT was higher than at 14, 21 and 28 days. In SHR, the BP and BTT were increased in 14, 21, 28 and 42 compared to day 7 and increased in 21, 28 and 42 compared to 14 days after surgery. At the 42 day the trabeculae were greater than those observed at 21 and 28 days after surgery. In DS of SHR, it was observed lower BP at 14, 28 and 42 days and smaller size of the trabeculae bone at 7 and 42 days than in DS of Wistar rats. At 28 day, the immunolabeling analysis showed strong labeling to RANKL and weak labeling to OPG in DS of SHR than in DS of Wistar. The treatment with atenolol did not alter the PB in Wistar or SHR during the stages analyzed, however, led to changes in BTT in both groups. In the DS of Wistar treated with atenolol, there was increased BTT at 28 and reduction to 14 day, compared to DS of untreated Wistar. In SHR treated with atenolol, the BTT increased to 28 and 42 days compared to untreated SHR. Together, the results showed that: 1 - The AH is characterized by a continuous increase in bone formation and trabecular thickness until the 42 postoperative day, 2 - AH in SHR is delayed compared to AH in normotensive rats, 3 – the increased bone resorption in DS of SHR could be associated to the delayed AH, 4-atenolol increases the BTT in DS during the remodeling phase, 5- In SHR, atenolol promotes improvement in AH, since it increases the BTT. Animal Reseach Ethics Committee (CEEA – FOA no. 2008-001397). **Support**: FAPESP

06.023

Endothelium contributes to the vascular relaxation induced by C-type natriuretic peptide (CNP) in aortas from renal hypertensive rats. Pernomian L[1], Bendhack LM[2] [1]FMRP-USP − Pharmacology, [2]FCF-USP − Physics and Chemistry

Introduction: Hypertension is associated with endothelial dysfunction, alterations on vascular smooth muscle reactivity to contractile agents and oxidative stress, with a decrease on endothelium dependent relaxation of arteries from 2K-1C (CALLERA, G.E. et al. Gen Pharmacol, 34: 379, 2000; RODRIGUES, G.J. et al. Nitric Oxide, 18: 176, 2008). C-type natriuretic peptide (CNP) has been described as a potent relaxing agonist of vascular smooth muscle from arteries and veins. In some vascular beds, this relaxation is endothelium-dependent (AMIN, J. et al. Hypertension 27: 684, 1996). The present study aimed to investigate in the endothelial cells isolated from renal hypertensive (2K-1C) and normotensive (2K) rats the basal production of NO, reactive oxygen species (ROS) and also the cytosolic Ca2+ concentration ([Ca2+]c). We have also studied the contribution of NO to the relaxation induced by CNP in 2K and 2K-1C aortic rings. **Methods**: Renovascular hypertension (2K-1C) was induced in Wistar rats and control rats (2K) were sham-operated. 2K-1C presented systolic blood pressure higher than 160mmHg, after 6 weeks of surgery. Rats were killed and thoracic aorta isolated for vascular reactivity studies and endothelial cells were isolated from the aorta for flow cytometry experiments by using selective fluorescent dyes to NO (DAF-2/DA 10 µmol/L, 20 min), Ca2+ (FLUO-3AM 10 µmol/L, 20 min) or ROS (DHE 2.5 µmol/L, 20 min), and the basal fluorescence intensity (FI) was measured. In denuded or intact endothelium aortic rings, cumulative concentration-effect curves to CNP (1 pmol/L-0.5 µmol/L) were performed in absence or in presence of non-selective NOS inhibitor L-NAME (100 mmol/L, 30 min). The pharmacological parameters efficacy (Emax) and potency (pD2) were analyzed. **Results**: Basal FI of DAF-2/DA (NO) in 2K cells was higher (1965.60+175.42, n=5) than in 2K-1C cells (1286.83+110.52, n=6, $P<0.05$). However, FI of FLUO-3AM (Ca2+) and DHE (ROS) was not different between 2K and 2K-1C endothelial cells. CNP induced relaxation of aortic rings from 2K and 2K-1C (Emax: 95.0+4.0% and 102.0+2.4%; n=7), respectively. Removal of the endothelium did not change the efficacy to CNP in 2K and 2K-1C aortas. On the other hand, the potency of CNP was lower in intact endothelium (E+) 2K-1C (pD2: 7.67+0.14) compared with 2K (pD2: 8.33+0.25, $P<0.05$). In addition, it was lower in 2K denuded aortas (7.79+0.15; n=9, $P<0.05$) than in 2K (E+). Endothelium removal did not change the CNP potency in aorta from 2K-1C. L-NAME had no effect in both 2K and 2K-1C (E+) aortic rings stimulated with CNP. Discussion. Basal [NO] is higher in the endothelial cells from 2K than 2K-1C rat aorta, without differences in [ROS] and [Ca2+]c. CNP induced maximum relaxation of aortas from 2K and 2K-1C rats, but with a decreased potency on 2K-1C rats probably due to lower [NO] common in endothelial dysfunction. Taken together, our results suggest that the endothelium contributes to CNP- induced relaxation of 2K and 2K-1C rat aortic rings, but it should not involve NO production via eNOS since L-NAME had no effect. Ethical Committee-FMRP, USP (No. 071/2009). **Financial support:** CAPES, FAPESP, CNPq.

06.024

Cellular mechanisms involved in the venodilation induced by nitric oxide donors. Paulo M, Vercesi JA, Biazzotto JC, Silva RS, Bendhack LM FCF-USP − Physics and Chemistry

Introduction: Veins are often overlooked when the contribution of the vascular system to systemic pathophysiology is considered. However, the venous circulation could also contribute to hypertension. Increases in mean circulatory filling pressure, a measure of venous tone, has been reported in the developmental stages of several experimental models of hypertension. In the present study we investigated the vasodilatation of inferior cava vein from normotensive (2K) and renal hypertensive rats (2K-1C) induced by the classic nitric oxide (NO) donor sodium nitroprusside (SNP) and the new NO donor Terpy synthesized in our laboratory. We also investigated the inhibitory effect of soluble guanylyl-cyclase (sGC) with ODQ (1µmol/L) and K+ channels activation by using the non-selective K+ channel blocker TEA (1mmol/L), small-conductance Ca2+-activated SKca selective blocker apamin (apa), voltage-dependent Kv selective blocker 4-aminopyridine (4-ap) and ATP-dependent KATP selective blocker glibenclamide (gli). **Methods:** We have analyzed the maximal relaxation (Emax) and potency (pEC50) of Terpy and SNP in cava vein which was incubated with L-NAME (30 min) in order to avoid the interference of the endogenous endothelial NO. Then, the veins were pre-contracted with endothelin-1 (ET-1) after pre-incubation with ODQ, TEA or K+ channel blockers. All these procedures were in accordance with the guidelines of the Animal Ethics Committee, University of São Paulo, Brazil (08.1432.538). **Results:** Vascular reactivity experiments showed that the potency of SNP was similar in 2K (pEC50: 7.53 ± 0.13 n=6) and 2K-1C (pEC50: 7.34 ± 0.26 n=7). On the other hand, the potency of Terpy was significantly higher in cava vein from 2K (pEC50: 6.10 ± 0.12 n=6) than in 2K-1C rats (pEC50: 5.42 ± 0.22 n=7, $P<0.05$). However, the Emax of both NO donors was lower ($P<0.001$) in cava vein from 2K-1C (SNP 58.7 ± 3.3% n=7, Terpy 56.7 ± 3.1% n=7) than in 2K rats (SNP 83.3 ± 2.4% n=6; and Terpy 70.3 ± 3.2% n=6;). The vasorelaxation induced by Terpy and SNP in 2K and 2K-1C veins was abolished after inhibition of sGC. The non-selective K+ channels blockade with TEA reduced Emax induced by Terpy in 2K (47.4 ± 2.6% n=6, $P<0.001$) and in 2K-1C (45.0 ± 1.1% n=6, $P<0.05$) and induced by SNP in 2K (52.1 ± 3.7%, n=6) and in 2K-1C (39.0 ± 1.6%, n=7, $P<0.05$). The selective K+ channel blockers 4-ap and Apa reduced pEC50 and Emax in 2K and 2K-1C veins stimulated with Terpy. However, gli had no effect. All the selective K+ channel blockers used reduced the potency of SNP in both 2K and 2K-1C veins. **Discussion:** Taken together, our results demonstrate that both NO donors induce relaxation in 2K and 2K-1C rat veins, which effect was lower in 2K-1C than in 2K. Relaxation induced by Terpy and SNP involves the activation of sGC and K+ channels. It seems that K+ channels activation is dependent of NO-cGMP-G-kinase pathway. Venodilation induced by Terpy involves Kv , SKCa activation and venodilation induced by SNP involves Kv , SKCa and KATP activation. Supported by FAPESP and CNPq.

06.025

Vasorelaxant effects to the essential oil of *Aniba canelilla* in isolated mesenteric artery rings from spontaneously hypertensive rats. Interaminense LFL[1], Ramos-Alves FE[1], Xavier FE[1], Pinto Duarte G[1], Magalhães PJC[2], da Silva JK[6], Sousa PJC[4], Leal-Cardoso JH[5], Maia JGS[3], Lahlou S[5] [1]UFPE − Fisiologia e Farmacologia, [2]UFC − Fisiologia e Farmacologia, [3]UFPA − Engenharia Química, [4]UFPA − Farmácia, [5]UECE − Ciências Biomédicas

Introduction: *Aniba canelilla* (H.B.K.) Mez (Lauraceae) is an aromatic plant abundant in the Amazon region, where it is commonly known as “casca preciosa”. This study investigated the effects of essential oil of *Aniba canelilla* (EOAC) in isolated mesenteric artery rings from spontaneously hypertensive rats. **Methods:** All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication 85-23, revised 1996). All procedures described here were reviewed by and had prior approval from local animal ethics committee (nº 23076.007185/2009-41). The rats were stunned and then exsanguinated the mesenteric artery were removed and immersed in cold Krebs-Henseleit solution. After removing adhering fat and connective tissue, the superior mesenteric artery was cut into ring (3 mm of length), which were suspended in a 5-mL organ baths containing Krebs-Henseleit solution continuously gassed with 95% O2 and 5% CO2 to maintain the pH at 7.4. **Results and Discussion:** In preparations with intact endothelium (n = 9), OEAC (0.1-1000 µg/ml) relaxed the phenylephrine-induced contractions in a concentration-dependent manner with an IC50 value of 11.1 ± 2.0 µg/ml. The first inhibitory effect of EOAC became significant at a concentration of 1 µg/ml while the maximal relaxation occurred at a concentration of 600 µg/ml. This vasorelaxant effect of EOAC remained unaffected by either vascular endothelium removal (IC50 = 12.4 ± 3.4 µg/ml, n = 4) or pre-treatment with 5 mM tetraethylamonium chloride, a potassium channel blocker (IC50 = 14.6 ± 1.3 µg/ml, n = 4). In endothelium-containing preparations incubated in Ca+2-free condition, EOAC (600 µg/ml, n = 4) completely abolished the KCl-induced contractions and at 100 µg/ml (n = 4) EOAC significantly reduced the contraction evoked by increasing concentrations of CaCl2. These data suggest that EOAC induced an endothelium-independent relaxation in rat isolated mesenteric artery, an effect that seems mediated through an inhibition of Ca2+ inward current rather than activation of potassium channels. **Financial support:** CNPq/UFPB

06.026

Maternal diabetes induces endothelial dysfunction in an age-dependent manner in resistance vessels from male offspring rats: identification of possible mechanisms involved.

Ramos-Alves FE, Queiroz DB, Pinto Duarte G, Xavier FE UFPE − Fisiologia e Farmacologia

Objectives: Offspring of diabetic mothers have an increased risk of developing metabolic and cardiovascular diseases. Although several studies have focused in the effects of maternal diabetes on metabolic state, the impact of maternal diabetes on cardiovascular function in the offspring is still poorly studied. In this study, we determined whether diabetes in pregnancy can affect vascular function in the offspring and the influence of age on these effects. **Methods**: Acetylcholine (Ach) relaxation was analyzed in small mesenteric arteries (~250 mm of internal diameter) from 3, 6 and 12 month-old male offspring of control rats (CO) and of rats rendered diabetic (DO) with streptozotocin. Results. In all ages evaluated, relaxation to Ach was decreased in arteries from DO compared to CO. In both CO and DO groups, relaxation to Ach was progressively diminished with age, being this effect higher in DO. In vessels from DO, the cyclooxygenase (COX)-1 and 2 inhibitor (indomethacin) or the selective COX-2 inhibitor (NS-398), but not the COX-1 inhibitor SC-560, normalized the Ach-induced relaxation. However, in vessels from CO group, acetylcholine responses remained unchanged in presence of these drugs. In arteries from DO group, thromboxane A2 (TxA2) synthesis inhibitor (furegrelate), TxA2 receptor antagonist (SQ29548), prostaglandin E2 (PGE2) receptor antagonist (AH6809) or prostaglandin F2a (PGF2a) receptor antagonist (AL8810) significantly increased relaxation to Ach. However, the effect produced by these drugs on response to Ach was smaller to that produced by indomethacin or NS-398. In these arteries, co-incubation of SQ29548, AH6809 and AL8810 increased relaxation to Ach in a similar manner to that produced by COX inhibitors. **Conclusions**: Results obtained suggest that perinatal diabetes in the rat result in age-related endothelial dysfunction in resistance arteries, reinforcing the concept of fetal programming of adult cardiovascular diseases. In addition, our results also suggest that this impaired endothelial function is associated with increased participation of contractile prostanoids from COX-2, probably the TxA2, PGE2 and PGF2a. **Financial Support:** Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE)

06.027

Cardiac dysfunction in experimental sepsis as assessed by the isolated and perfused mouse heart. Bóf ER, DalBó S, Ramos GC, Assreuy J UFSC − Pharmacology

Introduction: The systemic inflammatory response syndrome (SIRS) when associated with an infection can progress to sepsis and septic shock, which are important causes of death in ICUs. Death is often caused by a cardiovascular collapse and refractory hypotension, both appearing early in sepsis. Moreover, cardiac dysfunction is recognized as an important mediator of multiple organ dysfunction in sepsis. Here we characterize cardiac dysfunction in experimental sepsis induced by cecal ligation and puncture (CLP) in mice and also to evaluate the involvement of nitric oxide (NO) in this process. **Methods:** Cardiac function was evaluated using the methodology of the isolated and perfused heart (Langendorff preparation) 3, 6, 12 and 24 hours after Swiss female mice were submitted to CLP surgery. Parameters analyzed were systolic and diastolic tension, +dT/dt (speed of contraction), -dT/dt (speed of relaxation), coronary perfusion pressure, heart rate and the area under the curve (AUC; approximately the cardiac work). Hearts were also stimulated with isoprenaline, 3 and 24 hours after CLP. To study the effects of NO on functional cardiac alterations, the perfusion solution was supplemented with a non-selective NOS inhibitor (L-NAME) 24 hours after CLP and cardiac function was evaluated. All the procedures have been approved by our institutional Animal Ethics Committee (PP00320/CEUA/UFSC). Results Concerning the systolic tension, 3 hours after CLP procedure 38% of hearts had higher values than those presented by the control hearts (termed suprafunctional), 12% had lower values (subfunctional) and 50% had values similar to those presented by the control hearts (normofunctional). Six hours after CLP the suprafunctional subgroup amounted to only 21% of hearts evaluated, while the subfunctional subgroup represented 29%. Twelve hours after the CLP surgery, the normofunctional subgroup was the most numerous, accounting for 77% of hearts studied. Twenty-four hours after CLP there was a substantial increase in the number of subfunctional hearts, representing 40% of hearts studied. A similar profile was found for the parameters +dT/dt, -dT/dt and AUC. When stimulated with isoprenaline, the chronotropic and inotropic response of normofunctional hearts (3 and 24 hours after CLP), as well as subfunctional hearts assessed 3 hours after CLP, were similar to the response of control hearts. Subfunctional hearts assessed 24 hours after CLP, presented a significant reduction in the contractile capacity and chronotropism when compared with control. Suprafunctional hearts assessed 3 and 24 hours after CLP presented higher systolic tension. The infusion of L-NAME did not alter the basal activity of control and septic hearts evaluated 24 hours after CLP and also did not alter the inotropic and cronotropic responses to isoprenaline. **Discussion:** Our results demonstrate that: a) there are indeed significant changes in heart function induced by sepsis; b) this changes are evident from the beginning of the septic process but are more prominent at later stages of the condition; c) both contractile machinery and auto-rhythmic cells seem to be affected. As for the mechanisms underlying this condition our preliminary results indicate a limited role of NO in the observed changes. **Financial support:** CAPES, CNPq, FAPESC.

06.028

Sepsis-induced renal impairment to a second renal insult. Portella VG[1], Silva-Filho JL[1], de Rico TB[2], Landgraf SS[1], Vieira MAR[3], Takiya CM[4], Benjamim CF[2], Canetti C[1], Pinheiro AAS[1], Caruso-Neves C[1] [1]IBCCF-UFRJ − Ciências da Saúde, [2]ICB-UFRJ − Farmacologia, [3]ICB-UFMG − Fisiologia e Biofísica, [4]ICB-UFRJ − Anatomia e Histologia

Introduction: Sepsis is a clinical syndrome resulting from systemic inflammatory response of the host to infection, progressing to septic shock and multiple organ dysfunctions. The kidney is one of main organs affected during sepsis development in critically ill patients. Notably, these patients have lower life expectancy, because the affected organs during the septic process may not be fully recovered. The aim of this study is to verify whether animals previously subjected to severe sepsis induced by CLP (Cecal Ligation and Puncture), subsequently submitted to a new renal insult, using bovine albumin peritoneal injection, could develop more severe form of acute renal failure (ARF). **Methods:** Males Balb/c mice (DSBCICB028) were divided into two groups, S (Sham) =12 and CLP (septic) n=12. Following surgery, the animals were kept in metabolic cages for 14 days, urine was collected at days 0, 7 and 14 for proteinuria determination. From the 15th day the animals were divided into four groups: S Control (n = 6), S ARF (n = 6), CLP Control (n = 6) and CLP ARF (n = 6). Mice were subjected to i.p. administration of BSA (10g/kg/day) to the development of ARF model, and control animals were injected with saline (vehicle). After seven days of treatment, mice were sacrificed and their blood collected. All groups of mice were allocated in metabolic cages by 48 hours just after the albumin treatment to evaluate renal function. **Results and Discussion:** Before the albumin treatment, CLP mice have already showed an increase in proteinuria and urinary flow rate levels by 60 % in relation to S mice. After albumin treatment, urinary flow rate was increased in S ARF (0.7 uL.min-1) and CLP ARF (0.8 uL.min-1) when compared with respective controls: S control (0.2 uL.min-1) and CLP control groups (0.4 uL.min-1). The proteinuria level (mg/ 24 h) enhanced on all ARF mice in relation to the respective control mice (1.56 ± 0.23 S ARF mice; 0.24 ± 0.23 S control mice; 2.43 ± 0.04 CLP ARF mice and 0.98 ± 0.61 CLP control). It is worthy to mention that the increase in proteinuria in CLP ARF is higher than observed in S ARF group. Only septic mice showed an increase in serum creatinine (0.44 ± 0.06 CLP control mice and 0.40 ± 0.05 CLP ARF mice). The glomerular filtration rate (GFR) decreased in both CLP control and CLP ARF mice in relation to S ARF (0.30 ± 0.09 mL.min-1 S ARF; CLP control mice 0.10 ± 0.014 mL.min-1 and 0.12 ± 0.03 CLP ARF). The protein/urinary creatinine ratio (UP:C) was enhanced in S ARF, CLP control and CLP ARF mice being this increase more pronounced in the CLP ARF mice (0.56 ± 0.06 S control; 1.40 ± 0.50 S ARF; 1.08 ± 0.17 CLP control and 2.03 ± 0.29 CLP ARF). All results together suggest that CLP-induced severe sepsis impaired renal function and the kidneys become more sensitive to a second insult, which accelerate the progression of renal failure. This could correlate with the observation that pos-sepsis patients are more susceptible to a second insult, which leads to an end-damage organ and death. **Financial support:** FAPERJ, CAPES and CNPq

06.029

Effects of antioxidants treatment on cardiac dysfunction and MMP-2 levels in renovascular hypertension. Rizzi E[1], Castro MM[1], Ceron CS[1], Neto-Neves EM[1], Tanus-Santos JE[1], Gerlach RF[2] [1]FMRP-USP − Farmacologia, [2]FORP-USP − Morfologia

Introduction: Enhanced cardiac matrix metalloproteinase (MMPs) has been associated with cardiac hypertrophy1. Because MMPs are upregulated by increased formation of reactive oxygen species (ROS)2, we hypothesized that antioxidant approaches could attenuate the increases in MMP-2 levels and the cardiac dysfunction associated with two-kidney, one-clip (2K1C) hypertension. **Methods:** Sham-operated or 2K1C hypertensive rats were treated with vehicle, 18 mg/Kg/day of tempol (a superoxide dismutase mimetic) or 25 mg/Kg/day of apocyanin (an inhibitor of nicotinamide adenine dinucleotide phosphate oxidase – NADPH oxidase). The treatments were initiated two weeks after the surgery. Systolic blood pressure was monitored weekly by tail-cuff plethysmography. After 8 weeks of treatment the animals were anesthetized to evaluate systolic, diastolic and median blood pressure. Maximum and minimum values of first derivative of left ventricular pressure (± dp/dt) were evaluated. Left ventricle ROS and MMP-2 levels were determined using dihydroethidine and gelatin zymography, respectively. Experimental protocols followed standards and policies of the University of Sao Paulo's Animal Care and Use Committee (196/2008). **Results:** Tempol and apocyanin treatment attenuated 2K1C hypertension (176 ± 7.5 and 178 ± 8.0 mmHg, respectively, versus 206 ± 6.518 mmHg in hypertensive controls; both $p<0.05$). We found increased ± dp/dt in 2K1C rats compared with sham groups ($P<0.05$), which was decreased by approximately 33% in the presence of both antioxidants ($P<0.05$). Left ventricle ROS levels were increased in 2K1C rats when compared with sham groups ($P<0.05$). Treatment of 2K1C rats with both antioxidants was effective in attenuating hypertension-induced increases in ROS levels (both drugs $P<0.05$). MMP-2 levels was significantly higher in left ventricle from hypertensive rats ($P<0.05$) compared with sham groups ($P<0.05$). Tempol and apocyanin treatment did not affect the increases of MMP-2 levels found in hypertensive rats ($P>0.05$). **Discussion:** In conclusion, our results suggest that antioxidants can attenuate the cardiac dysfunction associated with 2K1C hypertension. . However, this effect was not accompanied of hypertension-induced MMP-2 levels increases. Schulz R. Ann. Rev. Pharmacol. Toxicol. 47:211-42; 2007. Viappini S. Mol. Cell. Cardiol. 40:907; 2006. **Financial support:** CNPq, CAPES and FAPESP.

06.030

Role of potassium channels in endothelium-dependent vasodilation in experimental periodontits in rats. Olchanheski Junior LR[1], Santos FA[2], Fernandes D[1] [1]UEPG − Ciências Farmacêuticas, [2]UEPG − Odontologia

Introduction: Clinical and epidemiological studies have shown that periodontitis, an infection of the oral cavity caused by Gram-negative bacteria, is a risk factor for cardiovascular diseases (CVDs). Endothelial dysfunction is the initial step in the development of CVD and has been demonstrate in subjects with periodontal disease. Potassium channels (KC) are key players in the control of endothelium-mediated vasorelaxation. Previous works have shown an up-regulation of KC in the impaired endothelium of hypertensive rats. Thus, the aim of the present study was study the role of KC in endothelium-dependent vasodilation in an animal model of peridontitis. **Methods:** All procedures were approved by ethic committee (23080.034301/2009-36). A ligature was placed around rat mandibular and maxilar molars to induce periodontitis. A simulated procedure was performed in the Sham group. Seven days after the effects of acetylcholine (Ach) or sodium nitroprusside (SNP) on blood pressure were evaluated. Tetraethylammonium (TEA, a non-selective blocker of KC, 100µmol/kg, i.v.) was administrated and a new dose-response curve to Ach and SNP were obtained. Results and discussion: There was no difference in vasodilator response to Ach or SNP between the sham and ligadure group. Additionally, TEA was note able to change hemodynamic response to either vasodilator agents. The results shown that, at least in evaluated times, there is no impairment in endothelium-dependent vasodilation in animals with periodontal disease. These data are different from those found in humans. Maybe the time of evaluation after placement of the ligature in animals has not been enough for the development of systemic changes. Further studies are needed. **Financial support:** Decit / SCTIE through the support of CNPq and Fundação Araucaria. CNPq.

06.031

Inhibition of MMP-mediated vascular changes in 2K1C hypertension by doxycycline is dose-dependent. Guimarães DA[1], Rizzi E[1], Ceron CS[1], Oliveira AM[2], Marçal DMO[5], Tirapelli CR[5], Gerlach RF[6], Tanus-Santos JE[1] [1]FMRP-USP − Farmacologia, [2]FCFRP-USP − Farmacologia, [5]EERP-USP − Farmacologia, [6]FORP-USP − Morfologia

Introduction: Metalloproteinases (MMPs) upregulation is associated with vascular remodeling in hypertension. Doxycycline, a non-selective MMP inhibitor plays important role in alterations induced by renovascular hypertension. We evaluated whether the treatment with three different doses of doxycycline, (3, 10 and 30 mg/kg/day) modified two kidney-one clip (2K1C) hypertension-induced changes in systolic blood pressure (SBP), vascular reactivity, aortic remodeling, and MMP-2 levels. **Methods:** 2K1C hypertension was induced by clipping the left renal artery with a silver clip (0.2 mm). SBP were assessed weekly throughout the experiment period by tail-cuff plethysmography. Aortic rings were isolated to assess endothelium dependent and independent relaxations. Morphometry of structural changes in the aortic wall were studied in hematoxylin/eosin sections. Aortic MMP-2 levels and its proteolytic activity were determined by gelatin and in situ zymography, respectively. Procedures were approved by the local Ethical Committee (protocol number: 140/2009). Results and discussion: All treatments attenuated the increases in SBP in hypertensive rats after the fifth week of the renal artery surgery (195.4 ± 3.9 mmHg versus 177.2 ± 6.2 mmHg, 176.3 ± 4.5 mmHg and 173 ± 5.1 mmHg in 2K1C and 2K1C+doxy 3, 2K1C+doxy 10 and 2K1C+doxy 30, respectively, all $P<0.01$). Only the treatment with doxycycline 30 mg/kg prevented the reduction in endothelium-dependent vasorelaxation (82.5 ± 0.04% vs 101.03 ± 1.65 % vascular relaxation, 2K1C and 2K1C+doxy 30, respectively, 10-5 M ACh, $P<0.05$), total MMP activity (32.71 ± 3.3 vs 22.24 ± 2.1 arbitrary units, 2K1C vs 2K1C+doxy30, respectively, $P<0.05$) and MMP-2 levels ($P<0.05$) found in the 2K1C group. While no significant differences were observed in pD2 obtained in response to ACh, 2K1C hypertension reduced the maximum effect by approximately 25% ($P< 0.01$), and this reduction was blunted only by the treatment with doxycycline at 30 mg/kg. We found no significant alterations in the response to sodium nitroprusside in all experimental groups. Doxycycline 30 mg/kg also prevented the increases in media thickness, and was associated with lower media/lumen ratio and cross sectional area in 2K1C hypertensive rats (all $P<0.05$). No significant changes were seen in Sham and Sham+treatment groups during the experiments in all studied parameters. Our results showed that treatment with doxycycline at 30 mg/kg, but not 3 and 10 mg/kg, was able to attenuate MMP-2-mediated vascular dysfunction and remodeling in 2K1C hypertension. These findings suggest that treatment with doxycycline 30mg/kg may be a promising therapeutic aim in the therapy or prevention of the alterations caused by hypertension. Supported by: FAEPA, FAPESP, CNPq, CAPES.

