Setor 06. Cardiovascular e Renal/ Cardiovascular and Renal

06.001

Cardiac dysfunction in experimental sepsis. Bóf ER, DalBó S, Assreuy J UFSC - Farmacologia

Introduction: Systemic inflammatory response syndrome (SIRS), when complicated with an infection, may evolve to sepsis and septic shock which is an important cause of death in ICU. In general, death is caused by a cardiovascular collapse and refractory hypotension that begins early in sepsis. The cardiovascular collapse include the decrease in the peripheral vasomotor tone resulting from the release of a great number of chemical mediators. The vascular dysfunction is more studied and comprehended than the heart dysfunction, although the latter is widely recognized as a very important player in sepsis organ dysfunction. The aim of the present work is to characterize the heart dysfunction in the experimental sepsis induced by cecal ligation and puncture (CLP) in the mice. In addition, the time course of this dysfunction was also studied. **Methods:** Swiss female mice were submitted to CLP surgery. Since some parameters were altered even in sham-operated animals, we used a group of naïve mice as controls. Cardiac function was evaluated 6, 12 and 24 hours after CLP surgery using the isolated Langendorff heart preparation. Hearts were continuously perfused (2 mL/min) with Krebs-Ringer solution at 37ºC and constant oxygenation (5% CO2 and 95% O2). After isolation, hearts were allowed a 30-minutes stabilization period. The parameters evaluated were: systolic and diastolic tension, +dT/dt (contractility rate), -dT/dt (relaxation rate), coronary perfusion pressure and heart rate. For each contraction, the area under the curve (AUC; approximately the cardiac work) was obtained. All the procedures have been approved by our institutional Animal Ethics Committee (PP003/CEUA/UFSC). **Results:** Six hours after the CLP procedure, isolated hearts presented a decreased diastolic tension (20%) and an increase in +dT/dt (19%), whereas the systolic tension, -dT/dt, AUC, coronary perfusion pressure and heart rate were unaffected. Essentially the same findings were obtained 12 hours after CLP, except coronary perfusion pressure that was increased (20%). The main alterations in the cardiac parameters were observed 24 hours after CLP procedure. Hearts showed a significantly lower systolic (13%) and diastolic tension (13%), -dT/dt (15%) and a decreased in the AUC (17%). As before, +dT/dt, coronary perfusion pressure and heart rate did not differ from values found in control animals. **Discussion:** Our results demonstrate that: a) there is indeed a profound collapse in heart function induced by sepsis and b) this collapse is evident from the beginning of the septic process but it is more evident at later stages of the condition. As for the mechanisms underlying this condition our results are preliminary. Nevertheless, it may be that excessive relaxation of sarcomere thus reducing the capacity of contraction and/or an impaired in calcium uptake to the sarcoplasmic reticulum thus reducing the rate of relaxation are implicated in the myocardial dysfunction. On the other hand, the workings of the rhythmic cells seem to be maintained during the course of sepsis. Taken together, it seems that sepsis-induced heart dysfunction is mainly carried out by a mechanical failure. **Financial support: CAPES, CNPq.**

06.002

Increased circulating levels of pro-inflammatory markers in metabolic syndrome patients. Gonçalves FM[1], Jacob Ferreira ALB[1], Gomes VA[1], Tanus-Santos JE[2], Gerlach RF[3], Casella-Filho A[4] [1]FCM-UNICAMP - Farmacologia, [2]FMRP-USP - Farmacologia, [3]FORP- USP - Morfologia, [4]InCor-FMUSP