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06.032

New nitrite-pro-drug releases nitric oxide in a tissue and enzyme-dependent way. Pereira AC[1], Lunardi CN[2], Biazzotto JC[1], Silva RS[1], Bendhack LM[1] [1]FCFRP-USP, [2]UnB

Introduction: Until recently, nitrite was considered an end product of NO metabolism. However, several studies have demonstrated that nitrite metabolism occurs in the tissues and blood to form NO. cis-[Ru(bpy)2(py)NO2](PF6) (RuBPY) is different from other NO donors because it has nitrite in its molecule that is converted to NO. Therefore, this study aimed to investigate the vascular mechanisms involved in the conversion of nitrite to NO. We have studied the vascular relaxation and cell membrane hyperpolarization induced by RuBPY as a pro-drug that releases NO. **Methods:** Male wistar rats (180g) were used in the experiments. NO release from the compound was measured by amperometry by using a selective sensor in Krebs solution in the absence or in the presence of phenylephrine (PHE), and in the presence of the aortic ring. NO was also measured by fluorescence intensity (FI) of the selective NO dye DAF-2DA by using confocal microscopy. The aortic rings were stimulated with RuBPY (5mM) in the presence or absence of the soluble guanylyl-cyclase inhibitor (1mM ODQ). Vascular relaxation was studied in aortic rings pre-contracted with 100nM PHE or 60mM KCl. Cumulative concentration-effect curves to RuBPY were constructed in the absence (Control) or after incubation with ODQ (1mM) or non-selective K+ channel blocker TEA (1mM). The values of maximum effect (ME) and the potency (pD2) of RuBPY were determined. In order to evaluate the membrane potential changes caused by RuBPY, we have used the membrane potential sensitive dye 1mM Di-4-Anepps excited at 458 and 514nm after addition of 5mM RuBPY. All the procedures were approved by the Ethics Committee of the University of São Paulo (CEUA 07.1.608.53.8). **Results:** NO was released from RuBPY only in the presence of the aortic rings. In the arteries loaded with DAF-2DA, RuBPY increased FI (1645.1 ± 388.32; n=5), which was decreased by ODQ (FI: 366.88 ± 120.33; n=7 $P<0.01$). RuBPY induced relaxation in the aortic rings pre-contracted with PHE (ME: 105.0 ± 1.14%; pD2: 6.54 ± 0.10 n=5), that was greater than in KCl-contracted arteries (ME: 60.5 ± 3.50%; pD2: 5.86 ± 0.10; n=6 $P<0.05$). ODQ reduced ME to 38.0 ± 3.62%; n=5 ($P<0.01$). In addition, TEA inhibited the relaxation induced by RuBPY (ME: 93.2 ± 1.92%; pD2: 5.83 ± 0.13; $P<0.05$ n=6). In PHE pre-contracted aortas, ODQ and TEA almost abolished the relaxation induced by RuBPY (ME: 5.3 ± 1.40%; n=7). The membrane potential measured with Di-4-Anepps showed an increased ratio of IF at 458nm/514nm, which means that RuBPY causes vascular smooth muscle cell membrane hyperpolarization. **Conclusion:** RuBPY releases a large amount of NO only in the presence of the aortic tissue and the conversion of nitrite to NO was dependent of sGC enzyme. The aortic rings relaxation induced by NO donor involves sGC, K+ channels activation and cell membrane hyperpolarization. K+ channels sensitive to TEA were directly activated by RuBPY. Supported by FAPESP and CNPq.

06.033

Effect of rosmarinic acid on the inhibition of angiotensin converting enzyme in normotensive and hypertensive rats. Ferreira LG, Celotto AC, Capellini VK, Albuquerque AAS, Evora PRB FMRP-USP − Cirurgia e Anatomia

Introduction: Hypertension is a common cardiovascular disease that affects millions of people worldwide (1). There are several ways to treat hypertension, such as the use of angiotensin I converting enzyme (ACE) inhibitors (2). In the last years, it has been demonstrated that some plant species can inhibit the ACE and the rosmarinic acid is one of the compounds in these species (3,4). The aim of this study was to verify if the rosmarinic acid was able to inhibit ACE activity in normal and hypertensive rats. **Methods:** The animals were divided into four groups: hypertensive (n = 10), normotensive (sham ,n = 10), normotensive + rosmarinic acid (n=6) and hypertensive + rosmarinic acid (n=6). Four weeks after the surgery, dose-response curves to angiotensin I were performed and the blood pressure was monitored (MP System 100 A, BioPac System, Inc., Santa Barbara, CA, USA). The hypertension was induced by a unilateral clip in the renal artery (2 kidneys - 1 clip). **Results:** The angiotensin I promoted an increase, dose-dependent, in the blood pressure and it was higher in hypertensive compared to normotensive rats (Table 1). When animals were treated with RA (5 minutes before AI dose-response curves, 25mh/Kg), the increase of blood pressure was lower in the hypertensive, but not in the normotensive group (Table 1). **Discussion:** The rosmarinic acid was effective to reduce the blood pressure in hypertensive animals. However in normotensive rats, the rosmarinic acid did not induce significant changes in blood pressure. Although the results are partial, rosmarinic acid has shown promise as ACE inhibitor, specially because its effect is hypertensive-specific, in other words rosmarinic acid don't change blood pressure in normotensive rats. This work was support by FAPESP and FAEPA-HC/FMRP. This work agrees with Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation COBEA by certification protocol number 162/2008. Table 1: Change in systolic blood pressure (DSBP, mmHg) in normotensive and hypertensive rats. Angiotensin I (µg/Kg) Normotensive Hipertensive Normotensive Rosmarinic acid Hipertensive Rosmarinic acid 0,03 4,63 ± 0,77 7,90 ± 2,24 -8,24 ± 1,14 -12,01 ± 6,18 0,10 14,21 ± 1,40 17,24 ± 2,01 13,84 ± 1,82 -2,28 ± 6,04 0,30 17,26 ± 1,53 27,12 ± 2,01 18,96 ± 2,30 1,95 ± 5,42 # 1,00 31,60 ± 1,83 44,68 ± 2,89 29,79 ± 6,38 15,23 ± 5,77 * # 3,00 40,94 ± 2,48 56,01 ± 2,63* 44,17 ± 4,34 12,21 ± 14,01 * # 10,0 51,27 ± 3,53 70,85 ± 3,04* 56,01 ± 4,82 39,86 ± 8,22 # * Indicates significant difference compared to normotensive and # indicates significant difference compared to the hypertensive. P <0.05 Two-way ANOVA with Bonferroni post-test. REFERENCES 1. Mion Jr, D. Arq. Bras. Cardiol., v.89, 24 p. 2007. 2. Kohlmann, J. Arq bras endocrinal metab v.43, 257p. 1999. 3. Apostolidis, E. Asia Pac J Clin Nutr, v.15, p.433. 2006. 4. Li, Q. L. Phytomedicine, v.15, p.386. 2008.

06.034

Modulation of cardiac and renal P-type ATPases in diet-induced atherosclerosis. Balter AS, Marques EB[1], Motta NAV[1], Brito FCF[1], Scaramello C[1] [1]UFF − Farmacologia Experimental

Introduction: Atherosclerosis is characterized by lipid and fibers deposition in blood vessels what may lead to myocardial ischemia and high blood pressure (BP) due to endothelial dysfunction. Ischemia leads to a rapid cessation of oxidative phosphorylation and contractile function, Na+/K+ATPase activity is reduced and persistent entry of Na+ leads to an intracellular Na+ accumulation (VIÉ et al. J Pharmacol Exp Ther, 330, p.696, 2009). In the myocardium, Na+ homeostasis is closely linked to intracellular Ca2+ handling via the Na+/Ca2+ exchanger, the principal mechanism for Ca2+ efflux from cardiomyocytes. An intracellular Ca2+ overload may damage and diminish the function of the myocardium via numerous potentially degenerative states (CHEN et al., Eur J Pharm, 603, p.86, 2009). The kidneys play a crucial role in the long-term regulation of systemic BP related to its ability to fine tune the level of Na+ excretion. The basolateral Na+ transport is one of the limiting steps to Na+ reabsorption and it is due to the classic Na+/K+ATPase and the ouabain-insensitive, furosemide-sensitive Na+ATPase (QUEIROZ-MADEIRA et al. Biochim Biophys Acta. Biom, 1798, p. 360, 2010). The aim of the present work is to establish a model of diet-induced atherosclerosis evaluating its consequence in cardiorenal function by the heart and kidney P-type ATPases activity/expression analysis. **Methods:** The use of animals was in agreement to the Animal Care and Use Committee of Fluminense Federal University (CEPA/UFF00116/09). Adult male Wistar rats (200g) were randomly divided into 2 groups: G1 (controls)– rats were fed with commercial chow for 30 days and G2 (atherosclerosis)– rats were fed with high-fat pro-aterogenic chow for 30 days. After this period, the animals were anesthetized and then hearts and kidneys were removed to prepare ultracentrifuged homogenates. P-type ATPases activity was determined according to the colorimetric method of Fiske & Subbarow. **Results:** Plasma cholesterol profile was statistically different between G1 (93,8 ± 11,4mg/dL, n=5) and G2 (163,4 ± 10,1mg/dL, n=5). Histological assays showed liver alterations in G2. Biochemical assays showed a decrease of cardiac Na+/K+ATPase activity in G2 (1364 ± 143nmolPi/mg, n=4) compared to G1 (3375 ± 170nmolPi/mg,n=2) without changes of renal Na+/K+ATPase (n=5) nor Na+ATPase activity (n=3). **Discussion:** SR Ca2+ATPase activity increase is associated to Ca2+ overload and tachyarrhytmias (Gyorke & Carnes, Pharmacol. & Ther.,v. 119, p. 340, 2008). Our data suggest that atherosclerosis may lead to a Ca2+ overload due to an impairment of cardiac Na+/K+ATPase as a consequence of myocardial ischemia. Evidences indicate that Na+/K+ATPase activity are reduced in failing human heart (Barwe et al. J. Mol. Cell. Cardiol., v. 47, p.552, 2009). Therefore, a recent concept for cardioprotection consists of ischemic Na+ accumulation and Ca2+ overload inhibition (VIÉ et al. J. Pharmacol. Exp. Ther., 330, p.696, 2009; CHEN et al., Eur J Pharm, 603, p.86, 2009). Cardiac Ca2+ATPase activity and western blot assays are being performed such as tests with new therapeutic potential compounds. **Financial support:** FAPERJ, CNPq, CAPES, PROPPI/UFF.

06.035

A new vasodilator compound (DCBPY-NO) presents cyclic activity in releasing nitric oxide by nitrite. Rodrigues GJ[1], Cicillini SA[2], Silva RS[2], Bendhack LM[2] [1]FMRP USP − Farmacologia, [2]FCFRP−USP

The ruthenium complexes have been studied as potent nitric oxide (NO) donors. The NO donor cis-[Ru(dcbpy)2(Cl)(NO)] (DCBPY-NO) and its “aquo” species cis-[Ru(dcbpy)2(Cl)(H2O)] (DCBPY-H2O) are synthesized in our laboratory. In physiological pH and temperature the compound DCBPY-NO is converted to the stable complex cis-[Ru(dcbpy)2(Cl)(NO2)] (DCBPY-NO2). The compound (DCBPY-NO2) induces vascular relaxation and generates NO in the vascular smooth muscle. After generate NO the compound DCBPY-H2O is formed and in the presence of nitrite it is converted to DCBPY-NO2. Therefore, this study aimed to verify the vasodilatation induced by the compound DCBPY and its cyclic activity of NO generation in the presence of nitrite. **Methods:** All the procedures were carried out in accordance with standards and policies of the University of São Paulo´s Animal Care and Use Committee (nº 030/2008). Aortic rings were isolated from rats and cumulative concentration-effect curves to DCBPY-NO2, DCBPY-H2O and sodium nitrite (NaNO2) were constructed in aortas pre-contracted with phenylephrine. To investigate whether the compound has a cyclic activity, the curves to DCBPY-NO2 and DCBPY-H2O were performed in the presence of 1μM NaNO2 that is the concentration that does not induce vasodilatation. In order to verify which is the NO specie generated by DCBPY-NO2 and NaNO2, we have used hydroxocobalamin (HCB, NO° scavenger) and L-cysteine (L-CY, NO- scavenger), and also Oxihemoglobin (HBO2) as extracellular NO scavenger. The potency (pD2) and maximum relaxant effect (ME) were determined. **Results:** Aortic rings were completely relaxed by DCBPY-NO2 (ME: 103.1 ± 1.2%; n=9) and NaNO2 (ME: 105.1 ± 1.4%, n=10) and higher potency was verified to DCBPY-NO2 (pD2: 5.71 ± 0.06, n=9, P<0.001) than NaNO2 (pD2: 4.52 ± 0.07, n=10). As expected, the aquo compound DCBPY-H2O had no effect, but in the presence of NaNO2 it induced lower relaxation (pD2: 4.99 ± 0.20, P<0.001, ME: 84.8 ± 9.2%, n=5, P<0.001) than DCBPY-NO2. The compound DCBPY-NO2 in the presence of NaNO2 presented greater potency (pD2: 6.01 ± 0.08, n=6, P<0.01) without changes in the ME (105.7 ± 1.0%, n=6) as compared to DCBPY-NO2 in the absence of NaNO2. Aorta relaxation induced by DCBPY-NO2 was almost abolished by NOo scavenger HCB (ME: 14.2 ± 2.0%, n=5; P<0,001) and HBO2 (ME: 14.1 ± 1.3%, n=5; P<0,001). NO- scavenger reduced the potency to DCBPY-NO2 (pD2: 5.03 ± 1.93; n=6; P<0.01) without effect in the ME (105.6 ± 1.9%; n=6). On the other hand, L-CY and HBO2 did not modify the relaxation induced by NaNO2. Otherwise, NOo scavenger reduced the potency (pD2: 4.03 ± 0.08, n=5, P<0.01) without effect on the ME. Conclusions: Our results suggest that: the compound DCBPY has a cyclic activity of NO generation in the presence of nitrite, and that nitrite is converted to NO° inside the cell to NaNO2 and in outside the cell to DCBPY-NO2. The compound is not a nitrite donor since the potency of DCBPY-NO2 is higher than the potency of NaNO2. Supported by FAPESP and CNPq.

06.036

The renal effects of l-amino acid oxidase from *Bothrops leucurus* venom in the rat perfused kidneys. Morais ICO[1], Marinho AD[2], Menezes RRPPB[3], Dantas RT[1], Torres AFC[2], Lopes KS[2], Meneses GC[2], Costa MFB[2], Jorge RJB[1], Alves RS[1], Toyama MH[4], Monteiro HSA[1], Martins AMC[3] [1]UFC − Fisiologia e Farmacologia, [2]UFC − Farmácia, [3]UFC − Análises Clínicas e Toxicológicas, [4]IB-UNICAMP

Introduction: L-Amino acid oxidases are flavoenzyme which catalyze the stereospecific oxidative deamination of an L-amino acid substrate to a corresponding a-ketoacid with the production of hydrogen peroxide and ammonia. These enzymes are widely distributed in many different organism and snake venom L-aminoácido oxidades (SV-LAAOS) are the best studied members of this protein family. Though the exact biological function of LAAO isolated from the venom of snakes is not completely understood, appear to be involved in allergic inflammatory response, endothelial cell damage in mammalian and induction of apoptosis (MORE, J. Venom. Anim. Toxins incl. Trop. v. 16, p. 60, 2010). **Methods:** Isolated kidneys from Wistar rats weighing 250 to 300g (n=6) were perfused with Krebs-Henseleit solution containing 6% of bovine serum albumin previously dialyzed for 120 minutes. The effects of LAAO (10µg/mL) were studied on glomerular filtration rate (GFR), urinary flow (UF), perfusion pressure (PP), renal vascular resistance (RVR) and percentage sodium (%TNa+), potassium (%TK+) and chloride (%TCl-) tubular transport at 60, 90 and 120 minutes of experiment. All data were analyzed by unpaired t test with level of significance of * $p<0.05$. In the treated group, the addition of venom occurred 30 minutes after the start of the experiment. The first 30 minutes were used as internal control. The experimental protocols were approved by the Federal University of Ceara Animal Research Ethical Committee, with license number 79/08. **Results:** LAAO from B. leucurus venom increased the PP at 60 min (PPCT=108.6 ± 1.5; PP60=131 ± 4.3* mmHg) and UF at 120 min (UFCT=0.133 ± 0.007; UF120=0.284 ± 0.04* mL/g-1.min-1). The glomerular filtration rate decreased at 60 and 90 min (GFRCT=0.793 ± 0.102; GFR60=0.296 ± 0.031*; GFR90=0.257 ± 0.022* mL/g-1.min-1). It was also observed a decrease on percentual tubular transport of sodium (%TNa+), chloride (%TCl-) and potassium (%TK+) at 60, 90 and 120 minutes (%TNa+CT=82.41 ± 2.21; %TNa+60=60.9 ± 3.34*; %TNa+90=51.46 ± 3.94*; %TNa+120=58.68 ± 3.14*); (%TCl-CT=80.68 ± 1.29; %TCl-60= 55.92 ± 1.69*; %TCl-90=47.53 ± 1.43*; %TCl-120=55.86 ± 0.72*); (%TKCT=75.26 ± 2.5; %TK+60=47.64 ± 3.72*; %TK+90=45.1 ± 5.39*; %TK+120=55.39 ± 3.93*). **Conclusion:** These results indicate that LAAO isolated of the B. leucurus venom caused nephrotoxicity in isolated kidney. **Financial support:** CNPq

06.037

Tempol attenuates the hemodynamic changes associated with acute pulmonary embolism. Santos Sousa O[1], Neto-Neves EM[1], Ferraz KC[2], Tanus-Santos JE[1] [1]FMRP-USP − Farmacologia, [2]UFJF − Farmacologia

Introduction: The importance of increased oxidative stress to the hemodynamic changes associated with acute pulmonary embolism (APE) are not well studied. Tempol is known as a scavenger of free radicals. In this study, we examined whether tempol attenuates the hemodynamic changes associated with APE. **Methods:** All animals received humane care and study protocols complied with the guidelines of the ethics committee for the use of experimental animals at the Faculty of Medicine of Ribeirao Preto (protocol 181/2009). Hemodynamic evaluations were performed in non-embolized sheep treated with saline (Sham group; n=4) and in sheep that were embolized with autologous clots and that received drug (Emb group, n=7; Emb+tempol group, n=7). Tempol (1 mg/kg/min) was infused intravenously over 30 min followed by a maintenance infusion for the duration of the study. The hemodynamic evaluations were carried every 15 min for two hours. The mean pulmonary arterial pressure (MPAP), mean arterial pressure (MAP), heart rate (HR), cardiac output (measured by thermodilution) and dp/dt max and min were assessed every 15 min. The pulmonary vascular resistance index (PVRI), systemic vascular resistance index (SVRI), and the cardiac index (CI) were calculated with standard formulae. **Results:** We found no significant hemodynamic changes in the Control group of sheep throughout the study period. However , APE increased mean pulmonary arterial pressure (MPAP) by 37 ± 6 mmHg, the pulmonary vascular resistance index (PVRI) by 558 ± 153 dyn.s.cm-5.m-2 and dp/dt max and min by 1143 ± 309 and -977 ± 184 mmHg/s respectively in Emb group. A significant decrease in MPAP (by 26 ± 6 mmHg) and dp/dt max and min (by 737 ± 140 and -649 ± 158 mmHg/s respectively; $P<0.05$ both) was observed with tempol. In parallel, tempol marginally attenuated PVRI (by 397 ± 143, respectively; $P>0.05$). No significant changes in the other hemodynamic parameters were observed in both groups. **Discussion:** These findings suggest that tempol may attenuate the pulmonary arterial hypertension produced by EPA. It is possible that the radical scavenging activities of tempol increase the bioavailability of NO in the pulmonary circulation as well as reducing the hypoxia signaling and therefore reduce pulmonary arterial pressure. Acknowledgments: FAPESP, CAPES and CNPq.

06.038

Impaired cardiovascular responsiveness to isoprenaline in rats subjected to high-salt intake. Crestani S[1], de Souza P[1], Bóf ER[2], Guarido KL[2], Assreuy J[2], Marques MCA[1], da Silva-Santos JE[2] [1]UFPR − Farmacologia, [2]UFSC − Farmacologia

Introduction: The β-adrenergic receptor, physiologically activated by adrenaline and noradrenaline, plays an important role in the regulation of cardiovascular function. Excessive ingestion of sodium has been associated with undesired cardiovascular effects, including hypertension. In this study, we investigated if in vivo changes in the β-adrenergic receptor system play a role in the cardiovascular effects of high salt-intake in rats. **Methods:** Male Wistar rats (21 days old) were subjected to food containing NaCl at 2, 4 and 8% for 6 weeks. The control group received regular food. At the end of the sixth week the rats were anesthetized with ketamine/xylazine (100/20 mg/kg, i.m.), the femoral vein and the carotid artery were isolated, and heparinized polyethylene catheters inserted for drug administration (vein access), and heart rate (HR), mean arterial pressure (MAP), systolic pressure (SP), and diastolic pressure (DP) measurement (arterial access). After the stabilization period (20 min) the animals were injected with isoprenaline (ISO, 0.1, 0.3, 1 and 3 nmol/kg), or sodium nitroprusside (SNP; 3, 10 and 30 nmol/kg). A 10 min interval was allowed between treatments. At the end of these experiments blood samples were collected, and subjected to Griess reaction for nitrate+nitrite (NOx) detection. In addition, the heart of these animals was removed and perfused (at a constant rate of 4 ml/min) in a Langerdorff system. The effect of isoprenaline (1, 3, 10 and 30 ρmol) in HR and contraction was evaluated in vitro. All protocols were approved by the Institutional Ethics Committee of UFPR (authorization number 345). **Results:** The basal MAP was not different in groups NaCl 2, 4 and 8% (73.5 ± 3.5, 78.3 ± 2.6, and 80.5 ± 4.6 mm Hg, respectively), when compared to control values (83.4 ± 4.2 mm Hg). Isoprenaline-induced hypotension remained unchanged in groups NaCl 2 and 4%, but was significantly reduced (~30-40%) in those animals from NaCl 8% group. For instance, ISO (1 nmol/kg) reduced MAP by 34.7 ± 1.6 and 20.8 ± 4.8 mm Hg, in control and NaCl 8% groups, respectively ($p < 0.05$, $n = 6$). Almost the same differences were found in DP, while the effects of ISO in SP have not been changed in NaCl groups, when compared to control. ISO also presented in vivo diminished effects in HR of rats from NaCl 8% group. When administered at 0.3 nmol/kg, it increased the number of beatings per minute by 95.7 ± 14.1 in control, and 55.6 ± 7.0 in NaCl 8% group. Interestingly, we have not found significant in vitro differences in the effects of ISO in isolated hearts from these same animals. On the other hand, the cardiovascular effects of SNP were similar in all groups. The plasmatic NOx level was reduced from 45.4 ± 7.6 μM in control animals to 25.9 ± 2.7 μM in NaCl 8% group. **Discussion:** Our findings show, for the first time, that high-salt treated animals (NaCl 8%) present in vivo reduced hypotensive and cardiac responses to isoprenaline, a β-adrenergic receptor agonist, as well as display smaller amounts of nitrate and nitrite in their plasma, an indicative of nitric oxide production. We are currently investigating the molecular events responsible for these in vivo variations of the cardiovascular effects of isoprenaline. **Financial support:** CAPES and CNPq (482214/2007-4).

06.039

High salt-intake increases the activity of angiotensin-converting enzyme in rats. Crestani S[1], Gasparotto Junior A[2], Marques MCA[1], da Silva-Santos JE[3] [1]UFPR − Farmacologia, [2]UNIPAR/UFPR − Farmacologia, [3]UFSC − Farmacologia

Introduction: Several studies and clinical trials have suggested a correlation between dietary salt intake, blood pressure regulation, prevalence and progression of hypertension. The renin-angiotensin system (RAS) plays an important role in controlling cardiovascular functions, including blood pressure. Nevertheless, the relationship between high ingestion of salt and the in vivo functionality of the plasmatic angiotensin-converting enzyme (ACE) have never been investigated. **Methods:** Male Wistar rats (21 days old) were exposed to food containing NaCl at 2, 4 and 8% for 6 weeks. The control group received regular food. At the end of the sixth week the rats were anesthetized with ketamine/xylazine (100/20 mg/kg, i.m.), the femoral vein and the carotid artery were isolated, and heparinized polyethylene catheters were inserted for drug administration and mean arterial pressure (MAP) measurement, respectively, using pressure transducers coupled to a MacLab System® (ADI Instruments, USA). After the stabilization period (20 min), the animals were injected with angiotensin I (AI), angiotensin II (AII; both at 3, 10 and 30 ρmol/kg), or bradykinin (BK; 3, 10 and 30 nmol/kg). A 10 min interval was allowed between treatments. The effects of these drugs on MAP were recorded. At the end of these protocols, blood samples were collected and the serum was separated and used to investigate the activity of ACE, which was measured by indirect fluorimetry. The Institutional Ethics Committee of UFPR approved these procedures (authorization number 345). **Results and Discussion:** The hypertensive effects of AI were significantly increased in rats subjected to NaCl 4 and 8%. For instance, the higher dose tested (30 ρmol/kg) increased MAP by 44.8 ± 5.9 mm Hg in control animals, and 68.3 ± 5.4 and 80.97 ± 4.2 mm Hg in NaCl 4 and 8% groups, respectively. We also observed increased responses to AII in our experiments; however, when compared to AI the enhancement seen for AII was significantly smaller, and was found only in NaCl 8% group (the hypertensive effect of AII at 30 ρmol/kg was 58.6 ± 2.8 and 75.4 ± 4.7, in control and NaCl 8% groups, respectively). The enhanced responses to AI were accompanied by increased ACE activity. The plasmatic activity of ACE was increased from 80.4 ± 2.0 to 90.9 ± 2.8 nmol/min/ml, in control and NaCl 8% groups, respectively ($p < 0.05$; $n = 7$-12). In addition, the average length of hypotension induced by bradykinin (3, 10 and 30 nmol/kg), which is degraded by ACE, was significantly reduced in NaCl 8% group, by 43, 52 and 44%, when compared to control values. **Conclusion:** These data disclose, for the first time, that rats treated with high amounts of salt present in vivo enhanced activity of the rennin-angiotensin system with augmented systemic responses to angiotensin, which can be, at least in part, attributed to an increased activity of the plasmatic angiotensin-converting enzyme. If extrapolated for humans, these findings can help us to understand the relationship between chronic exposition to NaCl and the incidence of cardiovascular diseases, such as hypertension. **Financial support:** CAPES and CNPq (482214/2007-4).