Introduction: The metabolic syndrome (MetS) is defined as a clustering of risk factors associated with increased risk for diabetes and cardiovascular diseases. This disorder is represented by insulin resistance, hyperglycemia, dyslipidemia, abdominal obesity, and hypertension. A chronic inflammatory state associated with impaired nitric oxide formation and endothelial dysfunction has been described in patients with MetS, thus leading to the development of atherosclerosis in these patients. Increased concentrations of pro-inflammatory mediators and growth factors also play a major role in the atherosclerotic process and may lead to clinical events. Little information exists about the level of inflammatory markers in patients with MetS. The aim of this study was investigate whether there are significant alterations in the circulating levels of inflammatory markers such as monocyte chemoattractant protein-1 (MCP-1), interleukin 6 (IL-6), and cell adhesion molecules including intercellular adhesion molecule (sICAM-1), and sP-selectin in MetS patients with those found in healthy controls. **Methods**: We studied 50 patients (age range: 18-80 years) including 25 patients with MetS selected from the routine outpatient clinic of the Heart Institute, and 25 healthy controls randomly selected from the local population and unrelated to the patients (approval of the ethics committee n° 547/05). The components of the MetS was defined according to the American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement: Abdominal obesity by waist circumference (men> 102 cm and women> 88 cm), Triglycerides Levels ≥ 150 mg / dL, HDL Cholesterol Levels (men <40 mg / dL and women <50 mg / dL), Blood pressure ≥ 130 mm Hg or ≥ 85 mmHg and Glycemia of fasting ≥ 110 mg / dL. Plasma from venous blood collected into EDTA tubes was stored -70^{o} C until assayed for sICAM-1, P-selectin, MCP-1 and IL-6 levels by ELISA. P values<0,05 were considered statistically significant. **Results**: We found significantly higher circulating concentrations of inflammatory markers and of cell adhesion molecules in MetS patients. The levels of sICAM-1, P-selectin, MCP-1 and IL-6 were higher in patients with MetS (80.63 ± 7.171 ng/ml, 48.48 ± 7.926 ng/ml, 137.1 ± 17.42 pg/ml, 5.403 ± 3.027 pg/ml, respectively) compared to controls (53.64 ± 9.124 ng/ml, 15.72 ± 2.802 ng/ml, 68.09 ± 8.707 pg/ml, 1.010 ± 0.3929 pg/ml, respectively; P<0,05). In addition, we found a correlation between the sICAM-1 levels and blood pressure, between the IL-6 levels and fasting glucose and between the sP-selectin levels and triglycerides. **Discussion**: We found higher levels of pro-inflammatory mediators and adhesion molecules in patients with MetS than in the control group. These findings may shed some light on mechanisms involved in the increased cardiovascular risk of MetS patients. **References:** Eckel, R.H. el al., *Lancet*, 365:1415, (2005). Grundy, S.M. et al., *Journal of American Heart Association,* 112:2735, (2005) **Support:** FAPESP and CNPq.

06.003

Anti-hipertensivos de ação central: efeitos sobre a hipertrofia do ventrículo esquerdo e alterações microcirculatórias de ratos espontaneamente hipertensos. Nascimento AR, Sabino BD, Lessa MA, Cruz FC, Tibiriçá E FIOCRUZ - Investigação Cardiovascular

Fundamentos: O aumento da resistência vascular sistêmica na hipertensão arterial é determinado essencialmente na microcirculação, resultando tanto de alterações funcionais quanto estruturais. Por outro lado, a hipertrofia do ventrículo esquerdo (HVE) é um achado comum na evolução clínica da hipertensão arterial e está diretamente associada com alto risco de morbi-mortalidade cardiovascular. **Objetivos**: No presente estudo, investigamos os efeitos do tratamento crônico com anti-hipertensivos de ação central sobre a densidade capilar funcional (DCF) cutânea e muscular esquelética (músculo grácil), assim como, a densidade capilar estrutural (DCE) muscular esquelética e miocárdica, e sobre a massa do ventrículo esquerdo (VE) de ratos espontaneamente hipertensos (SHR). **Métodos:** Ratos SHR machos com 12-14 semanas receberam tratamento oral com clonidina (CLO; 0,1 mg/kg/dia), rilmenidina (RIL; 1 mg/kg/dia), moxonidina (MOX; 10 mg/kg/d) ou veículo (grupo controle hipertenso) durante 4 semanas. Ratos Wistar-Kyoto foram utilizados como controles normotensos. Após o término do tratamento avaliou-se a DCF através de microscopia intravital por epi-iluminação com fluorescência. Para avaliação da massa do VE utilizou-se o método de Scherle. **Resultados:** O tratamento reduziu de forma similar a pressão arterial sistólica nos diferentes grupos de tratamento [197±4 mmHg para 122±5 mmHg (n=10), 193±7 mmHg para 119±3 mmHg (n=10) e de 191±4 mmHg para 127±4 mmHg (n=10), com CLO, RIL e MOX, respectivamente, $P<0,05$]. Foi observado aumento da DCF muscular esquelética de ratos SHR tratados com CLO, RIL ou MOX (512±14, 505±12 e 496±16 capilares/mm^2, respectivamente), comparados com o grupo controle hipertenso (294±24 capilares/mm^2, $P<0,05$). Observou-se aumento da DCF cutânea (533±24, 515±25 e 483±13 capilares/mm^2, tratados com CLO, RIL e MOX, respectivamente), comparados com o grupo controle hipertenso (332±16 capilares/mm^2, $P<0,05$). Os animais do grupo controle hipertenso apresentaram aumento da massa do VE, quando comparados com o grupo controle normotenso (2,40±0,09 g/kg e 1,69±0,04 g/kg, respectivamente, $P<0,05$). O grupo tratado com rilmenidina apresentou redução da massa do VE com relação ao grupo controle hipertenso (1,96±0,08 g/kg, $P<0,05$). **Conclusões:** O presente estudo demonstrou que o tratamento crônico com anti-hipertensivos de ação central resulta em aumento do número de capilares espontaneamente perfundidos na pele e no músculo esquelético de ratos hipertensos. Além disso, a HVE dos animais hipertensos foi parcialmente revertida pelo tratamento crônico com rilmenidina. **Apoio Financeiro:** FAPERJ, CNPq, FIOCRUZ.