06.040

Quercetin produces beneficial effects in renovascular hypertension. Neto-Neves EM, Montenegro MF, Ceron CS, Dias-Junior CAC, Castro MM, Tanus-Santos JE FMRP-USP – Farmacologia

Introduction: Hypertension is one of most serious diseases associated with structural and functional modifications of the vasculature. Accumulating evidences have suggested that the tissue damage observed during hypertension is caused by excessive generation of reactive oxygen species (ROS) that, in its turn, may degrade nitric oxide (NO). Quercetin, the most studied flavonoid, displays a great variety of pharmacological and biological properties. Several studies have demonstrated that quercetin antihypertensive effects are attributed mainly to its antioxidants properties. In the present study, we evaluated the effects induced by treatment with quercetin in blood pressure, nitric oxide production, vascular function and production of ROS during the renovascular hypertension. **Methods:** This investigation was conducted in accordance with the ethical guidelines of the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil (protocol number: 016/2010). After two weeks of surgery, sham-operated or two-kidney, one clip (2K-1C) hypertensive rats were treated with quercetin 10 mg/kg/day or vehicle for 21 days. Systolic blood pressure and body weight were monitored weekly. We assessed the vascular responses to acetylcholine and sodium nitroprusside on perfused hindquarter vascular bed of sham-operated or 2K-1C hypertensive rats treated with quercetin or vehicle. Plasma nitrite was determined using an ozone-based chemiluminescence assay and dihydroethidium (DHE), a sensitive superoxide ($O2^-$) probe, was used to evaluate in situ production of reactive oxygen species (ROS) in aortic tissues. Results No significant differences in body weight were observed among the experimental groups. Quercetin attenuated 2K-1C hypertension (165 ± 4 versus 191 ± 3 mmHg in hypertensive control group; $p < 0.05$). Treatment with quercetin produced significant increase in maximum relaxation effect obtained in response to acetylcholine in hindquarter bed of rats (from -43 ± -7 mmHg in the hypertensive control group to -64 ± -6 mmHg in the hypertensive quercetin-treated group; $p < 0.05$). Quercetin showed no effect on endothelial-independent responses to sodium nitroprusside ($p > 0.05$). Rats in 2K-1C hypertension group showed significantly lower plasma nitrite concentrations (364 ± 63 versus 590 ± 60 in Control group, $p < 0.05$). However, treatment with quercetin increased plasma nitrite concentrations (617 ± 107 nmoL/L, $p < 0.05$). Finally, quercetin attenuated the increased vascular formation of ROS in 2K-1C hypertension group (41 ± 3 versus 56 ± 4 mmHg in hypertensive control group; $p < 0.05$). **Discussion:** Our results suggest that quercetin was effective in lowering blood pressure, attenuating the endothelial dysfunction and the production of reactive oxygen species (ROS) during 2K-1C hypertension, possibly resulting in increased nitric oxide bioavailability. Acknowledgments: FAPESP, CAPES and CNPq.

06.041

Neonatal hyperleptinaemia possibly modulates cardiac function. Marques EB[1], Balter AS[1], Pereira-Toste F[2], Raimundo JM[3], Sudo RT[3], Zapata-Sudo G[3], Marques SA[4], Vieyra A[5], Scaramello C[1] [1]UFF − Farmacologia Experimental, [2]UFF − Ciências do Exercício, [3]UFRJ − Farmacologia Básica e Clínica, [4]UFRJ − Histologia e Embriologia, [5]IBCCF-UFRJ

Introduction: Previous studies showed cardiovascular diseases related to altered leptin activity (Schulze & Kratzsch, Clin. Chim. Acta,v. 362, p.1, 2005; Fernandes et al. Braz. J. Med. Biol. Res., v.40, p.1632, 2007;Triverdi et al., J. Cell Cycle, v.7, p.560, 2008) such as leptin resistance and metabolic changes in adult rats due to neonatal leptin treatment (Toste et al. Br. J of Nutrition, v.95, p.830, 2006). The aim of the present work is to study if neonatal leptin treatment programs cardiovascular function and may be involved in development of chronic diseases. **Methods:** Pups were divided into two groups: Leptin (L group) and Control (C group). During lactation (first 10 days) injections of leptin (8µg/100g sc) (L group) or saline (C group) were performed. After weaning, body weight and food intake were monitored. Rats (30, 90 and 150 days-old) were submitted to maximal effort ergometer test (initial rate 1Km/h raising 0.5 Km/h each 1min and 0.1% inclination each 2min). Rat hearts were removed for morphological, biochemical and functional analysis. Histological assays were performed using Hematoxilineosine (HE) and Gomori **Methods:** Heart tension was recorded before and after 3µM isoproterenol (Langendorff method). Cardiac P-type ATPases activity (PMCA, SERCA and Na+/K+ATPase) was determined according to the colorimetric method of Fiske & Subbarow. The use of animals was in agreement to the Animal Care and Use Committee of Fluminense Federal University (CEPA/UFF00123-09). **Results:** L group showed higher body weight/food intake. Morphological assays showed no difference between groups. L group (30 days-old) had worse performance in maximal effort ergometer test. Although no statistic difference were observed in heart isometric tension between L and C groups in the absence and in the presence of 3µM isoproterenol, it seems to be a minor increment of isometric tension by isoproterenol in L group, except in 30 days-old rats of L group. Biochemical assays showed a decrease of Na+/K+ATPase activity in 150 days-old rats but an increase of sarcoplasmatic reticulum (SR) Ca2+ATPase activity in all ages of L group. **Discussion:** Changes in body weight/food intake are described due to hyperleptinaemia in early life such as hormonal changes that are related to an increase of β1-adrenergic receptor expression in the heart (Toste et al. Br. J of Nutrition, v. 95, p.830, 2006; Trevenzoli et al. J. Physiol., v. 580, p.629, 2007). Evidences indicate that Na+/K+ATPase activity are reduced in the failing human heart (Barwe et al. J. Mol. Cell. Cardiol., v. 47, p.552, 2009). SR Ca2+ATPase activity increase is associated to Ca2+ overload and tachyarrhytmias (Gyorke & Carnes, Pharmacol. & Ther.,v. 119, p. 340, 2008). So our data suggest that neonatal leptin treatment may program cardiovascular function. **Financial support:** FAPERJ, CNPq, CAPES, PROPPI/UFF.

06.042

High-salt intake impairs the involvement of Rho-A/Rho-kinase and intracellular calcium in contractile responses of rat aortic rings. Crestani S[1], Marques MCA[1], da Silva-Santos JE[2]
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Introduction: The Rho-kinase (ROCK), activated by Rho-A, inhibits myosin phosphatase leading to Ca2+-sensitization, contributing to contraction induced by several agonists. High-salt intake is putatively associated with hypertension and cardiovascular diseases. The Rho-A/ROCK pathway has been associated with several models of hypertension, but its role in both structural and functional vascular changes that may be induced by sustained high-salt exposition, has been scarcely investigated. This study aimed to evaluate the functionality of ROCK in rat aortic rings isolated from rats exposed to high amounts of salt. **Methods:** Male Wistar rats (21 days old) were subjected to food containing NaCl at 2, 4, or 8% for 6 weeks. The control group received regular food. At the end of the sixth week the rats were killed and the thoracic aorta removed, cleaned and sectioned in rings. The rat aortic rings were transferred to baths containing Krebs' nutritive solution (37 °C, aerated with 95% O2/5% CO2), connected to force transducers and a MacLab System® (ADI Instruments, USA). A stabilization period of 60 min was respected between drug expositions. The functionality of endothelium was confirmed by the ability of acetylcholine (ACh, 1 μM) to relax the rings contracted by phenylephrine (PE, 1 μM). Concentration response curves to ACh or Y-27632 (both 1 nM-10 μM) were obtained in PE-contracted vessels. The vessels were also exposed to PE (1 nM-100 μM), either in the presence or absence of Y-27632 (1 μM, incubated for 15 min). Different rings were kept in a modified depolarizing Krebs's solution free of Ca2+, and were contracted by CaCl2 (1 to 100 mM). Some vessels were exposed to single concentrations of PE (1 μM) or caffeine (1 mM) in Ca2+ free solution. These procedures were approved by the Institutional Ethics Committee of UFPR (number 345). **Results:** The high-salt intake for 6 weeks did not result in any change in the maximal contraction (MC) induced by KCl and PE in rat aortic rings. In addition, the relaxation induced by ACh (a muscarinic agonist) and Y-27632 (a selective ROCK inhibitor) did not differ between groups. Incubation of Y-27632 reduced the MC elicited by PE from 1.93 ± 0.16 to 0.77 ± 0.15 g in control rings, but had no effect in vessels obtained from high-salt groups (e.g. the MC to PE was 1.66 ± 0.14 and 1.62 ± 0.17 g, before and after Y-27632 incubation, respectively, NaCl 8% group) ($p < 0.05$; $n \geq 6$). Caffeine-induced contraction (in Ca2+-free solution) was significantly reduced in vessels from high-salt treated rats (MC was 0.32 ± 0.2 g in control and 0.015 ± 0.005 g in vessels from NaCl 8% group). Similarly, PE-induced contraction in Ca2+-free solution was reduced in aortic rings from high-salt rats (MC was 1.46 ± 0.07 g in control, and 0.71 ± 0.15 and 0.76 ± 0.15 g in NaCl 4 and 8% groups, respectively). The contraction elicited by CaCl2 remained unchanged. **Discussion:** These data suggest that long-term exposition to high-salt amounts change the roles of Rho-A/ROCK and intracellular Ca2+ stores in the regulation of agonist-induced vascular contraction. This shift may be involved in the genesis of cardiovascular diseases, such as hypertension, related to salt ingestion in humans. **Financial support:** CAPES and CNPq (482214/2007-4).

06.043

A sulfonamide compound attenuates vascular smooth muscle contraction and lowers arterial pressure of normotensive and spontaneously hypertensive rats. Pontes LB[1], Raimundo JM[1], Sudo RT[1], Lima LM[2], Barreiro EJ[2], Zapata-Sudo G[1] [1]UFRJ − Farmacologia Básica e Clínica, [2]FF-UFRJ − LASSBio

Introduction: LASSBio-985 is a sulfonamide compound designed as a simplified structure of a non-selective PDE-4 inhibitor that presents vasodilatory effects in vitro. PDEs are enzymes responsible for the hydrolysis of cyclic adenosine 3',5'- monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP). Five different isozymes of phosphodiesterase are found in vascular smooth muscle (PDE1–5). The purpose of this study is to investigate the mechanisms of vasodilation induced by LASSBio-985 and its effect on blood pressure in Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). **Methods:** All animal protocols used in this study were approved by the Animal Care and Use Committee at Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro with license number DFBCICB 020. Aortic rings, with or without endothelium, from male Wistar, Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) were prepared for isometric tension recording. In some experiments, pharmacological tools were added to the preparation to study the involvement of some receptors or enzymes on LASSBio-985 vasodilator effect. Blood pressure was measured in WKY rats and SHR during intravenous infusions of LASSBio-985 (10 mg/kg/min) lasting 15 minutes. **Results:** LASSBio-985 caused concentration-dependent vasodilation in Wistar, WKY rats and SHR aortic rings, which was attenuated in endothelium-denuded vessels. Vasodilatory effects were also attenuated in endothelium-intact aortic rings that had been pre-treated with Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), a nitric oxide (NO) synthase inhibitor, and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a guanylate cyclase inhibitor. Vasodilation due to LASSBio-985 was also inhibited by sildenafil at 100 mmol/l, and SQ 22536, an adenylate cyclase (AC) inhibitor. To study the involvement of some endothelial pathways, atropine, diphenhydramine, HOE 140, naloxone, propranolol, indomethacin, and wortmannin were tested, but none inhibited the effects of LASSBio-985. The residual effect observed on endothelium-denuded aortic rings was abolished by nicardipine, a voltage-sensitive-Ca2+-channel blocker. Lastly, intravenous infusion of LASSBio-985 (10 mg/kg/min) significantly reduced systolic and diastolic pressures in both WKY and in SHR. CONCLUSION: LASSBio-985 is a compound with vasodilatory effects in vitro and likely acts through PDE1 inhibition. It also shows similar hypotensive effects in vivo in WKY rats and SHR. **Financial support:** CAPES, FUJB, INCT, FAPERJ, CNPq, PRONEX.

06.044

New NO donor induces relaxation of mesenteric resistance arteries and reduces resistance of mesenteric bed of normotensive and 2K-1C hypertensive rats. Araújo AV[1], Rodrigues GJ[1], Vercesi JA[2], Biazzotto JC[2], Bonagamba LGH[3], Machado BH[3], Silva RS[2], Bendhack LM[2]
[1]FMRP-USP − Farmacologia, [2]FCFRP-USP − Física e Química, [3]FMRP-USP − Fisiologia

Introduction: Nitrosyl ruthenium complexes such as [Ru(terpy)(bdq)NO]3+ (Terpy) are very attractive as NO donors. We previously reported that NO released from Terpy induces rat aorta relaxation due to soluble guanylyl-cyclase (sGC) and K^+ channels activation. However, it does not involve K+ channels activation in aortas from 2K-1C hypertensive rats. Although all blood vessels contribute in some extension to the regulation of blood pressure, the resistance vessels present the higher resistance and, therefore, they are the most involved in the regulation of blood pressure. Therefore, the present study aimed to verify the relaxation induced by the new NO donor (Terpy) and by the classic NO donor sodium nitroprusside (SNP), in mesenteric resistance arteries of normotensive (2K) and 2K-1C hypertensive rats, as well as the intracellular mechanisms of this relaxation. We also aimed to study their effects on the resistance of mesenteric bed. **Methods:** 2K rats were sham-operated and 2K-1C hypertension was induced by implantation of a silver clip in the renal artery of male rats. Concentration-effect curves for Terpy (100 nmol/L- 10mmol/L) or SNP (1nmol/L- 100 mmol/L) were constructed in endothelium-denuded mesenteric resistance arteries pre-contracted with 0.1mmol/L phenylephrine in the absence or in the presence of the soluble guanylyl-cyclase inhibitor (ODQ, 1mmol/L) or the non-selective potassium channel blocker tetraethylamonium (TEA 1mmol/L) or the selective K+ channel blockers: glibenclamide (3mmol/L, KATP), apamin (1mmol/L, SKCa) and 4-aminopyridine (1mmol/L, KV). The maximum effect (ME) and potency (pD2) of the NO donors were determined. For the experiments with the flowmeter, the rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) for the implantation of miniaturized Doppler probes around mesenteric artery and for catheterization of carotid artery and jugular vein. The velocity of blood flow through the changes in the frequency of the piezoelectric crystal (20 MHz) was determined. The changes in regional vascular resistance were calculated in each experiment considering the peak of changes in MAP as well as the peak of changes in regional blood flow. The doses used were 7mg/Kg for Terpy and 17.5mg/Kg for SNP. **Results:** Both SNP and Terpy induced relaxation with similar efficacy in 2K (Terpy 93.8 ± 1.2%, n=7, SNP 96.1 ± 0.7% n=6) and 2K-1C rats (Terpy 93.7 ± 1.4% n=8, SNP 90.2 ± 4.2% n=5). However, Terpy was less potent (pD2: 2K 4.45 ± 0.3; 2K-1C 4.51 ± 0.3) than SNP (pD2: 2K: 6.60 ± 0.2, 2K-1C: 7.28 ± 0.01) in both rat groups. In addition, the relaxation induced by Terpy and SNP was similar in mesenteric resistance arteries from 2K and 2K-1C. In the same way, Terpy and SNP induced a significant reduction in vascular resistance (%DVR) in mesenteric bed, which was not different between 2K (Terpy: 15.9 ± 4.17, n=13; SNP:37.1 ± 6.57%, n=15) and 2K-1C hypertensive rats (Terpy: 26.26 ± 3.2%, n=9; SNP: 41.65 ± 5.97. n=9). The relaxation induced by Terpy as well as SNP were almost abolished in the presence of ODQ, in arteries from 2K (ME: Terpy 5.10 ± 4.2%; SNP: 10.59 ± 5.4%) and 2K-1C rats (ME: Terpy 7.91 ± 2.67%; SNP: 14.61 ± 2.9%). The relaxation induced by Terpy was inhibited by all K+ channel blockers used in arteries isolated from 2K rats (ME: TEA 70.9 ± 6.3%, n= 6 ($P<0.05$); 4-AP: 75.6 ± 7.2% n= 8 ($P<0.05$); glibenclamide 75.3 ± 7.7%, n=4 ($P<0.05$), apamin 65.8 ± 6.1% n=7 ($P<0.05$)). In arteries of 2K-1C rats, the relaxation was inhibited by TEA (ME: 75.83 ± 7.6%), glibenclamide (ME: 80.45 ± 6.2%) and apamin (ME: 80.78 ± 6.9%). However, 4-aminopyridine had no effect (ME: 89.43 ± 8.2%). The relaxation induced by SNP was inhibited only by TEA in arteries from normotensive (ME: 71.7 ± 6.7%) and in hypertensive rats (ME: 75.8 ± 7.7%). **Conclusion:** The two NO donors induce vascular relaxation and reduces mesenteric bed resistance in a similar way in 2K and 2K-1C rats. The mechanisms involved in the relaxation induced by these NO donors involve activation of soluble guanylyl-cyclase and potassium channels. However, the relaxation induced by Terpy involves KATP, KV and SKCa in arteries from 2K rats, while KV does not appear to be involved in this relaxation I arteries from 2K-1C rats. On the other hand, the relaxation

induced by SNP involves TEA-sensitive potassium channels. This study was approved by Ethical Committee of the University of São Paulo (CETEA-FMRP, protocol number: 044/2008). Supported by: CNPq and FAPESP

06.045

Vasodilation induced by atrial natriuretic peptide (ANP) involves K_{ATP} channels activation in rat aorta. Andrade FA[1], Bendhack LM[2] [1]FMRP-USP, [2]FCFRP-USP

Introduction: ANP plays a key role in cardiovascular homeostasis and the vasodilatation induced by this peptide has been mainly attributed to the activation of the particulate guanylyl-cyclase enzyme (GCp), production of cGMP and activation of protein kinase-G (GK), which in turn can lead to activation of K^+ channels in order to induce vascular relaxation. It has been described that the receptors for ANP (GCp) could be also localized in the vascular endothelial cells. There are evidences that endothelium may contribute to vascular relaxation induced by ANP via NO-synthase (eNOS) activation and NO production. Therefore, this study aimed to investigate the contribution of NO produced in the endothelial cells and K+ channels to the rat aortic rings relaxation activated by ANP. We studied the effects of K+ channel blockers: non-selective tetraethylammonium (3mM TEA) and selective blockers of low conductance Ca2+-sensitive (SKCa, 0.5 µM Apamin), ATP-sensitive (KATP, 3µM glibenclamide) and voltage-sensitive (KV, 5 mM 4-aminopyridine). **Methods:** Cumulative concentration-effect curves were performed for ANP in denuded aortic rings pre-contracted with KCl (60mM) or phenylephrine (PHE, 100 nM) in the absence (control) or after incubation and in the presence of TEA, Glibenclamide, Apamin, or 4-Aminopyridine. Alternatively, cumulative concentration-effect curves were performed for ANP in intact endothelium aortic rings pre-contracted with PHE in the absence (control) or after incubation and in the presence of eNOS inhibitor L-NAME (100 mM). The potency (pD2) and maximal effect (EM) were determined. This study was approved by the Ethics Committee of the University of São Paulo (CETEA044/2008). **Results and Discussion:** There was no difference between the relaxation induced by ANP in denuded aortic rings (pD2: 9.08 ± 0.10, EM: 104.5 ± 1.0%, n= 7) and aortic rings with intact endothelium (EM: 105.4 ± 3.2% and pD2: 9.00 ± 0.15, n=7). The incubation with L-NAME did not change the relaxation induced by ANP in aortic rings with endothelium (EM: 109.8 ± 5.5%, pD2: 9.24 ± 0.16, n= 6). Relaxation induced by ANP was lower in aortic rings pre-contracted with KCl (EM: 49.7 ± 8.2%, pD2: 5.97 ± 1.03, n= 6, $P<0.05$) than in those pre-contracted with PHE (EM: 104.5 ± 1.0%, pD2: 9.08 ± 0.10, n= 7). The relaxation induced by ANP in PHE-contracted arteries was inhibited by TEA (EM: 93.2 ± 2.2%, pD2: 8.55 ± 0.21, n= 4, $P<0.05$) and Glibenclamide (EM: 98.7 ± 1.2%, pD2: 9.06 ± 0.12, n=6, $P<0.05$). However, incubation with the selective blockers 4-Aminopyridine and Apamin did not alter the relaxation induced by ANP. Taken together our data demonstrate that the GCp activated by ANP is not localized in the endothelial cells and NO produced by eNOS activation is not involved in the relaxation induced by ANP in rat aorta. On the other hand, K+ channels sensitive to TEA and glibenclamide contribute to the rat aortic rings relaxation induced by ANP, but not SKCa and Kv channels. **Financial support:** CAPES, CNPq and FAPESP.

06.046

Cardiovascular hyporresponsiveness in severe sepsis is related with augment of G-protein receptor kinase (GRK)-2 expression via a nitric oxide-dependent mechanism. Dal-Secco D[1], Olivon VC[2], Corrêa T[1], Celes MRN[3], Abreu A[3], Rossi MA[3], Oliveira AM[2], Cunha FQ[4], Assreuy J[1] [1]UFSC − Farmacologia, [2]FCFRP-USP − Física e Química, [3]FMRP-USP − Patologia, [4]FMRP-USP – Farmacologia

Introduction: Sepsis is a systemic inflammatory response resulting from the inability of the host to restrict local infection. From the cardiovascular point of view, septic shock is characterized by cardiac collapse and decreased peripheral resistance due to dilatation of systemic resistance vessels, generally induced by a large nitric oxide (NO) production from inducible NO synthase (iNOS). G protein–coupled receptor (GPCR) kinases (GRKs), specific kinases interacting with GPCR proteins, induce receptor phosphorylation and thereby signal GPCR desensitization in the continuing agonist presence. Then, an increased expression of GRKs could augment adrenergic receptor desensitization and in turn reduce cardiovascular responses. Thus, we hypothesized that the hyporesponsiveness observed in sepsis could result from signal receptor desensitization mediated by continuous and excessive adrenergic receptor activation via a NO-dependent mechanism. **Methods:** C57Bl/6 mice were submitted to cecal ligation and puncture (CLP) surgery and sham-operated animals as controls. The cardiovascular responsiveness activity was evaluated in aorta rings or cardiac ventricles. Aorta rings were contracted with phenylephrine (Phe; 1μM), whereas, ventricles were contracted with isoproterenol (Iso; 1μM). The tissues responsiveness was evaluated 6, 12 and 24 h after CLP surgery in the presence or absence of NO synthesis inhibitor (1400W; 100μM; 30min). GRK2 expression was analyzed on heart and aorta 6, 12 and 24 h after CLP from septic, 1400W (1mg/kg)-treated and sham mice by immunofluorescence analysis. The procedures have been approved by the Animal Ethics Committees of UFSC (PP003/CEUA). **Results:** The vascular responsiveness to vasoconstrictor Phe was significantly reduced in aorta rings from septic mice evaluated 6 (55%), 12 (57%) and 24 (78%) h after CLP. However, the 1400W incubation prevented this vascular hyporesponsiveness 6 and 12 h after CLP. The cardiac responsiveness to Iso was significantly reduced in ventricles from septic mice evaluated 12 (73%) and 24 (88%) h after CLP. Conversely, the 1400W incubation prevented this cardiac hyporesponsiveness 12 h after CLP. Moreover, high expression of GRK2 was detected in aorta 6 (65%), 12 (70%) and 24 (88%) h, and heart of septic mice 12 (52%) and 24 (63%) h after CLP. The 1400W-treatment reduced the GRK2 high expression on aorta (75%) and heart (79%) of septic mice. Finally, the treatment with 1400W enhanced significantly the survival rate of the septic mice (55%). **Discussion:** Our findings identify that NO seems to activate GRK2, which may induce adrenergic receptors desensitization to adrenergic agonists. Increased in the GRK2 expression is associated with impairment vascular response, contributing to severe cardiovascular hyporesponsiveness observed during septic shock. Moreover, NO synthesis inhibition improves output cardiovascular, and as consequence, enhances the survival of septic mice. Therefore, the results suggest that GRK2 could be a new potential target to sepsis pharmacotherapy. **Financial support:** CNPq, CAPES, FAPESP and FAPESC.

06.047

Vasorelaxant effect of Isotirumalin, a dihydroflavonol from *Derris urucu*, on rat aorta. Mendes LJ[1], Capettini LSA[2], Arruda MSP[3], Lemos VS[2], Côrtes SF[1] [1]UFMG − Farmacologia, [2]ICB-UFMG − Fisiologia e Biofísica, [3]UFPA − Química

Introduction: The present work aimed to investigating the vasorelaxant effect of isotirumalin, a dihydroflavonol isolated from *Derris urucu* (Leguminosae). **Methods and Results:** The vasorelaxant effect of isotirumalin was investigated in rat aorta in the presence and in the absence of a functional endothelium. The production of nitric oxide (NO) induced by isotirumalin was measured simultaneously with its vasorelaxation using carbon microsensors. In endothelium-intact aortic rings, isotirumalin induced a concentration-dependent vasorelaxation (pIC30 = 4.84 ± 0.24). This effect was abolished in endothelium-denuded aortic rings or in the presence of Nw-nitro-L-arginine-methyl-ester (L-NAME; 100 mM). In addition, isotirumalin (100 mM) induced a simultaneous and significant production of NO and vasorelaxation, whose where blunted in the presence of L-NAME. **Discussion:** The present results demonstrate that isotirumalin induced a vasorelaxant effect in rat aorta by a mechanism dependent of a functional endothelium and on production of NO. **Financial Support**: FAPEMIG e CNPq.

06.048

Echocardiography aspects of structural changes in different morphological and functional models of hypertension in SHR. Pereira DJ[1], Gazzoto AF[1], Pires NF[1], Moreira MM[1], Santos RC[1], Ludovico ND[1], Quinaglia TSS[1], Renno AL[2], Figueiredo VN[1], Moreno Junior H[1] [1]UNICAMP − Farmacologia cardiovascular, [2]UNICAMP − Farmacologia Bioquímica

Introduction: Refractory hypertension (HAR) has been considered a relatively rare manifestation of hypertension and the majority of the cases studied were really pseudo-refractoriness by poor adherence to treatment. But within the past decade, publications have increased exponentially in regards to HAR and several authors have described it as a syndrome based on biological mechanisms, when the lack of adherence to antihypertensive treatment is excluded. Thus, the hyperactivity of RAAS, endothelial dysfunction and plasma volume expansion linked to the constitutive hyperaldosteronism (aldosterone synthase polymorphisms) have been studied as mechanisms triggering and maintaining high levels of blood pressure in some hypertensive patients treated with three or more classes of antihypertensive drugs. The link in the pathophysiology of this condition can be obesity and metabolic syndrome, where adipocytes produce more of a factor that stimulates the adrenal glands to produce mineralocorticoids. Even with these known limitations an animal model should not be excluded from the study of structural and functional changes that mimic this condition. The objective was to study the effects on the blood pressure and cardiac abnormalities (echocardiography) caused by the superposition of different models of hypertension in rats (DOCA-Salt, 2K-1C and L-NAME) in SHR, this model is known as the most representative of hypertension in humans. The results presented here are preliminary of Phase-1, in the second phase we will evaluate the response of the blood press using the same group of animals from the experimental models of hypertension and include triple therapy with anti-HA (diuretics, ACEIs / ARBs and II of calcium channel blockers). **Methods:** This project was approved by the ethics committee of animal (CEEA) Unicamp protocol 2002-1, we divided 5 experimental groups: WKY control (WKY=5); SHR (n=5); SHR plus L-NAME 5mg/kg/day (SHR+L-NAME, N=5); SHR plus renal artery surgical stenosis (SHR+2R-1C, N=5); SHR plus DOCA (8mg/kg, SC, weekly) and high salt intake (SHR+DOCA-SALT, n=5). After 8 weeks, we obtained the echocardiographic and histologic data below: **Results:**: ECHOCARDIOGRAPHIC PARAMETERS GROUPS PAC (mmHg) SEPTUM (mm) LVDD (mm) LVPW (mm) LVSD (mm) LV MASS (mg) LVEF (%) MI (mg/g) WKY 98 ± 4.47 0.138 ± 0.013 0.592 ± 0.031 0.138 ± 0.008 0.336 ± 0.018 0.973 ± 0.581 81 ± 1.85 2.24 ± 0.228 SHR 138 ± 5.47* 0.162* ± 0.012 0.574 ± 0.023 0.164* ± 0.005 0.342 ± 0.021 1.490 ± 0.294 78 ± 5.62 2.44 ± 0.155 SL 158 ± 5.47* 0.178* ± 0.005 0.564 ± 0.015 0.178* ± 0.017 0.312 ± 0.017 1.102* ± 0.851 82 ± 2.98 4.08* ± 0.157 SD 166 ± 5.47* 0.164* ± 0.005 0.480* ± 0.023 0.162* ± 0.004 0.252* ± 0.040 1.094 ± 0.117 84 ± 6.64 3.08* ± 0.229 SK 150 ± 5.47* 0.170* ± 0.004 0.530* ± 0.057 0.160* ± 0.012 0.346 ± 0.019 1.090 ± 0.795 68 ± 18.39 3.18* ± 0.174 PAC – tail blood pressure, LVDD - Left ventricular diastolic diameter, LVPW - posterior wall of left ventricle, LVSD – left ventricular systolic diameter, LV Mass – left ventricular mass, LVEF – left ventricular ejection fraction, MI – mass index, $P < 0.05$ control group. **Discussion:** The results show significant increase in left ventricle (LV) mass and left ventricle mass index – MI - (body mass corrected) as the arterial pressure increases. In addition, the sum of another mechanism shows bigger LV mass and the association with the most mass increase was the SHR and L-NAME. These findings point to a better mechanism of resistant hypertensive rats with this last association. **Financial support:** FAPESP

06.049

Development and validation of analytical method for quantification of arsenic and antimony in liposomes. Reis PG, Souza J, Teixeira MC, Grabe-Guimarães A, Silva-Barcellos NM
UFOP − Farmácia

Introduction: Arsenic (As) and antimony (Sb) compounds have been used to treat endemic diseases such as cancer, leishmaniasis and schistosomiasis, besides their toxicity. Several studies are carried out for the development and characterization of nanocarriers´ systems such as liposome in order to minimize the toxicity of these drugs. However, there are no reference methods to quantify these semi metals within a liposomal matrix. Therefore, the validation of one analytical method for arsenic and antimony quantification in liposomal matrix, through inductively coupled plasma/optical emission spectrometry (ICP/OES), is proposed and presented here. **Methods:** The linearity, specificity, detection, and quantification limits as well as accuracy and precision parameters were determined according to the Harmonised Tripartite Guideline (ICH) norms and Brazilian Health Surveillance Agency (Resolution 899 of 2003). For the analyses it was used Spectrometer - Ciros CCD (Spectro) ICP/OES instrument. This project was approved by the local ethics committee (number 2009/11). **Results:** The analysis of samples containing only liposomal matrix produced data which were similar to the base line, demonstrating that the method is specific. The curves of As and Sb presented correlation coefficients of 0.9997 and 0.9996, respectively, demonstrating the linearity. The detection limits obtained were 0.222 mg.L-1 and 0.0578mg.L-1 for Sb and As, respectively. The quantification limit was 3.0mg.L-1 for both, with an adequate accuracy within 98.26 and 101.32% for Sb and 99.98 and 100.36% for As, and precision (CV lower then 5%). **Discussion:** The analytical spectrometric method was applied to quantify arsenic and antimony encapsulated in liposome formulations. The developed and validated method demonstrated to be simple, specificity, rapid, precise, accurate and reproducible. Additionally is important to point out that liposomes matrix are expensive and this developed method has an advantage of using small sample volumes, becoming an alternative tool for the quality controlling of arsenic and antimony containing liposome formulation. Acknowledgments: FAPEMIG, CNPq, UFOP.

06.050

Spontaneously hypertensive rats (SHR) under chronic treatment with sodium fluoride showed reduced fluoride and calcium concentrations in plasma and saliva. Picco DCR[1], Delbem ACB[1], Antoniali C[2] [1]FOA-UNESP − Odontologia Infantil e Social, [2]UNESP-Araçatuba − Ciências Básicas

Introduction: In spontaneously hypertensive rats (SHR) the salivary fluoride concentration is reduced compared with those observed in normotensive Wistar rats. This study examined possible alterations in absorption or distribution of fluoride that would be involved with less biodisponibility of fluoride in the saliva of SHR. **Methods:** Wistar rats and SHR were treated with 20 ppm of sodium fluoride (NaF) for 30 days. We analyzed the salivary flow, the concentrations of fluoride and calcium in saliva and plasma in treated (T) and untreated (UT) groups. Blood pressure was measured by plethysmography. The salivary flow was stimulated with pilocarpine (5 mg/kg) and the saliva was collected following standard procedures. Blood samples were collected by abdominal puncture to plasma attainment. The concentration of ionized fluoride was analyzed with ion-specific electrode and reference microelectrode coupled to an ion analyzer from a calibration curve. The calcium concentration was analyzed using a specific commercial kit (Katal Biotechnology) by spectrophotometry. The results were analyzed and compared between groups (ANOVA). **Results:** UT SHR showed lower salivary flow, lower concentration of fluoride in saliva and plasma and high concentration of calcium in saliva. The treatment with NaF did not alter the blood pressure of the animals, but decreased the salivary flow in Wistar rats. T SHR and Wistar had higher concentrations of fluoride in saliva and plasma than their UT controls. However, the fluoride concentration in the saliva and in plasma of SHR was still reduced when compared to the observed in Wistar rat. The treatment decreased the concentrations of calcium in saliva and plasma of SHR, but did not change it in Wistar groups. After treatment, SHR had lower concentrations of calcium in saliva than Wistar rats. **Discussion:** These results demonstrated that: 1) the concentration of fluoride in plasma and saliva is proportional to the amount of NaF ingested by the animals; 2) the processes of absorption and distribution of fluoride are altered in SHR; and 3) in SHR there is a negative interaction between fluoride and calcium that is not observed in Wistar rats. Ethics Committee in Animal Experimentation: 2008-006301. **Financial support:** CAPES, CNPq.

06.051

H_2O_2 -induced vasodilatation through neuronal nitric oxide synthase activation by a natural xanthone. Capettini LSA[1], Silva JF[1], Dos Santos MH[2], Nagem TJ[3], Côrtes SF[4], Lemos VS[1]
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Introduction: Neuronal nitric oxide synthase (nNOS) have been considerate an important enzyme in the control of vascular tone. We demonstrated very recently that nNOS contributes, together with endothelial NOS (eNOS), to maintenance of vascular homeostasis. In addition, nNOS has been proposed a new antiatherogenic factor in the mouse aorta. Xanthones are natural polyphenols known by your antihypertensive, antioxidant and vasorelaxant effects. In this work, we evaluated the vasorelaxant effect of 1,3-dihydroxy-7,8-dimethoxy-xanthone (DDX) in aorta from C57Bl/6J mouse. **Methods:** Experimental protocols were approved by the CETEA-UFMG (26/2007).Vascular reactivity was evaluated in organ bath system. NO and H2O2 measurements were performed simultaneously to organ bath experiments by the use of carbon microsensors. H2O2 production was confirmed by chemiluminescence. Endothelial NO and H2O2 production were determined by fluorescence microscopy. Total and phosphorylated eNOS and nNOS expressions were evaluated by Western blot. In vivo antisense-oligodeoxynucleotides (AS-ODN) technique was used to knockdown specifically eNOS and nNOS. **Results and discussion:** Our data suggest that DDX (0.1-500μM) induced vascular relaxation (Emax=98.2 ± 7.45%; pIC50%=5.2 ± 0.3μM) that was accomplished by a little increase in NO production and a strong increase in H2O2 production. DDX-induced vasodilation was abolished by endothelium removal and non-selective NOS inhibition with L-NAME (300μM). Catalase (2400U/mL), that decomposes H2O2, or L-AgNO2-L-DBu (1μM), a selective nNOS inhibitor impaired DDX-induced vasodilation (Emax=30.22 ± 10.23% and 34.29 ± 4.77%, respectively) and abolished H2O2 production. These data suggest an important role of nNOS-derived H2O2 on DDX-induced vasodilation. To confirm individual contribution of eNOS and nNOS on DDX-induced vasodilation, we used AS-ODN technique. eNOS AS-ODN treatment minimally altered vasodilation but impaired NO production induced by DDX. On the other hand, nNOS AS-ODN induced a significant reduction in vascular relaxation (~70%) and H2O2 production (~93%). Fluorescence microscopy and chemiluminescence method confirmed DDX-induced H2O2 production through nNOS activation in endothelial cells. In addition, DDX promoted dephosphorylation in nNOS-Ser852 indicating an activation of nNOS. These data can present a clinical significance once nNOS-derived H2O2 is an important endothelium-derived relaxant factor and nNOS has been proposed as an important anti-atherogenic factor. Conclusion. In conclusion, we demonstrated at first time that a natural polyphenol was able to induce endothelium-dependent vascular relaxation through nNOS activation and H2O2 synthesis. **Financial support:** CAPES, CNPq, FAPEMIG.

06.052

Consequences of acute or chronic stress on relaxation induced by angiotensin 1-7 in rat carotid. Banin TM[1], Olivon VC[1], Ramalho L[2], de Oliveira AM[1] [1]USP − Física e Química, [2]USP − Patologia

Introduction: Stress can alter the cardiovascular functions. The renin-angiotensin system (RAS), a major participant in control of these functions is affected in response to stress. Angiotensin 1-7 (Ang 1-7), is a component of the RAS and blood vessels are an important site for formation and biological action of Ang 1-7 (Santos, Brain Res Bull, 35, 293, 1994). Although the consequences of stress on the RAS have been extensively studied in the central nervous system, it is not clear how stress regulates the vasoactive activity of Ang 1-7 in the carotid. This study evaluated the consequences of acute or chronic stress on the actions of Ang 1-7 in isolated carotid of rats. **Methods:** Rats were subjected to immobilization stress for 3h in specifically designed plexiglass tubes. One group of rats was only immobilized once (acute stress) while in another group rats were immobilized (3h) daily for twenty-one days (chronic stress). After the session of acute or chronic stress, the animals were anaesthetized and killed by aortic exsanguinations in accordance with standards and policies of the University of São Paulo's Animal Care and Use Committee (number of the protocol: 09.1.204.53.6). Endothelium-intact and endothelium-denuded tissues were pre-contracted with phenylephrine, used in the concentrations of 10-4mol/L and 3X10-8mol/L, respectively, to induce contractions of similar magnitude. After reaching a stable and sustainable contraction, angiotensin 1-7 (10-10 to 3X10-6 mol/L) was added cumulatively to the organ bath. Experiments were conducted in the presence and absence of N G-nitro-l-arginine methyl ester (L-NAME) (10-4mol/L) or Indomethacin (10-5 mol/L). These studies allowed analyze the parameters of maximum effect (Emax) and Potency (pD2). Statistical significance was determined by using the one-way analysis of variance (ANOVA). In all cases, probability levels of less than 0.05 ($p<0.05$) were taken to indicate statistical significance. **Results:** Our results shows that angiotensin 1–7 induced concentration-dependent relaxation in endothelium-intact (Emax: 37,78 ± 2,34%) and endothelium-denuded (Emax: 47,80 ± 3,23g) carotid rings of control rats. In carotid from animals subjected to acute stress with endothelium the Emax for Ang 1-7 was 21,03 ± 4,05% and without endothelium was 14,21 ± 3,13%. On the other hand it was observed in the carotid from rats submitted to chronic stress with endothelium an Ang 1-7 Emax of 39,02 ± 5,88%, and without endothelium, of 32,6 ± 2,58%. The non selective NO synthase inhibitor, L-NAME, reduced the Emax for angiotensin 1-7 in all groups relation to control group in the absence of inhibitor. In preparations with endothelium pre-incubated with indomethacin, the groups stressed there were no differences in Emax in relation to control group in the presence of inhibitor. However in the carotid without endothelium of animals subjected to acute stress the Emax was 18,33 ± 5,91% and to chronic stress the Emax was 15,53 ± 5,53%, in presence of indomethacin. There was no statistical difference in pD2 among all the groups studied. **Discussion:** This study demonstrates that in conditions of acute or chronic stress the Emax for angiotensin 1-7 is significantly reduced. Results show that inhibition of NO synthesis by L-NAME in carotid arteries with and without endothelium inhibited the vasodilator effect induced by angiotensin (1-7). The inhibition of cyclooxygenase by indomethacin in carotids endothelium-denuded also inhibited the vasodilator effect induced by angiotensin (1-7). **Financial support:** FAPESP and CNPq.

06.053

Study of the cytotoxic effect of *Bothrops pauloensis* on MDCK cells. Jorge RJB[1], Marinho AD[2], Barbosa JPC[3], Abreu ML[1], Morais ICO[1], Menezes RRPPB[4], Lima Filho CF[5], Pessoa AWP[6], Santos LFL[6], Alves CD[3], Toyama MH[7], Martins AMC[4], Evangelista JSAM[6], Monteiro HSA[1], Moraes GB[6] [1]UFC − Fisiologia e Farmacologia, [2]UFC − Farmácia, [3]UNIFOR − Fisiologia e Farmacologia, [4]UFC − Análises Clínicas e Toxicológicas, [5]UECE − Ciências Fisiológicas, [6]UECE − Veterinária, [7]IB-UNICAMP

Introduction: The snakes from the genus Bothrops are the most important cause of snakebites in Brazil. The main complications in lethal cases are the acute renal failure, shock, acute respiratory failure, and sepsis. Bothrops pauloensis snake inhabits throughout the Brazilian territory, except in the Amazonian regions of Brazil (DOLEY R; KINI RM, Cell Mol Life Sci. v.66, p.2851, 2009). The aim of this work was to study the cytotoxic effect of the venom of B. pauloensis (VBp) on renal tubular cells Mardin-Darby Canine Kidney (MDCK). **Methods:** The MDCK cells were cultured in RPMI medium supplemented with 10% fetal bovine serum and penicillin / streptomycin, and incubated in a 5% CO2 at 37 °C for 3-5 days until reaching confluence state, and it was kept always aseptic conditions. Then, they were displaced with trypsin-EDTA (0.05%/ 0.02%), counted in a Neubauer chamber and plated to 1x105 cells/mL in 96-well plates. After 24h of incubation, the plate was washed with PBS and different concentrations (100, 50, 25, 12.5, 6.25 and 3.125µg/mL) of VBp was added. Then, it was added to 10µL of MTT (3-(4,5-Dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (2.5 mg/mL), and 4h later, 90µL of sodium dodecyl sulfate (SDS) at 10% was also added. The spectrophotometric reading at 570nm was performed 17h later. As a negative control PBS was used. The results were expressed as percent viability ± SEM in the control group. Three experiments were conducted in triplicate, and data were analyzed with GraphPad Prism 5.0, by ANOVA with Dunnet post-test, with significance at $p < 0.05$. To determine the IC50 it was used the method of nonlinear regression. The experimental protocols were approved by the Federal University of Ceará Animal Research Ethical Committee, license number of 68/08. **Results and Discussion:** The venom of B. pauloensis (VBp) was able to decrease cell viability under the study conditions, by showing cytotoxicity effect up to 3.12 mg/mL (IC50 = 4.18 mg / mL), and promoting an inhibition on the cell growth in a dependent-concentration. Other studies, with fractions of VBp will be conducted to ascertain which is the fraction involved in the role of the toxic effects in MDCK culture cells and also to investigate the mechanisms involved in cell death. **Financial support:** CNPq; CAPES and FUNCAP.

06.054

Important changes in hematologic and cardiovascular parameters produced by chronic treatment with etoricoxib in normotensive and spontaneously hypertensive rats. Baracho NCV FMIT − Farmacologia e Bioquímica

Introduction: and objective: Etoricoxib is a non- hormonal anti-inflammatory (NHAI) of second generation of selective COX-2 inhibitors.1 The objective of the present study was to evaluate the cardiovascular and hematologics effects produced by chronic treatment with two distinct doses of etoricoxib in normotensive Wistar rats and spontaneously hypertensive rats (SHR).2,3 **Materials and Methods:** This study was approved by the Research Ethics Committee of the Faculty of Medicine of Itajubá under protocol 017/07.Thirty normotensive Wistar rats and thirty SHR rats were used, allocated in six groups and receiving by gavage for sixty days, the following treatments: normotensive Wistar rats: I) Control group (Cn) - distilled water (n=10) , II) etoricoxib 10mg/kg (An) (n=10), III) etoricoxib 30mg/kg (ARn) (n=10) ; SHR rats: IV) distilled water (Ch) (n=10), V) etoricoxib 10mg/kg (Ah)(n=10), VI) etoricoxib 30mg/kg (ARh)(n=10). Mean arterial pressure (MAP) was measured by tail pletismograph , twice a week during the study. To the end of this period, under anesthesia, were collected blood samples by heart punction for blood parameters determination. **Results:** Wistar normotensive rats: Treatment with etoricoxib produced a significant increase of MAP in the An and ARn groups when compared to the control group (Cn) from day 28 (An vs Cn: Day 0 - 110,7 ± 4,3mmHg vs 112,4 ± 1,6mmHg p=0,35, Day 28 - 117,5 ± 2,6mmHg vs 114,4 ± 1,6mmHg p=0,01, Day 60 - 119,3 ± 1,4mmHg vs 114 ± 1,4mmHg p=0,0001; ARn vs Cn: Day 0 - 110,6 ± 2,1mmHg versus 112,4 ± 1,6mmHg p=0,27, Day 28 - 118,2 ± 1,7mmHg vs 114,4 ± 1,6mmHg p=0,003, Day 60 - 118,2 ± 1,7mmHg vs 114 ± 1,4mmHg p=0,0001). Etoricoxib administration produced important changes in blood parameters: hematocrite (An-Cn: 43 ± 2% vs 39 ± 2% p=0,001; ARn-Cn: 44 ± 2.7% vs 39 ± 2% p=0,001); red blood cells (An-Cn: 7,42 ± 0,454 x 106/mm3 vs 6,57 ± 0.441 x 106/mm3 p=0,001; ARn-Cn: 7,21 ± 0,399 x 106/mm3 vs 6,57 ± 0.441 x 106/mm3 p=0,01); and platelets (Cn-ARn: 533 ± 234 x 103/mm3 vs 835 ± 172 x 103/mm3 p=0,01). SHR rats: Treatment with etoricoxib produced a significant increase of MAP in the Ah and ARh groups when compared to the control group (Cn) from day 18 and 15, respectively (Ah-Ch: Day 0 - 181,6 ± 12,4mmHg vs 178,8 ± 15,2mmHg p=0,65, Day 18 - 191,5 ± 12,2mmHg vs 180,3 ± 7,1mmHg p=0,02, Day 60 - 205,4 ± 7,0mmHg vs 185,5 ± 11,4mmHg p=0,0002 ; ARh-Ch: Day 0 - 176.1 ± 13,6mmHg versus 178,8 ± 15,2mmHg p=0,68, Day 15 - 195,2 ± 9,6mmHg vs 182,5 ± 11,7mmHg p=0,01, Day 60 - 206,2 ± 13,4mmHg vs 185,5 ± 11,4mmHg p=0,001). The etoricoxib administration produced important changes in blood parameters: hematocrite: (Ah: 44,1 ± 2.2% versus 41,4 ± 2.7% p=0,02; ARn-Cn: 45,9 ± 4.1% vs 41,4 ± 2.7% p=0,009); hemoglobin (ARh-Ch: 15,3 ± 1.4g% vs 13,9 ± 1.1g% p=0,03). **Discussion:** Our data indicate that the chronic treatment with etoricoxib produces increase of MAP in Wistar normotensive rats and SHR rats and increase in hematocrite, red blood cells(An and ARn), hemoglobin (ARh) and platelets (ARn) when compared to the control group. **Financial support:** FAPEMIG and PDIC-FMIt 1- Araujo LF. Eventos cardiovasculares: um efeito de classe dos inibidores de COX-2. Arq. Bras. Card. 2005; vol. 3; p 222 2- Prozzi GR. Riesgo cardiovascular de los nuevos inhibidores de la COX-2: celecoxib – rofecoxib. Rev Soc. Odontol. Plata. 2002; vol. 15; p 42 3- Sánchez BM. Safety of etoricoxib, a new cyclooxygenase 2 inhibitor, in patients with nonsteroidal anti-inflammatory drug-induced urticaria and angioedema. Annals of allergy, asthma & immunology. 2005; vol. 2; p154

06.055

Hypotensive and diuretic effect of *Achillea millefolium* L. (Asteraceae) in rats. de Souza P[1], Gasparotto Junior A[2], Boffo MA[3], Lourenço EL[4], Stefanello MEA[5], Marques MCA[1], da Silva-Santos JE[6], Kassuya CAL[7] [1]UFPR − Farmacologia, [2]UNIPAR/UFPR − Farmacologia, [3]UNIPAR − Farmacologia, [4]USP − Toxicologia e Análises Toxicológicas., [5]UFPR − Química, [6]UFPA − Farmacologia Experimental e Pré-clínica, [7]UFGD − Ciências da Saúde

Introduction: Traditional uses of the *Achillea millefolium* L. (Asteraceae), popularly known as “mil-folhas”, include the treatment of cardiovascular diseases. In the present study, we assessed the hypotensive effect of hydroethanolic extract (HEAM), dichloromethane fraction (DCM), ethyl acetate fraction (AcE), butanolic fraction (BT) and dichloromethane-2 sub-fraction (DCM-2) in normotensive anesthetized rats, and the diuretic effect of HEAM and DCM-2 obtained from *A. millefolium* in rats. **Methods:** Aerial parts of *A. millefolium* were extracted with ethanol 90%, concentrated, filtered and the solution lyophilized to give the HEAM. It was suspended in EtOH-H2O (1:1) and then extracted with dichloromethane, ethyl acetate and 1-butanol, successively. DCM fraction was submitted to vacuum column chromatography on silica gel, eluted with solvents of crescent polarity, to give DCM-2 sub-fraction. These fractions were analyzed by NMR 1H. Male Wistar rats received the oral treatment with extract, fractions or vehicle and had the direct blood pressure measurement, where the anesthetized rats had the left carotid artery cannulated and connected to a pressure transducer coupled to a MacLab® recording system, and an application program (Chart, v 4.1) from ADI Instruments. Diuretic activity was determined in animals kept in metabolic cages. Urine was collected in a graduated cylinder and its volume was recorded at 2h intervals for 8h. Electrolytes (sodium - Na+ and potassium - K+) concentrations, pH, density and conductivity were estimated from a pooled urine sample of each pair of rats at the end of the experiment (8h). All procedures were approved by the Institutional Ethics Committee under protocol number 240. **Results and Discussion:** The oral administration, 3 hours prior, of the HEAM (100-300 mg/kg), DCM (20 mg/kg), DCM-2 (10-30 mg/kg), but not AcE (10 mg/kg) and BT (50 mg/kg) fraction, reduced significantly the mean arterial pressure (MAP) of anesthetized normotensive rats, with changes around 13 ± 1 mmHg for HEAM, 11 ± 1 mmHg for DCM and 10 ± 1 mmHg for DCM-2. The phytochemical analysis by NMR 1H of DCM and DCM-2 fractions revealed high amounts of flavonoids. Moreover, the single oral administration of HEAM (300 mg/kg) and DCM-2 (30 mg/kg) caused a significant increase in urinary excretion, about 37 ± 4 % for HEAM and 90 ± 6 % for DCM-2, compared to control group. The urinary excretion of electrolytes was also increased for both treatments, about 33 ± 7 to 51 ± 7 % for Na+ and 36 ± 7 to 49 ± 7 % for K+ excretion. There was no change in conductivity, density and pH of samples analyzed. The results of the present study did show that the extract and semi-purified fractions obtained from *A. millefolium* exhibit hypotensive and diuretic actions when orally administered, supporting its popular use as antihypertensive. Other studies are being conducted to assess these effects in experimental animal model of hypertension. **Acknowledgements**: CNPq and CAPES.