06.004

Atorvastatin improves the hemodynamics disarrangements of sheep with acute pulmonary thromboembolism (APT) treated with sildenafil. Uzuelli JA, Dias-Junior CAC, Neto-Neves EM, Tanus-Santos JE FMRP-USP - Farmacologia

Introduction: Sildenafil, an orally active inhibitor of cGMP phosphodiesterase-type-5, exerts pulmonary vasodilator effects and improves pulmonary haemodynamics in both animal models and clinic patients with APT. Atorvastatin, an HMG-CoA reductase inhibitor, besides has been shown to lower serum cholesterol levels and it also presents some pleiotropic effects. So we examined the effects of the pre-treatment with atorvastatin on haemodynamic disarrangements induced by APT in sheep and treated with sildenafil. **Methods:** Santa Ines sheep 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 020/2007). Santa Ines sheep (17.0±1kg and 87.0±1cm), were subjected to pre-treatment with atorvastatin (30mg/kg, s.c.), during 7 days. The animals were anesthetized and surgically prepared according to standard procedures. APT was induced with autologous dry clots (0.5g/kg), according to standard procedures, injected into the right atrium. Experimental groups were: Sham (n=6), Emb (n=6), Ator + Emb (n=6), Emb + Sild (n=6) and Ator + Emb + Sild (n=6). Haemodynamic evaluations were carried out for one hour after APT. Then, sildenafil (0.7mg/kg, i.v., over 30 minutes) or saline were injected into left femoral vein and the hemodynamic evaluations continued for more ninety minutes. It was measured the hemodynamic parameters: mean pulmonary artery pressure (MPAP), pulmonary vascular resistance index (PVRI), mean artery pressure (MAP), systemic vascular resistance index (SVRI), heart rate (HR) and cardiac index (CI). All the results are expressed as means±S.E.M. One-way analysis of variance (ANOVA) for repeated measures followed by the Dunnett multiple comparisons test were used to determine the changes in the hemodynamic. A probability value <0.05 was considered the minimum level of statistical significance. **Results:** The hemodynamic data from the Sham animals showed no significant changes throughout the study period. All groups study present the same basal conditions. APT produced increase MPAP in 30 mmHg in Emb group, from 12±2 mmHg (basal) to 42±5 mmHg fifteen minutes after APT, remaining constant during the experiment ($p<0.05$ versus Sham group). In Ator + Emb group, MPAP increased from 13±2 mmHg to 33±6 mmHg, showing an attenuation in relation to Emb group ($p<0.05$). Sildenafil infusion in Ator + Emb + Sild group, the MPAP decreased from 33±6 to 22±4 mmHg ($p<0.05$ versus Emb group and Ator + Emb group). PVRI presents similar statistical behavior to MPAP. While sildenafil has improved atorvastatin effects, the association produced slight systemic hypotension, 60 minutes after sildenafil infusion. MAP decreased from 80±10 mmHg to 66±5 mmHg ($p<0.05$ versus Ator + Emb group) and SVRI decreased from 1798 $dyn.s.cm^{-5}.m^{-2}$ to 1556 $dyn.s.cm^{-5}.m^{-2}$ ($p<0.05$ versus Ator + Emb group). HR and CI no showed significant changes throughout study period. **Discussion:** Our findings suggest atorvastatin attenuated pulmonary hypertension induced by APT and sildenafil infusion improved pulmonary vasodilator effects showed for atorvastatin. **Acknowledgments:** FAPESP, CAPES and CNPq.