06.056

Involvement of L-arginine/NO pathway on the vascular adaptive response of high fat-diet rats exposed or not to chronic stress. Bruder-Nascimento T[7], Campos DHJ[2], Cicogna AC[2], Cordellini S[1] [1]UNESP − Farmacologia, [2]UNESP − Clínica Médica

Introduction: Both stress and high fat diet are involved in cardiovascular diseases. However, the effects of the co-occurrence of these risk factors remain to be investigated. It was investigated the involvement of L-arginine/nitric oxide pathway on the vascular response of high fat diet rats exposed or not to chronic stress. **Methods:** The experimental protocol was approved by Ethical Committee for Animal Research-IB-UNESP (95/08 CEEA). Male Wistar rats were separated into control (C,n=8), stress (S,n=8), high fat diet (HFD=8) and stress/high fat diet (S/HFD,n=8) groups. C and S received a standard rat chow while HFD and S/HFD received a high-fat diet (4 flavors: vanilla, cheese, chocolate and bacon) for 15 weeks. Stress was immobilization 1h/day, 5 days/week during 15 weeks. Body weight (BW) and blood pressure (BP in mmHg) were measured once a week. After 15 weeks, the rats were killed and the index of adiposity (IA) was determined as follows: [epididymal fat+retroperitoneal fat+visceral fat]/[total body weight-sum of all adipose depots]x100. Concentration-response curves to noradrenaline (NA) were obtained in the absence and presence of acute inhibition of nitric oxide synthase (L-NAME, 3x10-4 M) in intact and denuded thoracic aorta of C, S, HFD and S/HFD rats. **Results:** Final BW and IA were not altered by stress but were increased after high fat diet isolated: BW(g): C 433 ± 40; S 435 ± 42; HFD 499 ± 38‡ and S/HFD 430 ± 36. IA (%): C 3,9 ± 1,1; S 4,0 ± 1,6; HFD 6,8 ± 1,9‡ and S/HFD 4,1 ± 1,3. Changes in BP was only observed in stress condition both isolated and associated (C 121 ± 7; S 154 ± 12†; HFD 128 ± 4 and S/HFD 155 ± 12†). Stress and HFD isolated induced a hyporreactivity to noradrenaline characterized by a decrease in aorta reactivity dependent on endothelium integrity. However, this effect was not potentiated by the association of these conditions. The presence of L-NAME abolished this reactivity alteration. Intact aortas [maximum response (g of tension): C 2,8 ± 0,2; S 0,9 ± 0,1*;HFD 1,7 ± 0,3* and S/HFD1,0 ± 0,1*, C/L-NAME 4,3 ± 0,4*#; S/L-NAME 4,5 ± 0,3#; HFD/L-NAME 4,0 ± 0,2*# and S/HFD4,5 ± 0,3#]. ‡P<0.05 related to C, S and S/HFD, †P<0.05 related to the respective non stressed group, *P<0.05 related to C, #P<0.05 related to the respective group in the absence of L-NAME. None of the procedures altered the response to NA in denuded aorta. **Conclusion:** These findings suggest that the aorta adaptive response to both chronic stress and HFD, individually or in association, involves an increased release and/or production of endothelial NO. The present study advances in the mechanisms of vascular adaptation in high fat-diet associated to stress and could lead to therapeutic advantages against cardiovascular diseases in this condition. Support: FAPESP

06.057

Comparative study of effects hemodynamic of diabetic cardiomyopathy is induced by L-name in rats. Gazzoto AF[1], Pereira DJ[1], Pires NF[1], Moreira MM[2], Santos RC[1], Figueiredo VN[2], Renno AL[3], Quinaglia TSS[2], Ludovico ND[2], Moreno Junior H[4] [1]UNICAMP − Farmacologia, [2]UNICAMP − Farmacologia cardiovascular, [3]UNICAMP − Farmacologia Bioquímica, [4]UNICAMP

Nitric oxide (NO) is a biological mediator that acts as a key molecule in many pathophysiological processes such as regulation of vascular tone, neurotransmission, learning, memory, and others. Because of its importance and involvement in physiological and pathological mechanisms, its regulation and synthesis have been extensively studied. Inhibition of NO by chronic oral administration of the nonspecific inhibitor of NO-synthase, the nitro-L-arginine methyl ester (L-NAME) results in hypertensive cardiomyopathy in rats. The renovascular hypertension [two kidneys and clip (2K1C)] and diabetes mellitus (DM) associated induce morphological abnormalities similar to those described in the model of cardiomyopathy induced by L-NAME in rats, but its hemodynamic effects are still controversial. This study evaluated the cardiac and vascular function in these two models of cardiomyopathy after eight weeks of treatment. We evaluate the following parameters: mean arterial pressure, cardiac output, peripheral vascular resistance, positive and negative derivar pressure in isolated heart. In all groups were observed increased mean arterial pressure, peripheral vascular resistance and reduced cardiac output after eight weeks in the control group. Wistar rats were divided into the following groups: Control; L-NAME: 60mg/kg/day; 2K1C+DM: streptozotocin (60 mg/kg) and one renal artery clipped. The following parameters were measured: mean arterial pressure, heart rate, cardiac output and peripheral vascular resistance. Positive and negative deriver pressure was also evaluated in an isolated heart. L-NAME and 2K1C+DM groups had increased mean arterial pressure (175.4 ± 29.7 and 158.7 ± 16.7 mmHg, respectively) and reduced cardiac output after the 8th week. Pressure vascular resistance was increased in both groups. A decrease in positive deriver pressure/deriver time was found in the 2K1C+DM (1895 ± 98 mmHg/s, $p<0.05$) vs. Control group (2534 ± 120 mmHg/s, $p< 0.05$). Negative deriver pressure/deriver time was decreased in the L-NAME and 2K1C+DM groups vs. Control group (1490 ± 104 and 1460 ± 94 mmHg/s, respectively vs. 2080 ± 92 mmHg/s, $p<0.05$). Decrease in positive deriver pressure/deriver time 2R1C+DM group compared to Control. The negative deriver pressure/deriver time decreased in L-NAME and 2R1C+DM groups. This study demonstrated that despite the morphological similarities between 2K1C+DM and L-NAME groups; the L-NAME group did not mimics the hemodynamic changes in hypertension-diabetic-renovascular model associated in rats.

06.058

Effect of potassium-sparing diuretics on rat corpus cavernosum smooth muscle reactivity. Claudino MA[1], Silva FH[1], Franco-Penteado CF[2], Takeshi FI[1], Lopes AG[3], Antunes E[1], Nucci G[1] [1]UNICAMP − Pharmacology, [2]UNICAMP − Hemocentro, [3]IBCCF-UFRJ − Fisiologia Renal

Introduction: Diuretics have been associated with sexual dysfunction in men, including decreased libido, difficult ejaculation and impotence, but these adverse mechanisms remain poorly understood. This study aimed to determine the effects of potassium-sparing diuretics on rat corpus cavernosum (RCC) smooth muscle reactivity. **Methods:** RCC was isolated and concentration-responses curves to phenylephrine (PE; 0.1–100μM) and potassium chloride (KCl; 1-300mM) were obtained in the absence or presence of amiloride (AMI), hexamethylamiloride (HMA) and benzamil (BEN; 10 and 100μM, respect.). Contractile responses induced by electrical-field stimulator (EFS) were also studied in the presence of potassium-sparing diuretics. Relaxant responses to AMI (0.01-100μM) were obtained in the absence and presence of L-NAME (100μM) or indomethacin (6μM). Concentration–response curves to CaCl2 (0.1-30mM) were constructed in the absence or presence of AMI (10 and 100μM). Gene expressions of potassium-sparing diuretic target proteins were also determined in RCC (COBEA 1595-1). **Results:** PE and KCl produced concentration-dependent contraction in the RCC. AMI and HMA (10μM) did not alter potency (pEC50) for PE (5.37 ± 0.03; 5.36 ± 0.02; respect.), compared to control (5.31 ± 0.01), but BEN (10μM) significantly reduced pEC50 to PE (3.38 ± 0.48). However, the maximal response (Emax) evoked to PE (3.65 ± 0.39mN) was significantly reduced by AMI (2.48 ± 0.29mN), HMA (2.21 ± 0.41mN) and BEZ (1.37 ± 0.11mN). Moreover, a higher concentration of AMI significantly reduced both pEC50 (1.23 ± 0.35) and Emax to PE (4.97 ± 0.03mM). HMA and BEN (100μM) almost abolished the PE-induced contraction. Both the pEC50 and Emax in the contractile responses to KCl (1.30 ± 0.03; 2.12 ± 0.25mN; respect.) were significantly reduced in the presence of 10μM of AMI and BEN (1.05 ± 0.02; 0.85 ± 0.16mN/ 1.04 ± 0.02; 0.57 ± 0.15mN, respect.), but HMA caused no change in the pEC50 (1.35 ± 0.05) and Emax (1.47 ± 0.31mN) to KCl. However, AMI, HMA and BEN, at higher concentrations, promoted significant reductions in both pEC50 (1.21 ± 0.06; 0.66 ± 0.31; 0.87 ± 0.12, respect.) and Emax to KCl (0.35 ± 0.01; 0.36 ± 0.06 and 0.47 ± 0.26mN; respect.). EFS-induced contraction responses were significantly reduced by both concentrations of AMI, HMA and BEN. AMI produced concentration-dependent relaxation responses in the RCC. Both the pEC50 (5.51 ± 0.23) and Emax (89 ± 4%) were significantly reduced by L-NAME (4.70 ± 0.29; 59 ± 7%, respect.), however, indomethacin did not alter AMI-induced relaxation. CaCl2 produced concentration-dependent contractile responses in the RCC and the pEC50 (2.35 ± 0.03) and Emax (3.06 ± 0.54mN), however these were not altered by AMI (10μM; 2.43 ± 0.01; 2.80 ± 0.15mN, respect.). Conversely, a higher concentration of AMI induced significant reductions both in pEC50 (2.08 ± 0.03) and Emax (0.54 ± 0.10mM). Real time PCR demonstrated, for the first time, the presence of genes encoding the Na+/H+ pump, epithelial Na+ channel and Na+/Ca+ exchange protein in RCC. **Conclusion:** Findings indicate that the targets of potassium-sparing diuretics are expressed in RCC, moreover, this diuretic class induced a reduction in contractile mechanism and a relaxation response in RCC, possibly involving changes in the pHi and [Ca2+]i. FAPESP/CNPq.

06.059

Cytotoxic effect of *Bothrops pirajai* on MDCK cells. Marinho AD[1], Jorge RJB[2], Barbosa JPC[3], Abreu ML[3], Rocha VHP[1], Morais ICO[2], Menezes RRPPB[4], Morais GB[5], Sampaio AM[8], Alves RS[2], Jorge ARC[2], Ximenes RM[2], Toyama MH[6], Martins AMC[4], Evangelista JSAM[7], Monteiro HSA[2] [1]UFC − Farmácia, [2]UFC − Fisiologia e Farmacologia, [3]UNIFOR − Fisiologia e Farmacologia, [4]UFC − Análises Clínicas e Toxicológicas, [5]UECE − Faculdade de Veterinária, [6]UNICAMP − Instituto de Biologia

Introduction: Snake venoms cause a variety of different biological effects as they are a mixture of simple and complex substances, such as biologically active peptides and proteins (DOLEY R; KINI RM, Cell Mol Life Sci. v.66, p.2851, 2009). *Bothrops pirajai* is an endemic species of southern Bahia State, Northeast Brazil, with its poison is still little studied, with effects known mitóxico, neurotoxic and changes in renal parameters.The aim of this work was study the cytotoxic effect of the venom of *B. pirajai* (VBpi) on renal tubular cells Mardin-Darby Canine Kidney (MDCK). **Methods:** The MDCK cells were cultured in RPMI medium supplemented with 10% fetal bovine serum and penicillin / streptomycin, and incubated in a 5% CO2 at 37 °C for 3-5 days until reaching confluence state, and it was kept always aseptic conditions. Then, they were displaced with trypsin-EDTA (0.05%/ 0.02%), counted in a Neubauer chamber and plated to 1x105 cells/mL in 96-well plates. After 24h of incubation, the plate was washed with PBS and different concentrations (100, 50, 25, 12.5, 6.25 and 3.125µg/mL) of VBp was added. Then, it was added to 10µL of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (2.5 mg/mL), and 4h later, 90µL of sodium dodecyl sulfate (SDS) at 10% was also added. The spectrophotometric reading at 570nm was performed 17h later. As a negative control PBS was used. The results were expressed as percent viability ± SEM in the control group. Three experiments were conducted in triplicate, and data were analyzed with GraphPad Prism 5.0, by ANOVA with Dunnet post-test, with significance at $p < 0.05$. To determine the IC50 it was used the method of nonlinear regression. The experimental protocols were approved by the Federal University of Ceará Animal Research Ethical Committee, license number of 68/08. **Results and Discussion:** The venom of *B. pirajai* (VBpi) was able to decrease cell viability under the study conditions, by showing cytotoxicity effect up to 3.12 mg/mL (IC50 = 1.42 mg / mL), and promoting an inhibition on the cell growth in a dependent-concentration. Other studies, with fractions of VBpi will be conducted to ascertain which is the fraction involved in the role of the toxic effects in MDCK culture cells and also to investigate the mechanisms involved in cell death. **Financial support:** CNPq; CAPES and FUNCAP.

06.060

Inhibition of MMP decreases mortality and right ventricular damage caused by acute pulmonary embolism in rats. Cau SBA[1], Barato RC[1], Gerlach RF[2], Tanus-Santos JE[1] − [1]FMRP-USP − Farmacologia, [2]FORP-USP − Morfologia

Introduction: Right ventricular failure is the major cause of mortality following acute pulmonary embolism (APE). Matrix metalloproteinases (MMP) were associated with the cardiovascular damage caused by APE. We investigated the effects of doxycycline, a MMPs inhibitor, on APE–induced 24–hr mortality rate and right ventricular damage in rats. **Methods:** All animal experiments were approved by Ethical Commission of Ethics in Animal Research (CETEA – FMRP/USP), protocol number 125/2007. Rats were randomly divided into four experimental groups: intraperitoneal (I.P.) vehicle administration 30 minutes before intravenous administration of saline (Vehicle + Control group) or a suspension of microspheres (21 mg/Kg) into the tail vein (Vehicle + APE group); doxycycline (30 mg/Kg; I.P.) administration 30 minutes before intravenous administration of saline (Doxy + Control group) or a suspension of microspheres into the tail vein (Doxy + APE group). All groups were followed up for 24 hrs. After 24 hrs, the surviving animals were killed and right ventricle (RV) was saved for biochemical dosages. **Results and Discussion:** Pretreatment with doxycycline was associated with a significant increase in 24–hr survival rate after APE (27.5% in Vehicle + APE group and 50% in Doxy + APE group; $p<0.05$). Gelatin zymography was performed. The 92 KDa–MMP-9 was not detected in RV from nonembolized animals while in embolized rats we found high levels (Vehicle + APE group: 0.33 ± 0.06 arbitrary units - A.U.; Doxy + APE group: 0.36 ± 0.08 A.U.; both $p<0.05$. The in situ gelatinolytic activity was increased in RV from animals of Vehicle + APE group (7.87 ± 1.12 A.U.; $p<0.05$) compared with Vehicle + Control group (4.09 ± 0.30 A.U.); however the treatment with doxycycline inhibited the increase in gelatinolytc activity in Doxy + APE group (4.09 ± 0.18 A.U.). Furthermore, doxycycline was effective in attenuating APE-induced increases in oxidative stress measured by a fluorimetric assay in right ventricular tissue (19.05 ± 1.04 A.U. in Vehicle + APE group to 28.34 ± 0.89 A.U. in Vehicle + APE group, $p<0.05$; to 22.62 ± 0.89 A.U. in Doxy + APE group). None of the evaluated parameters was significantly altered in Doxy + Control group compared with the Vehicle + Control group. Our results suggest that MMP inhibition increases 24-hr survival rate and blunt APE-induced increases in right ventricular MMP activity. **Financial support:** FAPESP, CNPq, CAPES.

06.061

Angiotensin (1-12) metabolism in cardiac perfusate of Wistar and spontaneously hypertensive rats. Becari C[1], Pereira HJV[2], Mesquita Jr O[1], Oliveira EB[1], Salgado MCO[1]
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Introduction: Identification of angiotensin (Ang) (1-12) as an intermediate precursor derived directly from angiotensinogen led us to explore whether the heart has the capacity to process Ang (1-12) into biologically active angiotensin peptides. **Methods:** The generation of Ang I, Ang II, Ang (1-9) and Ang (1-7) from Ang (1-12) was evaluated in cardiac perfusates of normotensive Wistar (NWR, n=4) and spontaneously hypertensive (SHR, n=4) rats. Hearts were recirculated with Krebs buffer for 2h, and proteolytic activities toward Ang (1-12) (30 nmol for 6 h at 37ºC) in cardiac perfusate were determined by C-18 HPLC analysis of the respective fragments formed in the absence or presence of inhibitors, such as the metalloendoproteinases inhibitor phosphoramidon (100 μM), the angiotensin converting enzyme (ACE) inhibitor captopril (10 μM), the serine protease inhibitor chymostatin (100 μM), and association of captopril and chymostatin. Expression of ACE and elastase-2 mRNA was determined by SYBR Green real time polymerase chain reaction (real time-PCR). **Results:** Ang I and Ang II were the major degradation products formed during incubation of Ang (1-12) with both coronary perfusates, although Ang (1-9) and Ang (1-7) were also recovered as minor products. The ACE inhibitor significantly decreased the formation of Ang I from Ang (1-12) (control vs. captopril: WNR: 2.18 ± 0.27 vs. 0.55 ± 0.22 nmol, *P<0.001 and SHR: 2.23 ± 0.29 vs. 0.35 ± 0.02 nmol, *P<0.001), but this inhibitor did not change the generation of others Ang (1-12) derived peptides. Interestingly, in the presence of chymostatin the formation of Ang II from Ang (1-12) was decreased only in SHR perfusates (control: 1.35 ± 0.2 vs. chymostatin: 0.7 ± 0.12 nmol, *P<0.05). Fosforamidon did not affect the metabolism of Ang (1-12) in cardiac perfusates of both groups. No difference in the expression of ACE mRNA was observed between hearts of WNR and SHR; however, elastase-2 mRNA expression was augmented in hearts of SHRs compared to that of normotensive rats. **Discussion:** These data demonstrate that Ang (1-12) is a substrate for the formation of Ang peptides, mainly Ang I and Ang II, by proteases from the rat heart. In addition, these data also show that a chymostatin-sensitive protease, most probably the rat elastase-2, contributed to generation of Ang II in the coronary perfusate obtained from SHR hearts. Supported by FAPESP.

06.062

Impaired Rho-A/Rho-kinase pathway contributes to vascular dysfunction in endotoxemic mouse. Corrêa T, da Silva-Santos JE UFSC − Farmacologia

Introduction: The Rho-A/Rho-kinase is responsible for Ca2+-sensitization in several tissues. Activation of Rho-kinase (ROCK) by Rho-A leads to phosphorylation and consequent inhibition of myosine phosphatase, enhancing muscle contraction. Increased expression and activation of this pathway has been widely associated with cardiovascular diseases, such as hypertension. Nevertheless, there are few studies regarding the role of Rho-A/Rho-kinase in the cardiovascular failure that takes place in septic conditions. We hypothesize that reduced functionality of this pathway may contribute to hypotension and vascular hyporeactivity to vasoconstrictors in septic shock. **Methods:** Male swiss mice (2-3 months) were injected with bacterial lipopolysaccharide (LPS 011-B4; 7.5 mg/kg, i.p.). Control animals received vehicle only (sterile saline, 0,1 ml/10g). At 6 or 18 h after LPS injection, the mice were anesthetized with ketamine/xylazine (100 and 15 mg/kg, i.m.), the femoral vein and carotid artery were cannulated using polyethylene (PE10) catheters. The venous access was used to inject heparin (2 IU) and all subsequent drugs. The arterial access was connected to a pressure transducer coupled to a PowerLab® system (ADI Instruments, Australia) allowing us to record both mean arterial pressure (MAP) and heart rate. After the surgical procedure, a resting period of 15 min was respected for blood pressure stabilization. LPS-treated and control mice (6 and 18 h groups) received a single injection of Y-27632 (0,1 mg/kg, i.v.), a ROCK inhibitor, or saline (100 µM). The effect of Y-27632 in MAP was recorded for 15 min. Then, the hypertensive effects of phenylephrine (PE; 3, 10 and 30 nmol/kg) or angiotensin II (AII; 3, 10 and 30 pmol/kg) were measured. In some experiments, a blood sample was collected and the plasmatic levels of nitrate + nitrite (NOx) were measured by means of Griess reaction. **Results and Discussion:** The animals treated with LPS 6 and 18 h before the experiments presented MAP of 76.5 ± 4.4 and 62.6 ± 6.9 mm Hg, respectively (MAP in control mice was 93.7 ± 3.9 mm Hg). Responsiveness to PE was also significantly reduced in LPS-treated mice (eg. the dose of 30 nmol/kg of PE increased MAP by 48.1 ± 4.8 mm Hg in control, and 21.6 ± 4.6 and 27.4 ± 5.2 mm Hg in LPS 6 and 18 h, respectively) ($p < 0.05$). In addition, plasmatic levels of NOx, an indicative of nitric oxide production, were substantially increased in plasma samples obtained both 6 and 18 h after LPS. Taken together, these data confirm the endotoxemic state induced by LPS administration. The hypotensive effect of Y-27632 was enhanced 6 h after LPS injection, but reduced at 18 h, when compared to control. The treatment with Y-27632 did not change the hypertensive effect of PE in control and LPS 6 h groups, but reduced it by ≈ 60% in LPS 18 h group (MAP increased 27.4 ± 5.2 and 10.7 ± 4.6 mm Hg, in the absence and presence of Y-27632 treatment, respectively). Similarly, the treatment with Y-27632 decreased the length of hypertensive effects of AII in LPS 18 groups, but had no effect in nonendotoxemic (control) mice. These data suggest that functionality of ROCK in vascular contraction under septic conditions is impaired. **Conclusion:** Hypotension and vascular hyporeactivity to vasoconstrictors in septic shock remain a clinically untreatable situation. Our data display, for the first time in vivo, that changes in Rho-A/Rho-kinase pathway may play an important role in this process. **Financial support:** CNPq (482214/2007-4).

06.063

Protein Disulfide Isomerase regulation of NADPH oxidase activity in vascular smooth muscle cells: effects on Angiotensin II redox signaling in hypertension. Camargo LL[1], Androwiki ACD[1], Ceravolo GS[1], Denadai-Souza A[1], Muscará MN[1], Carvalho MHC[1], Fortes ZB[1], Janiszewski M[2], Lopes LR[1] [1]USP − Farmacologia, [2]Hospital Israelita Albert Einstein − IEP

Introduction: The redox chaperone protein disulfide isomerase (PDI) was recently identified as a regulator of NADPH oxidase dependent ROS generation in VSMCs. However, the role of PDI in oxidative stress and in vascular alterations observed in hypertension is unknown. The aim of the present study was to investigate the role of PDI in ROS generation and contractile response to Ang II in the resistance vessels of SHR rats. **Methods:** The effect of inhibition of PDI in the modulation of vascular reactivity to Ang II was evaluated in isolated mesenteric arteriolar bed from SHR and Wistar rats (12-16 weeks-old). Preparations were treated with the PDI inhibitor, bacitracin (0.5mM), and the antioxidant, apocynin (300μm). PDI and Nox1 expression was assessed by immunohistochemistry and western blotting (WB) in mesenteric arteries from Wistar and SHR rats. To further investigate the mechanisms involved, VSMCs isolated from these arteries were cultured. Ang II-induced (10-7M) ROS production, c-Src activation and intracellular free calcium concentration ([Ca2+]i) were measured by dihydroethidium derived fluorescence (DHE, 5 μM), WB and fluo-3AM fluorescence, respectively. Experiments were done in the presence of the bacitracin (0.5 mM), antibody against PDI (anti-PDI, 1:1000), apocynin (30μM) or the thiol oxidant dithionitrobenzoic acid (DTNB; 0.5mM). **Results:** Vasoconstrictor responses to Ang II were increased in SHR (118.9 ± 3.92) as compared to Wistar rats (100.5 ± 6.75; $P<0.05$). PDI inhibition with bacitracin substantially suppressed contraction by approximately 44 % in Wistar (56.5 ± 9.33; $P<0.01$, n=6) and 62% in SHR (44.51 ± 8.79; $P<0.001$, n=5). Apocynin also reduced Ang II induced contraction in 30% in Wistar (72.79 ± 7.05; $P<0.05$; n=5) and in 41% in SHR (68.9 ± 8.55, $P<0.001$ n=6). Imunohistochemistry of the mesenteric arteries revealed an increased expression of PDI and NOX1 in SHR as compared to Wistar rats. The increase in PDI expression in SHR was confirmed by WB in mesenteric arteries (150%, $P< 0.05$, n=7) and in VSMCs (150% increase, $P< 0.05$, n=3) as compared to Wistar rats. PDI inhibition completely abolished Ang II dependent ROS generation and significantly reduced c-Src phosphorylation (Tyr 416) in both Wistar (169.0 ± 23.58 vs 93.3 ± 22.8, $P<0.01$, n=4) and SHR cells (249.7 ± 25.3 vs 66.6 ± 29.9, $P<0.001$, n=3). Furthermore, PDI inhibition resulted in a reduction of Ang II- induced [Ca2+]i responses in Wistar cells treated with bacitracin (33%, $P<0.001$) or anti-PDI antibody (31%, $P<0.001$). This inhibition was substantially increased in SHR cells, where bacitracin and anti-PDI reduced the [Ca2+]i responses in 62% and 58% ($P<0.001$). The Src inhibitor, PP2, also reduced the Ang II elicited [Ca2+]i responses in 20% in Wistar cells ($P<0.01$) and in 57% in SHR VSMCs ($P<0.001$). **Discussion:** Altogether our results show that PDI inhibition reduces Ang II mesenteric vasoconstriction which could be related to a decrease in ROS production leading to decrease in c-Src phosphorylation and [Ca2+]i responses in VSMC from Wistar and SHR rats. Therefore, PDI could play a role as an important regulator of NADPH oxidase in resistance arteries. Furthermore, the increase in PDI expression in SHR suggests that this protein could be a new player in the oxidative stress and vascular dysfunction associated to hypertension.