06.005

eNOS haplotype associated to hypertension does not affect susceptibility to cardiac hypertrophy. Lacchini R[1], Vasconcellos VB[2], Sales ML[3], Ferreira-Sae MC[3], Schreiber R[3], Nadruz Filho W[3], Tanus-Santos JE[2] [1]FCM-UNICAMP - Pharmacology, [2]FMRP-USP - Pharmacology, [3]FCM-UNICAMP - Internal Medicine

Introduction: Hypertension is one of the most important issues in health care system nowadays[1]. The progression of disease leads to cardiovascular remodeling process, which is characterized by increase in wall thickness of arterial vessels, end organ damage, cardiac hypertrophy, and other hazardous effects. Together, all this effects are responsible for the high morbidity and mortality of hypertensive disease. Nitric Oxide (NO) is the most important factor for endothelial dependent vasodilatation, and variations in eNOS gene (*NOS3*) are associated to variations in NO production[2]. The studied haplotypes are composed of three polymorphisms: $T^{-786}C$, intron 4 Variable Number of Tandem Repeats (VNTR) and Glu298Asp. Some of this haplotypes seem to be associated to hypertension, and others seem to have protective effects[3]. The objective of this work was to assess the influence of eNOS haplotypes on echocardiographic parameters on hypertensives. **Materials and Methods:** In this study were included 101 healthy volunteers and 173 hypertensive patients from Hospital de Clínicas of UNICAMP. Blood was collected and used for DNA extraction with salting out method. Polymorphisms $T^{-786}C$ and Glu298Asp were genotyped by Restriction Fragment Length Polymorphism, and Intron 4 VNTR by PCR. All patients were sent to echocardiography analysis as described elsewhere[4, 5]. This study was approved by Human Research Ethics Committee of FCM-UNICAMP (process number: 181/2005). Statistical analysis was made using χ^2, student´s t test and one way ANOVA with Tukey´s post test, when necessary. It was considered significant a $P<0.05$, except in haplotipic frequencies comparison, when P was corrected for multiple comparisons ($P<0.00625$). **Results and Discussion:** There were no differences in allelic and genotypic distributions between groups. On haplotypic distributions, however, there was na association between CAG haplotype (allele “C” of $T^{-786}C$; “a” of intron 4 VNTR; “G” of Glu298Asp) and hypertension phenotype. There was an association of CBG haplotype and the healthy group, thus suggesting an protective effect against hypertension. There were no changes in arterial pressure and echocardiographic parameters measured between the haplotypic groups, suggesting that eNOS heliotypes probably don’t have an important role on cardiac hypertrophic process. 1. Chobanian A. V., et al., *Hypertension*, vol. 42, p. 1206, 2003; 2. Metzger I. F., et al., *Free Radic Biol Med*, vol. 43, p. 987, 2007; 3. Sandrim V. C., et al., *J Hypertens*, vol. 24, p. 2393, 2006; 4. Borges M. C., et al., *Hypertension*, vol. 47, p. 854, 2006; 5. Sales M. L., et al., *J Hum Hypertens*, vol. 21, p. 504, 2007. **Financial support:** FAPESP, CNPq, CAPES

06.006

Participação da endotelina no aumento das ações venocontráteis da fenilefrina (PHE) e da angiotensina II (ANG II) induzido pelo exercício em veias porta isoladas de ratos treinados. Chies AB[1], Rossignoli PS[2], Silva OG[3] [1]FAMEMA - Ciências Fisiológicas, [2]UNESP-Botucatu - Farmacologia, [3]UNIMAR- Farmácia