06.064

Endothelium potentiates vasodilator effect of nitric oxide donor with gold nanoparticles in aortas from normotensive but not from renal hypertensive rats. Silva BR[1], Vercesi J. A[2], Moraes JB[2], Silva RS[2], Bendhack LM[2] [1]FMRP-USP − Farmacologia, [2]FCFRP-USP − Física e Química

Introduction: Nitric Oxide (NO) plays an important role in the control of the vascular tone. NO can be produced by NO-synthase activation or it can be released from NO donors. It has been described that vascular endothelium can modulate the vasodilatation induced by some NO donors. The present study aimed to investigate the relaxation induced by the complex AuNPs-{Ru-4PySH}n in aortas from normotensive (2K) and renal hypertensive (2K-1C) rats and to investigate the role of the endothelial contribution to the contractile response induced by phenylephrine (PHE) and relaxation induced by the NO donor. **Methods:** Initially, to evaluate the influence of the contractile agent on the vasodilator effect of the NO donor, we performed cumulative concentration-effect curves for PHE in 2K and 2K-1C aortic rings with or without endothelium. After that, cumulative concentration-effect curves for AuNPs-{Ru-4PySH}n were constructed in 2K and 2K-1C aortic rings with or without endothelium pre-contracted with 100nM PHE. We have analyzed the efficacy (Emax) and potency (pD2) of the phenylephrine in inducing contraction (grams of tension) and of the AuNPs-{Ru-4PySH}n in inducing vasodilatation represented as percentage of PHE contractile response reversal. The experimental procedures were approved by the Ethics Committee of the University of São Paulo (N° 156/2009). RESULTS AND **Discussion:** PHE induced similar contraction in normotensive and renal hypertensive rats denuded aortic rings (2K Emax: 2.5 ± 0.2g; pD2: 7.93 ± 0.10; n=8 and 2K-1C Emax: 2.6 ± 0.2g; pD2: 7.89 ± 0.08; n=9). However, in intact endothelium, PHE was less potent in 2K and 2K-1C aortic rings than in denuded aortas, while the maximum effect was reduced only in hypertensive rats rings (2K/ E+ Emax: 2.2 ± 0.1g; pD2: 7.44 ± 0.03; n=5 and 2K-1C/E+ Emax: 1.2 ± 0.2g; pD2: 7.54 ± 0.06; n=7; $p<0.001$). In addition, in intact endothelium aortic rings, the Emax to phenylephrine was higher in 2K than in 2K-1C. The complex AuNPs-{Ru-4PySH}n induced vasodilatation in a concentration-dependent way in 2K and 2K-1C aortic rings with or without endothelium. In denuded aortic rings the vasodilatation induced by AuNPs-{Ru-4PySH}n was more potent in hypertensive (Emax: 110.9 ± 6.3%; pD2: 6.29 ± 0.04; n=4; $p<0.05$) than in normotensive (Emax: 99.1 ± 1.0%; pD2: 6.10 ± 0.04; n=4). In 2K aortic rings the presence of the endothelium potentiated the vasodilation induced by AuNPs-{Ru-4PySH}n (Emax: 97.4 ± 2.9%; pD2: 6.24 ± 0.02; n=4; $p<0.05$), but the endothelium had no effect in the vasodilatation induced by AuNPs-{Ru-4PySH}n in 2K-1C aortic rings. Our results demonstrated that although PHE induced similar contraction in normotensive and renal hypertensive rat aortic rings without endothelium, the vasodilatation induced by AuNPs-{Ru-4PySH}n was more potent in hypertensive than in normotensive rat aortic rings. Our results suggest that the endothelium negatively modulates the contractile response to PHE in normotensive and hypertensive rat aorta. It seems that the endothelium potentiates the vasodilatation induced by AuNPs-{Ru-4PySH}n in normotensive, but not in hypertensive rat aortic rings. Supported by FAPESP, CAPES and CNPq.

06.065

Vascular response in acute lung injury induced by paraquat. Aires RD[1], Capettini LSA[1], Pinho JF[2], Côrtes SF[3], Pinho V[4], Lemos VS[1] [1]UFMG − Fisiologia e Biofísica, [2]UFMG − Fisiologia e Farmacologia, [3]UFMG − Farmacologia, [4]UFMG − Bioquímica e Imunologia

Introduction: Paraquat (PQ) is a toxic herbicide that induces acute lung injury (ALI) and pulmonary hypertension in humans and animals. Although vascular disorders are present and contribute to increased mortality in ALI patients, there is little data available on vascular responsiveness after accidental or intentional exposure to paraquat. The present study aimed to investigating the vascular response of the rat aorta in rats treated with a dose of paraquat that induces ALI. **Methods:** 10-12 weeks old Wistar rats received an i.p. injection of PQ (20 mg/Kg) or vehicle (saline). Changes in isometric tension in rat aorta were recorded on a myograph. Concentration response curves to phenylephrine and acetylcholine were performed in the presence or absence of endothelium. Inhibitors of different isoforms of nitric oxide synthase (NOS) were used when necessary. All experimental protocols were approved by the animal ethics committee of Federal University of Minas Gerais (protocol # 051/08). Results and discussion: PQ-induced ALI did not alter the relaxant response of aortic rings to acetylcholine (Emax= 95,62 ± 3,57% and 95,82 ± 1,54%, control and paraquat, respectively), but greatly diminished contractile response to phenyleprine (Emax= 11,20 ± 0,2579 mN/mm and 6,57 ± 0,2631 mN/mm, control and paraquat respectively). Endothelium removal (Emax=12,33 ± 0,3585 mN/mm and 12,15 ± 0,5066 mN/mm, control and paraquat, respectively) or pre treatment of aortic rings with the non selective NOS inhibitor L-NAME (300 µM) restored contraction of aortic from PQ poisoned rats to the same level as those not exposed to paraquat (Emax= 14,13 ± 1,248 mN/mm and 12,99 ± 0,8749 mN/mm, control+L-NAME and paraquat+L-NAME, respectively), indicating a role for nitric oxide produced by endothelial cells. Pre-treatment with the selective inducible nitric oxide syntase (iNOS) inhibitor L-NIL (10 µM) also restored the impaired contraction (Emax= 11,38 ± 0,6153 mN/mm and 11,97 ± 0,5162 mN/mm, control+L-NIL and paraquat L-NIL, respectively) suggesting a role for iNOS. **Conclusion:** Our findings suggest that paraquat-induced ALI enhances endothelial iNOS-derived nitric oxide production decreasing responsiveness of aorta to vasoconstrictors. Support: FAPEMIG/Capes/CNPq.

06.066

Expression of protein disulfide isomerase is associated with increased reactive oxygen species generation in target organs in hypertension: possible interaction with NADPH oxidase. Androwiki ACD[1], Camargo LL[1], Dias AA[1], Janiszewski M[2], Lopes LR[1] [1]ICB-USP − Farmacologia, [2]Hospital Israelita Albert Einstein − IEP

Introduction: Reactive oxygen species (ROS) are important players in the pathophysiology of cardiovascular diseases such as hypertension. Although several sources of ROS may be involved, a family of NADPH oxidases appears to be the primary source of ROS in cardiovascular system and especially important for redox signaling processes. Growing evidence shows an increased activity and protein expression of NADPH oxidase isoforms, NOX1, NOX2 and NOX4 in heart, kidney and lung during hypertension. Nevertheless, the mechanisms that regulate oxidase-mediated ROS generation in the cardiovascular system are incompletely understood. We recently demonstrated that protein disulfide isomerase (PDI), a dithiol disulfide oxidoreductase chaperone from the endoplasmic reticulum, modulates NADPH oxidase dependent ROS generation in vascular smooth muscle cells (VSMCs). However, the role of PDI in NADPH oxidase ROS generation in hypertension remains unknown. The aim of the present study was to investigate the role of PDI in NADPH oxidase derived ROS generation in mesenteric arteries, aorta, heart, kidneys and lungs of spontaneously hypertensive rats (SHR). **Methods:** Samples of isolated resistance mesenteric arteries, aorta, heart, kidneys and lungs, were isolated from SHR and Wistar rats. Liver tissue which is known to have high expression of PDI and is not a target organ in hypertension, was used as an experimental control. ROS production was assessed by dihydroethidium derived fluorescence (DHE, 5 μM). The expression of PDI and NOX isoforms was analysed by Western Blot and immunohistochemistry. **Results:** PDI expression was increased in the heart (138%; $P<0.05$, n=6), lungs (158%; $P<0.01$, n=6) and mesenteric arteries (150%; $P<0.05$, n=7) from SHR as compared to Wistar rats. These organs also presented higher ROS generation. In contrast, aorta and kidney showed no difference in PDI expression in both strains. However, interestingly, the generation of ROS in the kidney and aorta was significantly higher in SHR as compared to Wistar rats. **Discussion:** Results show an increase in PDI expression and ROS generation in hypertension target organs in SHR animals, which can indicate an association between PDI and NOX dependent ROS generation. Furthermore, this interaction is observed in the heart, lungs and mesenteric arteries which have mostly NOX 1 and NOX 2 but not in the in the kidney which contains mostly NOX 4, suggesting that PDI interaction is dependent on the NOX isoform. Additionally, the fact that SHR aortas have a similar PDI expression as Wistar suggests that PDI and NOX interaction in hypertension is dependent on the vascular bed. In summary, our results are indicative of a novel role for PDI in oxidative stress and possibly functional alterations in different target organs in hypertension.

06.067

Comparison of the action mechanisms of sodium nitroprusside and $[Ru(terpy)(bdq)NO^{+}]^{3+}$ in normotensive and hypertensive rats (SHR). Munhoz, FC[1], Pereira AC[2], Bonaventura D[2], Bendhack LM[2], Antoniali C[1] [1]FOA-UNESP − Ciências Básicas, [2]FCFRP-USP − Física e Química

Introduction: The aim of the present work was to compare the vasorelaxation induced by sodium nitroprusside (SNP) and the new NO donor [Ru(terpy)(bdq)NO+]3+ (TERPY) in aortic rings of Spontaneously Hypertensive Rats (SHR) and normotensive wistar rats (control). **Methods:** All the experimental protocols were approved by the Animal Research Ethics Committee (CEEA) of School of Dentistry of Araçatuba (proc. number 001776-2010). Aortic rings from SHR and control rats were connected to an isometric force transducer (Letica Scientific Instruments; Barcelona–Spain) to measure isometric tension in the vessels. The rings were placed in an organ chamber containing Kreb´s solution at pH 7.4, gassed with carbogen at 37°C. Aortic rings were initially stretched at a tension of 1.5 g for 60 min for stabilization. Denuded aortic rings from SHR (Systolic Pressure>150mmHg) and normotensive control were pre-contracted with phenylephrine and concentration-response curves for TERPY (1nM-100mM) and SNP (0.01nM-1uM) were constructed. In another set of experiments, the non-selective K+ channel blocker TEA (1mM) or the selective inhibitor of guanylyl-cyclase (sGC) (ODQ 1mM) were incubated for 30 min before performing the concentration-response curves. Potency (pD2) and efficacy (maximum relaxation, ME) of the vasodilator effects of SNP and TERPY were compared between SHR and control rats. Differences were considered significant when $P<0.05$. **Results and Discussion:** The present data shows that SNP is more potent than TERPY in inducing vasorelaxation of rat aortic rings. The maximum effect of vasorelaxation induced by SNP was similar in control aortic rings (ME:105.9 ± 3.3%; n=6) and in SHR (ME:118.8 ± 7.3%; n=6, $P<0.05$). However, the potency of SNP was lower in control (pD2: 7.97 ± 0.07; n=6) than in SHR (pD2: 8.74 ± 0.15; n=6, $P<0.05$) aortic rings. On the other hand, the vasodilator effect evoked by TERPY was not different between control (ME: 103.5 ± 1.1%, pD2: 6.60 ± 0.08, n=8) and SHR (ME:106.3 ± 4.8%, pD2: 6.25 ± 0.07, n=5). Blockade of K+ channels with TEA reduced the relaxation induced by SNP and TERPY in control rat aorta (SNP + TEA, ME: 98.7 ± 0.4%, n=14; pD2: 7,29 ± 0,08; n=5; TERPY + TEA, ME: 98.7 ± 1.4%; n=14; pD2: 5.39 ± 0.10; n=14) but it did not change the relaxation induced by both NO donors in SHR aortic rings. Inhibition of GCs with ODQ reduced the aortic relaxation induced by SNP in control (ME: 36.1 ± 10.8%, pD2: 6.22 ± 0.20, n=8) and in SHR aortic rings (ME: 90.4 ± 3.1%, pD2: 7.18 ± 0.13, n=5). Therefore, ODQ almost abolished the relaxation induced by SNP in control aortic rings. On the other hand, ODQ reduced the potency of TERPY in a similar way in control rat aortic rings (pD2: 4.89 ± 0.08) and SHR aortic rings (pD2: 4.90 ± 0.40). In summary, in normotensive control rat aorta the vasodilatation induced by TERPY and SNP is due to K+ channels and sGC activation. Otherwise, SNP and TERPY do not activate K+ channels in hypertensive rat aorta. The inhibition of sGC abolishes the relaxation induced by SNP in normotensive but not in hypertensive rat aorta. Supported by CNPq and FAPESP.

06.068

Protein disulfide isomerase regulates reactive oxygen species generation and vascular reactivity to angiotensin II in rat aorta. Dias AA[1], Camargo LL[2], Ceravolo GS[1], Androwiki ACD[1], Carvalho, MHC[1], Laurindo FRM[2], Janiszewski M[3], Lopes LR[1] [1]ICB-USP − Farmacologia, [2]InCor-HC-FMUSP, [3]Hospital Israelita Albert Einstein − IEP

Introduction: Angiotensin II (Ang II) reactive oxygen species (ROS) generation is involved in the modulation of vascular resistance. NADPH oxidase is a major source of ROS in the vascular system and consists of membrane-bound subunits (NOX and p22phox) and cytosolic subunits (p40phox, p47phox e p67phox). We previously demonstrated that the redox chaperone of the endoplasmic reticulum protein disulfide isomerase (PDI) regulates NADPH oxidase dependent ROS generation in aortic vascular smooth muscle cells. PDI also closely associates with the catalytic subunit of the vascular NADPH oxidase (NOX). More recently we showed that PDI overexpression in aortic VSMCs leads to spontaneous, AngII independent NADPH oxidase activation. However, the mechanisms through which PDI regulates NADPH oxidase activity and its role in Ang II redox dependent processes such as contraction remain unknown. Thus, the goal of the present study was to investigate the role of PDI in ROS generation and vascular reactivity to Ang II in rat aorta. MATERIAL AND METHODS PDI localization was analyzed by immunohistochemistry. Rats were anesthetized and rings from the thoracic aorta (~4mm in length), free of fat and connective tissue, were mounted in an isolated tissue chamber containing Krebs-Henseleit solution and cumulative curve-effect (CCE) to AngII (1nM to 1mM) were obtained in aortic rings with (E+) and without endothelium (E-). To analyze the role of PDI and ROS in Ang II response a PDI inhibitor, bacitracin (BAC; 1 mM, 15 min), antioxidant enzymes superoxide dismutase (SOD; 150U/ml, 30 min) and catalase (CAT; 300U/ml, 30 min) and NADPH oxidase inhibitor apocynin (APO; 30Mm, 30 min), was added to the aorta preparations before Ang II stimulation. ROS production in aorta rings from Wistar rats was assessed by analysis of dihydroethidium (DHE) derived fluorescence. RESULTS PDI expression was observed in intima, media and adventitia aorta layers. Ang II caused a dose-dependent increase in contraction in both E+ and E- aortic rings. The contraction induced by Ang II was reduced in the presence of the PDI inhibitor BAC in E+ (0,74 ± 0,04 vs 0,4 ± 0,03; $p<0.001$) and E- (1,6 ± 0,05 vs 0,9 ± 0,1;$p<0.001$) aortic rings. Since bacitracin inhibitory effect on Ang II response was endothelium-independent, E- rings were used in future experiments. Contractile response induced by Ang II was redox modulated, since it could be reduced by pre incubation with the NADPH oxidase inhibitor APO (1,0 ± 0,08; $p<0.001$), or the antioxidant enzymes SOD (0,94 ± 0,1; $p<0.001$) and CAT (1,16 ± 0,05; $p<0.01$) At the same time, bacitracin also reduced ANG II dependent ROS generation. **Discussion**: These results suggest that PDI modulates Ang II dependent ROS generation and vascular reactivity to Ang II in aortic rings. Therefore, PDI may be a new player in the regulation of vascular tone.

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06.069

Pharmacological characterization of the contractile response of the dorsal penile vein

Carioletti GH[1], Linder AE[2] [1]UFSC − Farmacologia, Centro de ciências Biológicas, [2]UFSC − Farmacologia

Introduction: Penile erection occurs by increasing the blood flow into the cavernosal sinoids and by restriction of the venous outflow from the penis. Venous occlusion is, therefore, an important mechanism to inhibit venous flow and thus maintaining the penis in the erectile state (Neves et. al., 2004). Thus the dorsal penile vein is connected to a number of factors related to erectile function. However, little is known about the reactivity of the penile veins. Our study aimed to evaluate the contractile response of the dorsal penile vein in rats stimulated with different agonists. **Methods:** Dorsal penile veins were isolated from Wistar rats, and endothelium-denuded rings from these vessels were placed in an isolated organ system, for measurements of isometric force (experimental protocol approved by the ethical committee PP00306). Concentration-effect curves induced by serotonin (5-hydroxytryptamine, 5-HT) (10-9M to 10-5M) were constructed in the absence and in the presence of the 5-HT2A/2C receptor antagonist, ketanserin (3x10-8M). Concentration-effect curves induced by phenylephrine were constructed in the absence and in the presence of the α1-adrenergic antagonist, prazosin (10-9M). Vehicle or antagonists were added 30 min before the start of the concentration-effect curves induced by the agonists. Preliminarily, the contribution of the endothelium to 5-HT-induced contraction was also evaluated. To this aim, the presence of the endothelium was assessed by the ability of acetylcholine (ACh) to relax penile dorsal vein rings contracted with 5-HT (10-7M). Tissues that relaxed more than 50% to ACh were considered as (partially) endothelium intact. Sensitivity to an agonist was expressed as pD2=-log EC50, where EC50 is the effective concentration of the agonist that induced 50% of maximal response and it was obtained by non linear regression. **Results:** Phenylephrine and 5-HT induced contractions dependent on the concentration in the dorsal penile vein. The concentration-effect curve induced by 5-HT in the presence of vehicle (pD2 6.57 ± 0.15, N=9) was shifted to right in the presence of ketanserin (pD2 4.37 ± 0.86, N=6, $P < 0.05$). The concentration-effect curve induced by phenylephrine in the presence of vehicle (pD2 6.15 ± 0.15, N= 6) was also rightward shifted in the presence of prazosin (pD2 5.14 ± 0.33, N= 5, $P < 0.05$). Preliminary data indicate that endothelium-intact dorsal penile vein rings were more sensitive to 5-HT (pD2 5.55, N=2) than those in which the endothelium had been removed. **Discussion:** These data suggest that the contractile response induced by 5-HT is mediated by 5-HT2A/2C receptor activation in the dorsal penile vein of rats. Furthermore, in this vessel, phenylephrine induces contraction mediated by α1-adrenergic receptors. Our data also suggest that endothelial factors modulate the contractile response induced by 5-HT in the dorsal penile vein of rats. **Conclusion:** We suggest the involvement of the 5-HT2A/2C and the α1-adrenergic receptors in the penile dorsal vein contraction with consequent contribution to venous occlusion and, thus to maintenance of penile erection. **References**: Neves G et al. Quim. Nova, 6: 949, 2004. This work was supported by FAPESC /CNPq (005/2009) and Funpesquisa.

06.070

The contribution of endothelial factors to serotonin-induced contraction in the rat jugular vein.

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Introduction: Serotonin (5-hydroxytryptamine; 5-HT) can be captured by peripheral veins in addition to interact with vascular receptors to alter muscle tone. mRNA expression for the 5-HT2A, 5-HT2B and 5-HT7 receptors were found in the rat jugular vein (Linder et al, 2010). Whereas the 5-HT2A receptor is involved in 5-HT-induced vascular contraction, it has been shown that 5-HT induces relaxation mediated by 5-HT2B and 5-HT7 receptors activation in an endothelium-dependent and -independent fashion, respectively (Watts and Cohen, 1999; Ishine et al., 2000). Therefore, 5-HT can activate receptors in both the endothelial and smooth muscle cells. We hypothesized that endothelial factors released by serotonergic receptor activation in the endothelium may be involved in the contractile response induced by 5-HT in the rat jugular vein. **Methods:** The contractile response induced by 5-HT in rings of the jugular vein of rats was measured by isometric tension recordings in isolated organ chamber (approved by the animal ethics committee PP00306). Concentration effect curves stimulated with 5-HT (10-9 to 10-5M) were performed in the absence and in the presence of the 5-HT2A/2C receptor antagonist (ketanserin 10-9 and 3x10-8M), and of the inhibitors of nitric oxide synthesis (L-NAME 10-4M) and prostanoid synthesis (indomethacin 10-5M). The response to 5-HT was assessed 30 minutes after the incubation with vehicle or with the compounds under study. The integrity of vascular endothelium was evaluated by the ability of acetylcholine (10-5M) to induce relaxation of the jugular vein rings contracted with 5-HT (10-7M). Only vessels that relaxed more than 65% to acetylcholine were considered in this study. The values were measured as mg of contraction and pD2=-log EC50, (where EC50 is the effective concentration of the agonist that induced 50% of maximal response) was obtained by linear regression. **Results:** 5-HT induced concentration dependent contractions of the rat jugular vein. The concentration effect curves induced by 5-HT (pD2 6.81 ± 0.16) were right-shifted in the presence of ketanserin 10-9M (pD2 5.84 ± 0.19 $P < 0.05$) and ketanserin 3x10-8M (pD2 3.75 ± 0.80 $P < 0.05$). The concentration effect curves to 5-HT were not altered in the presence of L-NAME (pD2 6.85 ± 0.13) and indometacin (pD2 6.72 ± 0.022). **Discussion:** These results support the 5-HT2A receptor as the receptor activated by 5-HT to induce contraction of the jugular vein from Wistar rats, as it has been proven to jugular vein from Sprange Dawley rats (Linder et. al. 2010). These results suggest that nitric oxide and prostanoids derived from the endothelium do not modulate 5-HT-induced contraction in the rat jugular vein. Whether 5-HT activates endothelial serotonergic receptors and, moreover, whether 5-HT is able to induce relaxation in the rat jugular vein are questions that remain to be answered. References: Linder, AE et al. J. Pharmacol. Exp. Ther., 2010, in press; Ishine, T et al. Am. J. Physiol. 278: H907, 2000; Watts, SW and Cohen, ML Neurotransmissions 15: 3, 1999. Supported by CNPq (PIBIC/CNPq-BIP/UFSC), FAPESC / CNPq (005/2009), Funpesquisa.

06.071

Hypothalamic obese and non-obese rats express a similar functional muscarinic M_3 subtype in the conductance artery. Scolaro LL[1], Oliveira JC[2], Ambiel CR[3], Mathias PC[2], Alves-do-Prado W[1] [1]UEM − Farmacologia, [2]UEM − Biologia Celular e Genética, [3]UEM − Ciências Fisiológicas

Introduction: Hypothalamic obese rats, obtained by neonatal treatment with monosodium L-glutamate (MSG) (Von Diemem et al. Acta Cir Bras, 21: 425, 2006), were used in the present study, which investigated whether the expected hypotensive effect is absent in MSG-obese rats because of a lower responsiveness of muscarinic receptors in the vascular tissue of these animals or because the muscarinic receptor subtype expressed in endothelial tissue in MSG-obese rats is functionally different from the M3 subtype found in non-obese animals. **Methods:** The Ethical Committee for Animal Experiments of the State University of Maringa approved the animal protocols (PRO 049/2009 CEAE). Neonate male Wistar rats were subcutaneously injected during the first 5 days of life with MSG (4 g/kg body weight) or equimolar saline solution. After obtaining the arterial pressure values (through polyethylene catheter (PE 10) into the femoral artery, the aortic ring preparation was mounted and isometric tension was recorded. **Results:** Mean arterial blood pressure in obese rats (90.0 ± 3.25 mmHg) was slightly lower than in non-obese rats (94.4 ± 1.06 mmHg) but was not significantly different ($p > 0.05$) from the control. Such data are similar to those previously reported (Tokarev et al. Physiol Res, 46: 165, 1997). The EC50 values for acetylcholine-induced relaxation in obese rats (2.27 ± 0.26 nM) and non-obese rats (2.37 ± 0.32 nM) were similar to the intact aortic ring preparations precontracted with norepinephrine. The non-selective blocker of muscarinic receptor Atropine, the selective antagonist for M3 muscarinic receptor 4-DAMP, and the selective antagonist for M1 muscarinic receptor pirenzepine induced similar parallel rightward displacements of the log [concentration]-response curve of acetylcholine-induced relaxation in preparations isolated from both types of animals. The pA2 values for atropine, 4-DAMP, and pirenzepine in obese (atropine: 10.1 ± 0.1, slope 1.2 ± 0.2; 4-DAMP: 9.2 ± 0.1, slope 1.2 ± 0.6; pirenzepine 7.3 ± 0.1, slope 1.1 ± 0.1) animals were not significantly ($p > 0.05$) different from controls (atropine: 10.0 ± 0.1, slope 0.9 ± 0.2; 4-DAMP: 9.2 ± 0.1, slope 0.9 ± 0.5; pirenzepine 7.4 ± 0.2, slope 1.2 ± 0.2). Methoctramine, a selective blocker of M2/M4 muscarinic receptors did not produce any effect on the log [concentration]-response curve for acetylcholine in obese and non-obese rats. **Conclusion:** These data suggest that mechanisms others than the functional activity of M3 receptors in conductance arteries in MSG-obese rats contribute to impair the reduction in arterial blood pressure in MSG-obese animals. **Financial support:** PRONEX

06.072

Phenylephrine relaxes rat jugular vein. De Prá MA, Linder AE UFSC − Farmacologia

Introduction: Studying the vascular reactivity in veins, we realized that phenylephrine, an α1-adrenergic agonist, causes relaxation of the rat jugular vein and contraction of the rat dorsal penile vein, both previously contracted with serotonin (5-hydroxytryptamine; 5-HT). According to the literature, this observed relaxation may be mediated by β-adrenergic receptors1,2. It has also been reported the involvement of α2-adrenergic receptors located on the endothelium from different vessels mediating vasodilation3. However, it is not entirely clear which are the receptors and the mechanisms of action responsible for the relaxation induced by phenylephrine. The aim of this study is to characterize the putative receptors and the possible mechanisms involved in phenylephrine-induced relaxation of the rat jugular vein. **Methods:** Jugular veins were isolated from Wistar rats and mounted in an organ isolated bath for isometric tension recording. The vessels were contracted with 5-HT (10-7 M) and a concentration-effect curve to phenylephrine (10-9M – 10-5M) was performed after the plateau of the contraction induced by 5-HT was reached. The responses were evaluated in the presence and absence of prazosin (10-8M), an α1-adrenergic antagonist. Similar experimental protocols were also performed in the dorsal penile vein for comparisons. Sensitivity to phenylephrine was expressed as pD2=-log EC50, where EC50 is the effective concentration of the agonist that induced 50% of maximal response and it was obtained by non linear regression. This experimental protocol was approved by the ethical committee PP00306. **Results:** 5-HT induced a contractile response in both the jugular and the dorsal penile veins. In these vessels contracted with 5-HT, whereas phenylephrine induced relaxation of the rat jugular vein, it caused additional contraction of the dorsal penile vein. In the rat jugular vein, no differences in the concentration-effect curves induced by phenylephrine were observed between those constructed in the absence (pD2 (vehicle) =6,59) and in the presence of prazosin (pD2 (prazosin)= 6,34; $P > 0,05$). Nevertheless, in the dorsal penile veins, the concentration-effect curves induced by phenylephrine was shifted to the right in the presence of prazosin (pD2 (prazosin)= 6,09 vs pD2 (veículo) = 4,97; $P < 0,05$). **Discussion:** These data suggest that the relaxation induced by phenylephine in the rat jugular vein involves an adrenergic receptor other than the α1-adrenergic receptor. On the other hand, the α1- adrenergic receptor mediates the contractile response induced by phenylephrine in the dorsal penile vein. Further studies will be performed to elucidate the receptors and mechanisms involved in this α1-adrenergic agonist-mediated relaxation of the rat jugular vein. References: 1) Cohen, ML et al. J. Pharmacol. Exp. Ther.,205: 400, 1978. 2) Duckles, SP et al. J. Pharmacol. Exp. Ther., 236: 71, 1985; 3) Rascado RR and Bendhack LM Vascul. Pharmacol. 42: 63, 2005. Supported by CNPq, FAPESC/CNPq (005/2009), Funpesquisa.

06.073

Blood renin activity and vasoactive peptides concentrations in rats with different angiotensin I converting enzyme (ACE) phenotypes. Peixoto HS, da Silva RM, Tanae MM, Souccar C, Lapa AJ, Lima-Landman MTR UNIFESP − Farmacologia

Introduction: Hypertension is a prevalent chronic disease with around 1 billion of hypertensive patients all over the world. Several genetics and environmental factors have been investigated and implicated in its genesis indicating the multifactorial and polygenic nature of the disease. The Renin-Angiotensin System (RAS) plays an important role in blood pressure control and specifically angiotensin I converting enzyme (ACE) polymorphisms have been implicated in this pathology (O'Donnell. Circ. vol 97, p1766. 1998). In previous work we have demonstrated that Wistar rats could be grouped, according to their plasmatic ACE phenotype, in animals with high (hACE), intermediate (iACE) and low (lACE) ACE activities (Ninauhaman et al, Phytomedicine. Vol 4, p 321. 2007). It was also verified that this phenotype has a clear heritance determination (De Oliveira SS. Dissertação (Mestrado), UNIFESP/EPM, 90 f, 2008) and, although being normotensive, they respond differently to ACE inhibitors (Oliveira SS. VII International Symposium of Vasoactive Peptides. Abstract P-28: 73, 2008). Objective: Considering the importance of the RAS in blood pressure control and the benefits of ACE inhibitors therapy, this work aimed to compare the plasmatic renin activity and the vasoactive peptides (Bradikinin – BK, Angiotensin I – Ang I, Angiotensin II – Ang II) concentrations in aged (14 months old), males (M) and females (F) rats with hACE and lACE phenotypes. **Methods:** The plasmatic renin activity was measured indirectly by radioimmunoassay (RVR-EX-125, Zen Tech S.A) and expressed in ng/mL/h. The concentration of the vasoactive peptides was determined by HPLC technique (Danser et al,. Hypertension. Vol. 24. p 37. 1994) and the results were expressed in µg/mL. All datas were expressed as mean ± standard error and compared by ANOVA followed by the Bonferroni test ($p<0.05$) (CEP 0049/10). **Results and Discussion:** The plasmatic renin activity did not differ in M and F rats with high and low ACE phenotypes (hACE = 174.1 ± 16.4, n=6; lACE = 254.8 ± 28.6, n=6 and hACE = 162.3 ± 26.5, n=6; lACE = 151.8 ± 10.9, n=6, respectively) as well as the plasmatic Ang I concentration (M: hACE = 0.451 ± 0.03, n= 11; lACE = 0.377 ± 0.02, n=10 and F: hACE = 0.560 ± 0.02, n=10; lACE = 0.479 ± 0.09, n=10). The BK concentration was not different between hACE and lACE phenotypes, but, higher in F than in M (M: hACE = 0.290 ± 0.07, n= 11; lACE = 0.304 ± 0.05, n=11 and F: hACE = 0.621 ± 0.04, n=8; lACE = 0.664 ± 0.05, n=11). In contrast, the Ang II levels were higher in M than in F (M: hACE = 0,073 ± 0,004, n=11; lACE = 0,069 ± 0,002, n=12 and F: hACE = 0,031 ± 0,002, n=8; lACE = 0,031 ± 0,003, n=12). Our results show that the difference in plasmatic ACE activity is not accompanied by changes in the blood concentration of vasoactive peptides. The balance between Ang II and BK, as well as other RAS components such as renin and Ang I concentrations is not affected by the ACE phenotype. The observed sex difference in Ang II and BK concentrations could not be attributed to the enzymatic activity of plasmatic ACE since it does not vary between sexes (Peixoto, IASH, 2009). Based on the above results we can conclude about a possible gender related influence on the plasma concentration of these vasoactive peptides. Further studies to confirm this suggestion are currently being performed. **Financial support:** CNPq, CAPES, FAPESP.

06.074

Atorvastatin seems inhibit the MMP-9 expression more pronouncedly than sinvastatin in human endothelial cell culture. Izidoro-Toledo TC[1], Guimarães DA[1], Gerlach RF[2], Tanus-Santos JE[1] [1]FMRP-USP − Pharmacology, [2]FORP-USP − Morphology

Introduction: Statins possess anti-inflammatory properties and may downregulate matrix metalloproteinases (MMPs) expression, thus contributing to restore cardiovascular homeostasis in cardiovascular diseases. This study aimed at comparing the effects of two different statins (simvastatin and atorvastatin) on MMP-9 expression in human umbilical vein endothelial cells (HUVEC) culture cell line, commercially available. **Methods:** A preliminary viability assay by Thiazolyl Blue Tetrazolium Bromide (MTT) was performed to choose the concentrations of statins and phorbol 12-myristate 13-acetate (PMA), a known MMP inducer, which were non-toxic for the cells. HUVECs were incubated with simvastatin or atorvastatin 1 micromolar (or vehicle), and with PMA at 100 nanomolar for 24 h. MMP-9 levels were assessed by Gelatin Zymography. Gelatinolytic activities in samples from media culture were detected by densitometry using ImageJ Program. MMP-9 was identified as a band at 92 KDa and its levels were normalized by the number of cells. **Results:** Media culture from vehicle-treated HUVECs showed undetectable MMP-9 levels. While treatment with PMA increased MMP-9 levels to 258 ± 12 arbitrary units (AU), atorvastatin and simvastatin attenuated this increase significantly (to 138 ± 4 AU and 170 ± 13 AU, respectively; both $P<0.001$), thus showing that atorvastatin more clearly inhibited MMP-9 upregulation than simvastatin ($P<0.01$). Conclusions: These preliminary findings suggest that although both statins attenuated PMA-induced MMP-9 upregulation, atorvastatin is probably more potent than simvastatin. This study was supported by CNPq, CAPES, Faepa.

06.075

Hydrogen sulfide improves vascular hyporesponsiveness and survival in severe polymicrobial sepsis in mice. Balsanelli LS, Dal-Secco D, Assreuy J UFSC − Farmacologia

Introduction: Sepsis is a systemic inflammatory response resulting from the inability of the host to restrict local infection. From the vascular point of view, severe sepsis is characterized by marked hypotension with vascular hyporesponsiveness to vasoconstrictor agents, as consequence. Endogenous hydrogen sulfide (H2S) is synthesized in various types of mammalian cells from L-cysteine in a reaction catalyzed by two enzymes, cystathionine-gamma-lyase and/or cystathionine-beta-synthase. The H2S role during a septic process is still controversial. The previous studies have demonstrated that H2S aggravates sepsis-associated systemic inflammation, contributing with the augment of septic mice mortality. On the other hand, recently, it was also showed that H2S restores neutrophil migration to the infectious focus and improves survival outcome in severe sepsis. Thus, the aim of this study was to investigate the potential role H2S on vascular hyporesponsiveness observed in severe polymicrobial sepsis in mice. **Methods:** The experimental consisted of female Swiss mice submitted to cecal ligation and puncture (CLP) surgery and sham-operated animals as controls. The vascular responsiveness activity was evaluated in aorta rings placed in a bath filled with 5.0 mL Krebs-Henseleit solution (pH 7.4; 37 ºC, 95% O2 and 5% CO2). After an equilibration period (60 min) the preparations were contracted with phenylephrine (Phe; 1µM), and the integrity of the endothelial layer was confirmed with acetylcholine (1µM). Aortic responsiveness was evaluated 6, 12 and 24 h after CLP surgery. Just in 24 h, after CLP surgery, the aortic responsiveness was evaluated in the presence or absence of the H2S donor, sodium hydrosulfide (NaHS; 3.10-5 M; 30 min). The survival was analyzed until 72 h after CLP surgery in mice pretreated with PBS (0.2 mL) or NaHS (10-100 µmol/kg). The procedures have been approved by the animal ethics committees of UFSC (PP003/ Animal Use Ethics Committees-CEUA-UFSC). **Results:** The vascular responsiveness to vasoconstrictor Phe was just significantly reduced in aorta rings from septic mice evaluated 24 h after CLP (0.3 ± 0.01 g, P=0.02, N=10) compared to SHAM (control group; 0.45 ± 0.05 g, N=10). However, the NaHS incubation prevented this vascular hyporesponsiveness evaluated 24 h after CLP surgery (0.4 ± 0.01 g, P=0.01, N=10). Furthermore, the pre-treatment with NaHS enhanced significantly the survival rate of the septic mice (40%, P=0.03, N=10). **Discussion:** The results of present study identify that H2S has an important role on the vascular response during sepsis. H2S prevents the vascular hyporesponsiveness to vasoconstrictor in septic mice, and as consequence, enhances the survival of septic mice. Therefore, the results suggest that H2S donor drugs could be a new potential target to sepsis pharmacotherapy. **Financial support:** CNPq, CAPES and FAPESC.

06.076

Changes in rat's vascular reactivity in response to neonatal induced hyperleptinaemia. Motta NAV[1], Marques EB[1], Louback LS[2], Miranda ALP[2], Scaramello C[1], Brito FCF[1] − [1]UFF − Fisiologia e Farmacologia, [2]FF-UFRJ − LASSBio

Introduction: Leptin, an adipocyte-derived hormone, is an important regulator of energy metabolism with relevant vascular effects. Leptin may increase blood pressure through enhanced sympathetic activity and it also exerts direct depressor effects on vascular tone by stimulating endothelial production of nitric oxide in a dose-dependent manner (Zanetti M et al, Atherosclerosis 2004; 175: 253–259).Ex vivo incubation of aortas from sympathectomized rats in the presence of leptin results in endothelial-dependent vasorelaxation and this effect is mediated by stimulation of the AKT-endothelial NO synthase phosphorylation pathway (Lembo G et al, Diabetes 2000;49:293-297). The aim of the present work is to study if neonatal leptin treatment develops vascular alterations. **Methods:** Pups were divided into two groups. Leptin group, injected daily with leptin (8µg/100g sc) and Control group injected saline daily, for the first 10 days of lactation. The animal groups were studied at one, three and five months of age. Rings (4mm long) from thoracic aorta artery were used for assessing vascular reactivity, where they were suspended in organ chambers containing Krebs Bicarbonate solution (pH 7.4; temperature 37°C; composition in mmols/L:4.2 KH2PO4; 14.9 NaHCO3; 119 NaCl; 4.7 KCl; 1.6 CaCl2; 1.2 MgSO4.7H2O; 11.5 glucose). The maximal contraction of each ring was determined by cumulative addition of phenylephrine (10-10 to 10-6 mol/l). Acetylcholine (10-9 to 10-5 mol/l) was added cumulatively during a maximal contraction to phenylephrine. **Results:** No differences were observed in phenylephrine dependent aorta contraction at the different ages studied. However, acetylcholine EC50 was statistically different for Leptin group (1.36x10-7M) compared to Control group (6.53x10-7M) at 1 month of age. There was no difference at 3 and 5 months of age. **Conclusion:** The higher potency of acetylcholine in Leptin group at 1 month of age agrees with previous works that shows leptin decreasing blood pressure via NO production. Possible adaptations can explain the lost of this activity at the other ages studied. **Financial support:** FAPERJ, CNPq, CAPES, PROPPI/UFF.

06.077

Temporal evaluation of 2K1C model of renovascular hypertension: metalloproteinases and oxidative stress. Ceron CS[1], Rizzi E[1], Oliveira AM[3], Guimarães DA[1], Cau SBA[1], Marçal DMO[1], Gerlach RF[1], Tanus-Santos JE[1] [1]FMRP-USP − Farmacologia, [2]FORP-USP − Morfologia

Introduction: Increase of oxidative stress and metalloproteinases (MMPs) that lead to structural and functional vascular changes have been observed at the end of many chronical studies of renovascular hypertension. In this work we examined the temporal changes induced by raise of arterial blood pressure in the metalloproteinase levels and oxidative stress observed in the two kidney-one clip (2K1C) hypertension model. **Methods:** 2K1C hypertension was induced in male Wistar rats by clipping the left renal artery. The same surgical procedure without the placement of the arterial clip was carried out in sham rats. Systolic blood pressure (SBP) was monitored weekly by tail-cuff plethysmography. At the end of two, four, six and ten weeks of hypertension, animals were anesthetized and killed to realize the experiments. Aortic MMP-2 levels and gelatinolytic activity were determined by zymography gelatin and in situ zymography respectively. The aortic superoxide production was evaluated with dihidroetidium (DHE), and the formation of reactive oxygen species was measured in plasma as thiobarbituric acid reactive substances (TBARS). This study was approved by Animal Ethics Committee of FMRP/USP (nº 160/2009).**Results:** SBP increased in the hypertensive rats during all the weeks of the study (130 ± 3.4 mmHg for sham versus 170 ± 2.3 mmHg in the second, 180 ± 2.2 mmHg in the fourth 195 ± 5.2 mmHg in the sixth and 200 ± 5.4 mmHg in the tenth week of hypertensive rats; all $P<0.05$). Aortic MMP-2 total levels increased by 40% in hypertensive rats at sixth and tenth week (both $P<0.05$), while the gelatinolytic activity increased by 10% in the fourth week, and 25% in the sixth and tenth week in the hypertensive rats (all $P<0.05$). Aortic superoxide production increase from seconds to the tenth week (all increase of 20%, $p<0.05$) and the TBARS was increase in the sixth and tenth week (increase of 30%, both $P<0.05$) in the hypertensive rats. **Discussion:** These results suggest that oxidative stress levels were the first to become evident in the 2K1C hypertension. Probably this increase of oxidative stress is the responsible for the increase in MMP levels and activity. These MMPs are involved in chronic processes such as angiogenesis, remodeling and atherosclerosis, thus a comprehensive understanding of MMPs biology is necessary for the combat the alterations observed in cardiovascular diseases. **References**: Chow AK, Cena J, Schulz R. Acute actions and novel targets of matrix metalloproteinases in the heart and vasculature. Br J Pharmacol. 2007 Sep;152(2):189-205. Supported by: FAPESP, CNPq, CAPES.

06.078

Evaluation of cilostazol effects in hypercholesterolemic diet fed rats. Motta NAV[1], Canal PF[1], Amorim LEO[1], Reis RC[1], Miranda ALP[2], Brito FCF[1] − [1]UFF − Fisiologia e Farmacologia, [2]FF-UFRJ − LASSBio

Introduction: Cardiovascular disease is widely recognized as the most common underlying cause of mortality in all prosperous countries, varying with time and population but more prominently with increased prosperity and longevity. The pivotal role of atherosclerosis as the primary lesion in macrovascular disease has been the subject of discussion and theory since the 19th century work of Virchow and Rokitanski and has been regarded as a response to injury or inflammatory process for many years (Russell & Proctor, 2006, Cardiovascular Pathology, 15: 318-330). Atherosclerosis is characterized by endothelial lesions, adhesion of mononuclear leukocytes, migration and proliferation of smooth muscle cells, as well as extracellular matrix deposition (Ross, 1999, N Engl J Med, 340: 115-126). Cilostazol is a PDE3 inhibitor which presents antiplatelet and vasodilatation properties. Studies have demonstrated that cilostazol diminished the concentration of remaining VLDL and chylomicrons, increased HDL and diminished triglycerides in patients as well as it is able to reduce many inflammatory markers (Wang et al., 2003, Atherosclerosis, 171: 337-342; Rosa et al., 2006, Arq Bras Cardiol, 87: 221-225). The aim of this study is to evaluate the actions of cilostazol in hypercholesterolemic diet fed rats to contribute for the elucidation of its effects. **Methods:** Male albino Wistar rats weighing (150 – 200g) were divided into three groups consisting of 8-10 rats each. Group I (control) served as the control group and they were fed with commercial standard chow ad libitum for 30 days and were then administered vehicle p.o. (0.1 ml/ Kg) for 15 days. Group II (atherosclerosis) received the hypercholesterolemic diet for 30 days and were then administered vehicle p.o. for 15 days. Group III (cilostazol) received a hypercholesterolemic diet for 30 days and were then administered cilostazol 30 mg/kg p.o for 15 days. At the end of the experimental period, all the animals were killed by cervical decapitation under anesthesia. From each animal, blood samples were collected, the thoracic aorta, and liver were excised. Then, we evaluated the lipidic profile, the serum lipid peroxidation, the vascular reactivity and histological changes. We have compared the results for the groups (one-way ANOVA) and tested the statistical significance of differences between groups by Bonferroni´s test ($p < 0.05$). **Results and Discussion:** At the dose employed (30 mg/ kg), cilostazol was able to reduce total cholesterol and LDL levels when compared to atherosclerosis group, 94 ± 3.8 mg/dL x 65 ± 5.1mg/dL and 39 ± 4.1 mg/dL x 15 ± 5.4 mg/dL, respectively. It was also able to reduce lipid peroxidation as evaluated by TBARS assay, when compared with atherosclerotic group, 51 ± 1.4 nmol/mL x 86 ± 3.0 nmol/mL respectively, and presenting a level similar to control group. In preliminary studies when we evaluated the vascular reactivity, using rings from thoracic aorta artery, no statistical differences were observed in phenylephrine dependent aorta contraction or in acetylcholine induced relaxation. At histological analyses the intima of the aorta was found to be thickened in the atherogenic diet-fed rats compared with normal rats. On the other hand, in rats receiving cilostazol treatment, the intima of the aorta was found to be less thick than that of atherosclerosis group. These results contribute to elucidate the role of cilostazol as a useful drug at anti-atherogenic therapy besides its antiplatelet properties. Ethics Comission approval number 0016/09 - CEPA/UFF 03/2009. **Financial support:** FAPERJ, CAPES, PROPPI/UFF.

06.079

Cardiovascular and neuroendocrine changes after blood volume expansion with hydroxyethyl starch 450/0.7 during experimental septic shock induced by cecal ligation and perforation (CLP). Santiago MB[1], Andrade CAF[1], Antunes-Rodrigues J[2], Giusti-Paiva A[1]
[1]UNIFAL − Ciências Biológicas, [2]FMRP-USP − Fisiologia

Introduction: In the present study we evaluated the effects of intravenous infusion of isotonic and hypertonic hydroxyethyl starch solutions on mean arterial pressure and on electrolytes, vasopressin levels and gases in the blood during experimental septic shock induced by cecal ligation and perforation. **Methods and Results:** Male Wistar rats (250 – 300 g) with jugular vein and femoral artery catheters were anesthetized with tribromoethanol (250 mg/kg) and submitted to cecal ligation and perforation (CLP) or sham surgery (SS). Mean arterial pressure (MAP) was monitored for 24 hours and blood parameters (Na+, osmolality, hematocrit, pH, pO2, pCO2, O2 saturation, HCO3-) were evaluated at 0, 6 and 24 hours after CLP or SS. Volume expansion with isotonic hydroxyethyl starch solution (Iso-HES) (HES 6% in NaCl 0.9%; 4 ml/kg, 8ml/kg, 16ml/kg), hypertonic hydroxyethyl starch solution (Hyper-HES, HES 6% in NaCl 7.5%; 4 ml/kg) or hypertonic saline (HS, 7.5% NaCl; 4 ml/kg) were performed 6 hours after CLP or SS. MAP was measured for 30 minutes, blood parameters were measured at 30 min, and plasma vasopressin was assessed 5 minutes after volume expansion (CEUA 162-2008). CLP decreased MAP (76.9 ± 2.8 vs. SS: 103.6 ± 1.9 mmHg) and this decrease last for 24 hours (69.0 ± 4.0 mmHg vs. SS: 99.4 ± 2.7 mmHg). Intravenous infusion of Iso-HES decreased hematocrit but did not change MAP or other blood parameters. However, both infusions of Hyper-HES and HS increased MAP (18.3 ± 2.5 and 16.4 ± 1.2 mmHg, respectively, vs. CLP; $p < 0.05$), increased vasopressin release (10.5 ± 1.7 and 11.9 ± 1.5 pg/ml, respectively, vs. CLP: 4.2 ± 0.2 pg/ml; $p < 0.001$), increased plasma sodium (148 ± 1.3 and 147 ± 0.9 mEq/l, respectively, vs. CLP: 139 ± 0.7 mEq/ml; $p < 0.001$) and also increased plasma osmolality (309.7 ± 1.7 and 306 ± 4.1 mOsm/kg H2O, respectively, vs. CLP: 281 ± 3.5 mOsm/kg H2O; $p<0.05$). There were no other changes in blood parameters after administration of hypertonic solutions. **Conclusion:** Volume expansion with hypertonic solutions (Hyper-HES and HS), but not with isotonic solution (Iso-HES), produced an increase in MAP during septic shock induced by cecal ligation and perforation, and this effect is mediated by vasopressin release. Supported by: FAPEMIG, CAPES, CNPq, FINEP

06.080

Pharmacological characterization of a new steroidal cardiotonic isolated from *Physalis angulata* leafs. Gomes VM[1], Pessoa ODL[2], Santos CF[3], Fonteles MC[4], Lessa LMA[5], Nascimento NRF[6] [1]UECE − Fisiologia, [2]UFC − Química Orgânica, [3]UECE − Medicina, [4]Mackenzie − Fisiologia e Farmacologia, [5]ISCB-UECE, [6]UECE − Medicina Veterinária

Heart failure (HF) is one of the most common diseases in cardiovascular system. This complication is frequently associated with hypertension and diabetes. The current used therapeutics in the treatment of HF involves intrinsic mechanisms that may promote adverse effects. Our preliminary data demonstrated that a compound isolated from a steroidal mixture obtained from *Physalis angulata* leaf extract, codified F9, promoted a significant positive inotropic effect when compared to control. The present study was designed to investigate the pharmacological mechanisms involved in the F9 action, by guinea pig atrial preparation. Male Guinea pigs “Cavia porcellus” were sacrified by cervical displacement, followed by thoracotomy and heart exposition. The right atria was extracted and immediately immersed in 37 ºC Krebs-Henseleit solution (95% O2 e 5 % CO2).The cardiac tissue was assembled in a organ bath system coupled to four channel polygraph to isometric record. The evaluated parameters were contraction force and heart rate. Dose-response curves were performed with F9 (0, 1 - 100 µg/mL; n = 4), in the presence of 10-6 M Propranolol (β-adrenergic antagonist) or 10-7 M Verapamil (voltage dependent calcium channel blocker). The intracellular cAMP generation in guinea pig atria was also evaluated by imunoenzymatic method. F9 promoted a positive inotropic effect at a concentration-dependent way (∆190,35 ± 0,44%; n=5), with no significant alterations in heart rate. This effect was not inhibited after incubation with either propranolol (∆ 220,23 ± 35,39 %; n=5) or verapamil (∆ 164,58 ± 21,59 %, n=4). Moreover, the intracellular cAMP contend did not changed after treatment with F9. Our data indicate that neither β-adrenergic or voltage dependent calcium channels are involved in the F9 action mechanism in guinea pig atria. Besides, the cAMP pathway also seems not to be involved. Therefore, further studies will be designed to expand the knowledge about the F9 inotropic effect.

06.081

Blood arterial pressure and vasoactive peptides concentration after nephrectomy in rats with different ace phenotypes. da Silva RM, Tanae MM, Peixoto HS, Souccar C, Lapa AJ, Lima-Landman MTR UNIFESP − Farmacologia

Introduction: The renin-angiotensin-aldosterone system (RAS) controls the cardiovascular and renal functions (Carey et al., Endoc. Rev. 24: 261, 2010). Drugs that act on this system are important therapeutic tools in hypertension control. In previous work, it was demonstrated that Wistar rats could be divided in 3 phenotypes according to its plasmatic ACE activity: rats with high (hACE), intermediate (iACE) and low (lACE) activities (Ninahuaman et al., Phytomedicine 14: 314, 2007). Despite the different phenotypes, the blood pressure (BP) of these animals is normal, but the sensitivity to captopril is higher in lACE rats (De Oliveira SS. – Dissertação (Mestrado), UNIFESP/ EPM, 90f , 2008). Objective: Considering the importance of the kidney in BP control, this work aimed to study the influence of the ACE phenotypes (hACE and lACE) on BP and on blood concentrations of bradykinin (BK), angiotensin I (AI) and angiotensin II (AII) in sham-operated (S) and nephrectomized (NF) rats. **Methods:** ACE plasmatic activity was spectrofluorimetricaly determined and expressed in nmol/min/mL (Carmona et al. - Nature **Methods:** 1(4): 1971, 2006). BP was measured by the tail-cuff method. The hACE and lACE animals were submitted to left unilateral nephrectomy (NF). The S group was submitted to a posterior incision without kidney removal. Blood AI, AII and BK concentrations were determined by high performance liquid chromatography and expressed in ng/mL (Danser et al., Hypertension 24: 37, 1994). All data were expressed as mean ± standard error and compared by ANOVA followed by the Tukey test ($p<0.05$) (CEP 1347/09). **Results and Discussion:** BP in the hACE and lACE groups were 139.3 ± 1.2 mm Hg, n=14 and 138.9 ± 1.1 mm Hg, n=14, respectively. Twenty five days after surgery BP did not change in neither ACE phenotype group. The ACE activities in hACE and lACE rats, previous to surgery, were 69.9 ± 5.2 nmol/mim/mL (n=14) and 34.2 ± 2.1 nmol/mim/mL (n=14), respectively. After surgery, ACE plasmatic activity was maintained according to the phenotypic profile of each group. In the hACE, BK concentration in S and NF groups were increased by 292% and 146% (S=206.9 ± 33.3 ng/mL, n=6; NF= 199.3 ± 39.2 ng/mL, n=6, respectively) and in the lACE by 652% and 762% (S=140.7 ± 26.9, n=6; NF= 138.2 ± 14.9 ng/mL, n=6, respectively). In hACE, AI levels in S (676%) and NF (944%) groups were higher than those obtained in the preoperative period (hACE: S=262.9 ± 19.9 ng/mL, n=8; NF= 288.4 ± 19.5 ng/mL, n=6). In the lACE group, AI levels were 1004% and 1317% higher after surgery in S and NF, respectively. The concentration of AII was affected only in the NF group increasing 225% and 166% after kidney removal in hACE and lACE rats, respectively (hACE: NF=78.3 ± 9.8 ng/mL, n=6; lACE: NF=31.2 ± 2.5 ng/mL, n=5, before surgery). The results indicate that surgery itself affected the blood concentration of BK and AI since the postoperative values increased in both, S and NF groups. Nephrectomy, in its turn, increased the concentration of AII, which could result from a compensatory action of the remnant kidney. Despite the increased concentration of AII, BP was not affected by 25 days nefrectomy which could be due to the increased BK level after surgery. **Financial support:** CNPq, CAPES, FAPESP

06.082

Cardiovascular responses to bothropstoxin. Rodrigues MAP, Dias L, Smaal A, Rennó AL, da Silva DA, Lorenzetti R, Hyslop S, Hyslop S UNICAMP − Farmacologia

Introduction: *Bothrops* venoms produce systemic effects, including hypotension and cardiovascular shock. We have previously shown that *Bothrops jararacussu* (jararacuçu) venom is cardiotoxic in rat atria. In this work, we sought to identify the venom component(s) responsible for this activity. **Methods:** Venom was fractionated by gel filtration on Superdex 75 and the peaks tested for atrial toxicity (see below). The active peak was further fractionated by ion exchange on SP-Sepharose and tested in right atria isolated from male Wistar rats anesthetized with isoflurane. The atria were mounted (resting tension: 1 g) in 10 ml organ baths containing modified Krebs-Henseleit solution at 37°C and continuously aerated with 5%CO2-95%O2. Changes in contractile force were measured isometrically and atrial rate was calculated from this record. Creatine kinase MB (CK-MB) levels were measured with commercial kits. At the end of the experiments, the atria were fixed and processed for histological analysis. Male Wistar rats (300-400 g) were anesthetized with urethane (1.2 g/kg, i.p.) and a carotid artery was catheterized for continuous blood pressure measurements. The active peak was administered via a femoral vein in 100 ml that was washed in with a further 100 ml of 0.9% NaCl. Changes in cardiovascular parameters were monitored for 120 min. The results were compared statistically using Student´s t-test or ANOVA followed by the Tukey-Kramer test; $p<0.05$ indicated significance. The experiments were approved by the institutional Committee for Ethics in Animal Research (protocol nos.1433-1 and 1739-1). **Results:** Venom (0.2 mg/ml) caused a progressive decrease in contractile force and atrial rate within 60 min. Of the five peaks obtained by gel filtration only peak III reproduced the effects of the venom by reducing contractile force and atrial frequency. Ion exchange chromatography of peak III resulted in three additional peaks, the last (most basic) and largest of which (peak III-3) reproduced the reduction in contractile force and atrial frequency caused by venom and peak III. Venom, peak III and peak III-3 also caused atrial contracture (increase in baseline tension). Peak III-3 caused myonecrosis and CK-MB release, effects also seen with venom and peak III. In contrast to the effects seen in vitro, the i.v. administration of peak III-3 (0.5 and 1.5 mg/kg) did not significantly alter heart rate, blood pressure or respiratory rate. Peaks III and III-3 contained PLA2 activity. SDS-PAGE of peak III-3 in the absence and presence of reducing agents (DTT or b-mercaptoethanol) revealed a protein with a molecular mass of ~28 kDa and 14 kDa, respectively, which agreed with data for bothropstoxins I and II. Reverse phase HPLC indicated the presence of two major peaks in peak III-3 corresponding to bothropstoxins I and II. **Discussion:** These results identify bothropstoxins as the major components responsible for the atrial toxicity of *B. jararacussu* venom and indicate that these toxins have cardiotoxin-like activity. The lack of effect in vivo indicates that these toxins do not contribute to the hypotension and respiratory failure caused by *B. jararacussu* venom. **Financial support:** CAPES, CNPq, FAPESP

06.083

Cardiovascular alterations caused by *Bothrops alternatus* snake venom in anesthetized dogs. Rodrigues MAP[1], Dias L[1], Smaal A[1], Rennó AL[1], Moreno Junior H[2], Mello SM[3], Hyslop S[1] [1]UNICAMP − Farmacologia, [2]UNICAMP, [3]UNICAMP − Controle de Intoxicações

Introduction: Bothrops snake venoms have long been known to cause hypotension. However, the hemodynamic and cardiac alterations involved have not been extensively investigated. In this work, we examined the cardiovascular alterations caused by *Bothrops alternatus* snake venom in dogs. **Methods:** Male mongrel dogs (10-20 kg) were sedated with sodium thiopentone (30 mg/kg, i.v.), anesthetized with 2% isoflurane and cannulated for the measurement of hemodynamic parameters. Cardiac output (CO) and derived parameters were determined by thermodilution using a Swan-Ganz catheter. Venom (0.3 and 1 mg/kg, i.v., in 1 mL) was administered via the left femoral vein and systemic blood pressure was recorded from the left femoral artery. **Results:** Venom (0.3 mg/kg, i.v.) significantly ($p<0.05$) decreased the mean arterial blood pressure and CO after 5 min (from 116 ± 4 to 39 ± 3 mmHg and from 4.8 ± 0.5 to 2.5 ± 0.3 L/min, respectively; n=9-12, mean ± SEM). Blood pressure recovered over the following 30-40 min but did not reach pre-venom levels; CO showed no recovery. There was also an abrupt decrease in left and right ventricular systolic work and systolic indices and volume that persisted until the end of the experiment. There were no significant changes in heart rate, systemic vascular resistance or pulmonary vascular resistance. A dose of 1 mg/kg produced similar but more pronounced cardiovascular alterations and death occurred within 30 min. There were no significant changes in heart rate, ECG, pulmonary hemodynamics, blood gas levels (pO2, pCO2, HCO3, SBC and SBE) and metabolic parameters (blood pH, lactate, glucose, creatine kinase, Na+ and K+); however, there was a significant increase in plasma lactate dehydrogenase at 2 min post-venom (from 121 ± 17 to 793 ± 33 IU/L) with a return to basal thereafter. There were no significant changes in the control group. There were no histological alterations in cardiac tissue, but microaneurysms and epithelial desquamation were seen in renal tubules. Circulating venom concentrations (determined by ELISA) decreased rapidly after administration (0.3 mg/kg, i.v.), but venom was still detectable after 240 min. **Discussion:** These results show that in dogs B. alternatus venom produces marked hypotension and a direct cardiac action, with few metabolic alterations. **Financial support:** FAPESP

06.084

The participation of AT_1 receptor in the Ang II-increased contraction in the contralateral carotid artery after balloon catheter injury. Olivon VC[1], Mestriner FL[2], Cunha FQ[2], de Oliveira AM[1] [1]FCF-USP − Física e Química, [2]FMRP-USP

Introduction: The AT1 receptor is evolves in the very important physiological actions of Ang II, includes in the vascular system and mediates most of the actions of Ang II closely associated with the regulation of vascular tone and extracellular fluid volume. The AT1 receptor belongs to the G protein-coupled receptor (GPCR) superfamily, typically activates phospholipase C, as well as activation by tyrosine kinases. AT1 receptor mediates the contractile response by phospholipase C-dependent mechanisms leading to an increase in intracellular calcium. Fifteen days after balloon catheter injury the Emax of Ang II was increased in the contralateral artery as compared to Emax obtained in arteries from intact animals. AIM: Based in this information, the aim of this study was investigated the participation of the AT1 receptor in the increased of Ang II response in contralateral carotid artery, fifteen days after balloon catheter injury. **Methods:** In the vascular reactivity experiments to Ang II were used carotid artery rings endothelium-intact from arteries from control Wistar rats (control group) and contralateral arteries from Wistar rats that underwent balloon catheter injury surgery (contralateral group). To investigate AT1 response, were constructed Ang II curves in presence of different concentrations of losartan (AT1 selective antagonist, 10-9 mol/L, 10-8 mol/L, 10-7 mol/L, 10-6 mol/L) or in presence of L-NAME (NOS non-selective inhibitor, 1 mmol/L) + losartan (AT1 selective antagonist, 0,1 µmol/L) or in presence of PD 123,319 (AT2 selective antagonist, 1 µmol/L) + A-779 (Mas selective antagonist, 1 µmol/L) + HOE-140 (B2 selective antagonist, 1 µmol/L) Western blotting and imunohistochimistry were realized to AT1 receptor. All procedures were in accordance with the standards and policies of the Animal Care Committee of this institution (licenses number: 06.1.1019.53.5). **Results:** In the presence of endothelium-intact, Emax to Ang II was increased in the contralateral to balloon catheter injury (0,38 ± 0,01g) when to compared control group (0,27 ± 0,01g). Cumulative concentration-effect curves to Ang II were antagonized by losartan in different concentration-dependent showed unsurmountable antagonistic characteristic in both groups. In presence of L-NAME + losartan, surmountable antagonistic characteristic was abolish. Schild plot resulted in a linear regression with a slope of 0,75 to control group and 0,78 to contralateral group, different from unity. The pA2 value indicated increased in the antagonist potency in the contralateral group (pA2 value = 8,63) to compare to control group (pA2 value = 7,95). In presence of AT2, Mas and B2 selective antagonist receptors, concentration-effect curves for Ang II showed increased in the Ang II Emax in the control group (0,39 ± 0,03g) and reduction in the contralateral group (0,27 ± 0,03g). AT1 receptor expression was similar in the contralateral artery when to compare to control group. Data are shown as means ± E.P.M. Anova and Newman-Keuls's post test, $P<0.05$. CONCLUSION: AT1 receptor not contributed to Emax-Ang II increased in the contralateral artery to balloon catheter injury. However, the results suggested that AT2 receptor or Mas receptor or B2 receptor could modulate this increase because in the presence of the antagonists receptors, the Ang II response in the contralateral artery was similar to artery from control animals. **Financial support:** FAPESP and CNPq.

06.085

Renin inhibition with aliskiren did not prevent the vascular remodeling found in 2K1C hypertension. Oliveira AM[1], Castro MM[1], Marçal DMO[1], Rizzi E[1], Ceron CS[1], Guimarães DA[1], Gerlach RF[2], Tanus-Santos JE[1] [1]FMRP-USP − Farmacologia, [2]FORP-USP − Morfologia

Introduction: Renin-angiotensin system participates of effective form on vascular modifications present in hypertensive process. Metalloproteinases of extracellular matrix (MMPs), also participate of alterations present in cardiovascular system during hypertension. In this context, this study aimed at examining whether upregulation of matrix metalloproteinases (MMPs), specifically MMP-2, associated with vascular dysfunction and remodeling in 2K1C hypertension model, could be attenuated by pharmacologic inhibition of renin and AT1 receptor or in association. **Methods:** Renovascular hypertension (2K1C) was induced in wistar rats treated with aliskiren (50 mg/kg/day) or losartan (10 mg/kg/day), or both, given by gavage. Treatments started 2 weeks after 2K1C hypertension was induced. After 4 weeks of treatment, sham-operated and hypertensive rats were killed by decapitation. Their thoracic aorta were harvested and cleaned of connective tissue to histological, biochemical and structural analyses. **Results:** We found similar reduction in systolic blood pressure (SBP) in animals treated with 2K1C+aliskiren (156.8 ± 2.36 mmHg) or 2K1C+losartan (158.5 ± 1.99 mmHg) when compared with 2K1C+vehicle (207.7 ± 1.92 mmHg) after 6 weeks ($p<0.05$). The association (145.9 ± 1.76 mmHg) of drugs attenuated more effectively hypertension when compared with each drug alone ($p<0.05$). While we found that losartan (M/L: 10.95 ± 1.35% and CSA: 11.431 ± 1.178 um2) and the association (M/L:11.32 ± 0.76% and CSA: 12.567 ± 995.2 um2) prevented the increases in media to lumen (M/L) ratio and aortic cross-sectional area (CSA) compared with 2K1C+vehicle group (M/L:14.68 ± 1.15% and CSA: 17.410 ± 1.771um2) ($p<0.05$), aliskiren produced no such effect (M/L:12.97 ± 1.28% and CSA: 16.735 ± 1.632 um2) ($p>0.05$). Interestingly, we found increased in situ gelatinolytic activity in the media of thoracic aortas, which colocalized with increased aortic MMP-2 levels in vehicle-treated hypertensive animals compared with sham-operated animals and with animals receiving drugs ($p<0.05$), except aliskiren alone. **Discussion:** Our results suggest that inhibition of AT1 receptor in 2K1C hypertension attenuates the vascular hypertrophy and decreases MMP-2 levels. Conversely, the renin inhibitor did not prevent the vascular alterations found in 2K1C hypertension. In this regard, previous studies have shown that direct inhibition of prorenin/renin by aliskiren beyond of induce a significant increase in circulating levels of prorenin/renin, not prevent the binding of renin/prorenin to prorenin receptor. Prorenin receptor activation result in upregulation of profibrotic molecules such as transforming growth factor-β1 (TGF-β1), collagens and fibronectin. Therefore, the possible activation of prorenin receptor by renin/prorenin may, at least in part, explain the effect of aliskiren found in this study.

06.086

Renal cyclooxygenase expression in rats treated with *Bothrops alternatus* venom. Rennó AL[1], Penteado CF[2], Linardi A[1], Hyslop S[1] [1]UNICAMP − Farmacologia, [2]UNICAMP − Hemocentro

Introduction: Prostaglandins produced by cyclooxygenase (COX) have an important role in renal physiology, where they regulate renal blood flow and glomerular filtration. Two major isoforms of COX are known: COX-1 which is constitutively expressed and COX-2 which is inducible. COX-2 is induced in a variety of pathologies, including inflammatory processes and nephropathies. Bites by Bothrops snakes produce renal damage and acute renal failure in animals and humans, but the molecular mechanisms are poorly understood. In this work, we investigated the profile of COX-1 and COX-2 expression in renal tissue of rats injected with Bothrops alternatus venom. **Methods:** Male Wistar rats (~250 g) were injected with B. alternatus venom (0.8 mg/kg, i.v.) and killed with an overdose of isoflurane 1, 3, 6, 24, 48, 72 h and 7 and 15 days post-venom. Urine samples were collected prior to killing at each interval and analyzed for protein content and hematuria or hemolysis. Both kidneys were subsequently removed and one was used for histological analysis (sections stained with hematoxylin-eosin) and immunohistochemistry (with primary antibodies to COX-1 and COX-2) whereas the other was frozen in liquid nitrogen and stored -80°C until processed for western blotting, immunohistochemistry and real-time PCR for COX gene expression. The experimental protocols were approved by an institutional Committee for Ethics in Animal Experimentation (CEEA/UNICAMP protocol no. 1740-1). **Results:** Histological analysis revealed microaneurysms, tubular necrosis and epithelial desquamation during the first 24 h post-venom. These alterations coincided with proteinuria and hematuria in the same period. Venom significantly increased COX-2 gene expression only at 3 h post-venom (0.84 ± 0.39 arbitrary units; mean ± S.D.) when compared to saline-treated (control) rats (0.08 ± 0.12; n=6 each). In contrast, western blotting revealed a significant increase in COX-2 protein expression at 3 h (0.82 ± 0.14), 6 h (0.79 ± 0.04) and 24 h (0.73 ± 0.07) post-venom when compared to saline-treated (control) rats (0.46 ± 0.04) (n=6 each; $p<0.05$; ANOVA followed by Bonferroni's test). Immunohistochemistry confirmed the enhanced COX-2 expression in renal cortex and medulla in 3 h, 6 h and 24 h post-venom. In contrast, there were no significant alterations in COX-1 gene and protein expression at any of the time intervals examined. **Discussion:** Bothrops alternatus venom increases COX-2 gene and protein expression in rat renal cortex and medulla during the first 24 h post-venom. This time-scale coincided with the period of greatest histological and functional damage. Although COX-2 may be involved in a local renal inflammatory response to envenoming, enhanced COX-2 expression may also represent a renoprotective response to counterbalance venom-induced damage and maintain renal blood flow and glomerular filtration. **Financial support:** CNPq, FAPESP

06.087

Effects of high-fat diet during six week on biochemical parameters and arterial blood pressure of Wistar rats. Milet-Morais MM[1], Silva OA[1], Figueiredo TG[2], Guedes GS[2], Xavier FE[3], Rabelo LA[2], Pinto Duarte G[1] [1]UFPE − Fisiologia e Farmacologia, [2]UFAL − Fisiologia e Farmacologia, [3]UFES − Fisiologia

Introduction: An exposure to high fat diet during an extended period of time can promote some metabolic alterations, based on this, the present study intend to show the effects of a six week exposure to a high fat diet on biochemical parameters and the consequences on arterial blood pressure of Wistar rats. **Materials and Methods:** All the procedures were approved by the human and animal ethical committee of the Universidade Federal de Pernambuco (23076.007185/2009-41). Eight-week-old male Wistar rats with similar body weights (221.3 ± 9.3 g) were divided into the following groups: Control Group (C- Group), which received free access standard rodent chow (Labina®), and HFD group, exposure to high fat diet with 58.4% of the total calories derived from fat, during six weeks. The index weight gain between the C- Group and HFD were compared and the serum levels of total cholesterol, high-density lipoprotein (HDL), triglycerides (TG), and glucose from all groups were determined with an overnight fast. The animals were anesthetized and a catheter was implanted in femoral artery to obtain the arterial pressure values. Data were expressed as means of eight animals/group ± SEM. The groups were compared using unpaired Student's t-test. The level of significance adopted was $p< 0.05$. **Results:** The index of weight gain results shows no significant difference between the C-group and the HFD group. There was also no significant differences on serum levels of biochemical parameters in the HFD group right after six week of diet exposure when compare with C-group. However, there was a significant ($p<0,01$) increase on HFD group triglycerides levels after six months. The Mean Arterial Pressure (MAP) levels were significantly ($p< 0,05$) increased in the group HFD right after the last day of exposure to the high fat diet. The results showed that an exposure to this high-fat diet, for a short time, can induce alteration in MAP and only triglycerides values were later altered. **Financial support:** CNPq, FACEPE

06.088

Cardiovascular effects produced by chronic treatment with L-arginine in hypertensive rats. Baracho NCV[1], Silva GF[2], Bernardes DSV[1], Oliveira RCS[1] [1]FMIT − Farmacologia e Bioquímica, [2]FMIT

Introduction: and objective: The nitric oxide (NO) is an important vasodilator and is formed from the amino acid L-arginine by nitric oxide synthase (NOS). The NO is produced by a wide variety of cell types including epithelial, nerve, endothelial and inflammatory. The endothelial uses the nitric oxide to control the relaxation of the smooth muscle of the vessel wall, causing it expands thus increasing blood flow and lowering blood pressure. In medical literature there are studies that demonstrate the hypotensive effect of L-arginine, but there are no studies showing the effect of their combination with Angiotensin Conversion Enzyme (ACE) inhibitors. The aim of this study was to evaluate the effect of supplementation of L-arginine and L-arginine associated to the enalapril maleate in mean arterial pressure (MAP) of hypertensive rats. **Materials and Methods:** This study was approved by the Research Ethics Committee of the Faculty of Medicine of Itajubá under protocol 07/08 and received financial support from FAPEMIG. Forty-eight male, adult, Wistar rats were used, allocated in six groups (n=8), according to the treatment. The animals were submitted to surgery for induction experimental hypertension by modified Grollman model. Fifteen days after surgery the hypertensive rats (MAP>140mmHg) received daily by gavage the fllowing treatments for thirty days : (1) Control, (2) L-Arginine 500mg/Kg, (3) Enalapril Maleate 2,5mg/Kg, (4) Enalapril Maleate 5,0mg/Kg, (5) L-Arginine 500mg/Kg + Enalapril Maleate 2,5mg/Kg and (6) L-arginine 500mg/Kg + Enalapril Maleate 5,0mg/Kg. Mean arterial pressure was measured by tail pletismography twice a week. At the end of experimental period, the rats were anesthetized (Ketamine/Xylazin, I.P) and their blood were analyzed. **Results:** Treatment with L-Arginine produced a significant reduction in MAP from the third day when compared to the Control group (154 ± 11,6 vs 136,8 ± 6,5 mmHg, p <0,05). After start, the antihypertensive effect of L-Arginine remained stable during the following days of the experimental period. As expected, the treatment with Enalapril Maleate 2,5 mg/Kg and 5,0 mg/Kg also significantly reduced the MAP from the sixth day as compared to controls (153,6 ± 10,5 vs 121,7 ± 14,6 mmHg, p <0,01 and 153,6 ± 10,5 vs 122,8 ± 9,5 mmHg, p <0,01, respectively) and remained through the experimental period. The association between L-Arginine and Enalapril Maleate 2,5 mg/Kg produced a significant reduction in MAP when compared with Control from the sixty day (154 ± 11,6 vs 122,3 ± 11,3 mmHg, p <0,01) and from the third day using Enalapril Maleate 5,0mg/Kg (154 ± 11,6 vs 125,4 ± 13,7 mmHg, p <0,01) and remained through the experimental period. **Discussion:** These data indicate that the treatment with L-arginine and L-Arginine associated with enalapril maleate 5,0mg/Kg reduces the MAP faster and significantly compared with the other groups. 1. Novaes MRCG. Effects of dietetic supplementation with L-arginine in cancer patients. A review of the literature. Arch Latinoam Nutr. 1999; Vol.4, p. 301. 2. McConell GK. Effects of L-arginine supplementation on exercise metabolism. Curr Opin Clin Nutr Metab Care. 2007; Vol.10; p. 46. 3. Bescós R. , Effects of dietary L-arginine intake on cardiorespiratory and metabolic adaptation in athletes. Int J Sport Nutr Exerc Metab. 2009; Vol. 19; P. 355

06.089

Cardiovascular effects produced by chronic treatment of sodium cyclamate in hypertensive rats. Pereira AC, Zaroni ACE, Tavares JD, Furtado GS, Baracho NCV, Irulegui RSC FMIT

Introduction: Hypertension is the biggest problem in Brazil's healthy public service. The use of sweeteners has been growing in the last decades, mainly among diabetics and hypertensive population. Studies show that sodium cyclamate acts harmfully on many organs, between them liver and kidneys. Besides, the sodium cyclamate increases water reabsorption by the kidneys, which contributes for the increase of the blood pressure. The aim of this study was to determine the effect of chronic treatment with cyclamate, on the blood pressure of hypertensive rats. This project was approved by Comitê de Ética em Pesquisa (CEP - FMIt), protocol PAN 09/08 (15/09/09). **Methods:** Thirty-three female, adult, Wistar rats were used. The animals were submitted to surgery for induction experimental hypertension by modified Grollman´s model. Fifteen days after surgery, the hypertensive rats (MAP > 140mmHg) were allocated in three groups and received daily gavage for 120 days, according to the treatment as follow: 1) Distilled water (control group, n = 12); 2) Sodium cyclamate 500mg/kg (n = 9) and 3) Sodium cyclamate 1120mg/kg (n = 12). Mean arterial pressure (MAP) was measured by tail plestimography twice a week. At the end of experimental period, the rats were anesthetized (ketamine and Xylazin, IM), and their blood were analyzed. **Results:** The rats receiving 1120mg/kg cyclamate presented a significant increase of MAP in days 32, 39 and 81, when compared to control group (day 32: 172,3 ± 6,2 vs 165,8 ± 7,1, $p < 0,05$; day 39: 175,0 ± 6,1 vs 167,1 ± 8,9, $p < 0,04$; day 81: 173,1 ± 7,3 vs 165,8 ± 6,7, $p < 0,3$). Similarly, 500mg/kg cyclamate administration produced a significant increase of MAP in days 56 and 81, when compared to control group (day 56: 171,1 ± 10,3 vs 162,5 ± 10,7, $p < 0,02$; day 81: 175,9 ± 9,5 vs 165,8 ± 6,7, $p < 0,01$). Also, 1120mg/kg cyclamate administration produced a significant increased of MAP in days 1 and 39, when compared to 500mg/kg group (day 1: 183,3 ± 6,6 vs 175,4 ± 8,1, $p < 0,03$; day 39: 175 ± 6,1 vs 165,8 ± 7,1, $p < 0,01$). After these days, the hypertensive effect on both doses didn't remain stable during the following days of the experimental period. **Discussion:** These data indicate that the chronic administration of sodium cyclamate would produce increase in MAP in specific days. **Financial support:** PDIC-FMIt and FAPEMIG. **Bibliographic references**: Sanson SC. Proc Soc Exp Biol Med. 172. 111. 1983. Dórea EL. Rev Soc Bras Hipert. 7. 86. 2004. Castro AGPF. Arq Bras Endoc Metab. 46. 280. 2002. Zats RA. Lab Anim Sci. 42. 198. 1990. Johns C. Hypert. 28. 1064. 1996.