Introdução: Em estudos anteriores realizados em preparações de veia porta de ratos observamos, após um período de treinamento, que as respostas vasomotora à PHE e à ANG II obtidas em animais estudados após uma sessão de exercício é maior em comparação aos animais mantidos em repouso. Esse fenômeno parece envolver um balanço entre o óxido nítrico e prostanóides vasodilatadores e algum mecanismo local vasoconstritor não identificado. Assim, objetivamos estudar a participação da endotelina neste aumento de resposta vasomotora de veia porta à PHE e à ANG II induzido pelo exercício. **Métodos:** Trabalho aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Medicina de Marília (registro nº 662/07). Ratos Wistar adultos ♂ foram treinados em esteira, 5 dias/semana por 10 semanas (velocidade correspondente a 60% da capacidade máxima do animal). Ao final desse período, esses animais treinados e sedentários (pesando entre 300-350g), em repouso ou reexpostos a uma sessão de exercício, foram anestesiados com tribromoetanol (250 mg/kg, i.p.) e exanguinados para retirada de segmentos (5mm) de veia porta. Preparações circulares de veia porta foram montadas em cubas para estudo de órgão isolado com solução de Krebs-Henseleit, pH 7.4, aerado com mistura carbogênica (95% O_2 e 5% CO_2) e aquecido a 37°C. Contrações (isométricas) sob tensão basal de 0,5g foram registradas através de sistema PowerLab®. Curvas concentração-resposta foram obtidas em preparações não tratadas e tratadas com BQ-123 (10^{-6}M) ou BQ-788 (10^{-6}M), respectivos antagonistas seletivos de receptores de endotelina subtipos ET_A e ET_B. Comparações (n de 6) foram feitas entre valores de pD_2 (- log da EC_{50}) e de resposta máxima em gramas (Rmax) por meio de Análise de Variância de uma via (ANOVA). Diferenças significativas quando $P<0,05$. **Resultados:** O exercício não modificou significativamente o Rmax tanto da ANG II quanto da PHE em veias porta isoladas de ratos treinados, pré-tratadas com o BQ-123. O exercício físico também não modificou significativamente o Rmax da ANG II em preparações de veia porta obtidas de ratos treinados, pré-tratadas com o BQ-788. Por outro lado, na presença de BQ-788, o Rmax para PHE em preparações de veia porta obtidas de ratos treinados foi significativamente diminuído, ao invés de ser aumentado, pela exposição ao exercício físico (0,99±0,14g para 0,53±0,7g; $P<0,05$). Essa redução de Rmax, nestas preparações, foi acompanhada por redução significativa de pD_2 (6,52±0,16 para 5,67±0,19; $P<0,05$). **Discussão:** O bloqueio seletivo dos receptores ET_A suprimiu o aumento de resposta contrátil à PHE e à ANGII induzido pelo exercício, verificado anteriormente em preparações de veia porta obtidas de rato treinado. O mesmo acontece em relação à ANG II na vigência do bloqueio dos receptores ET_B. Além disso, o bloqueio dos receptores ET_B não apenas previne o aumento, mas também propicia uma redução da resposta contrátil à PHE induzida pelo exercício em preparações de veia porta obtidas de rato treinado. Estes dados sugerem que a endotelina possivelmente participa das modificações de resposta vasomotora induzida pelo exercício em veia porta de animais treinados. **Apoio Financeiro:** FAPESP (processo nº 05/51550-4).

06.007

Papel do organocalcogênio disseleneto de difenila como um novo agente antiaterogênico. Hort MA[1], Netto PM[1], Oliveira J[2], Bem AF[2], Ribeiro-do-Valle RM[1]
[1]UFSC - Farmacologia, [2]UFSC - Bioquímica

Introdução: O disselento de difenila (DD) é um composto orgânico de selênio, que apresenta importantes propriedades antioxidantes atuando como mimético da enzima glutationa peroxidase. Diversas propriedades farmacológicas deste composto foram descritas, como atividades antiinflamatória, antinociceptiva, anti-úlcera, neuroprotetora e antioxidante. Recentemente foi demonstrado que este composto é capaz de inibir a oxidação da LDL humana *in vitro.* Este trabalho teve por objetivo investigar o efeito do DD sobre o processo aterosclerótico *in vivo.* **Métodos**: Camundongos machos C57BL/6 (LDLr -/-) com 3 meses de idade foram divididos em 4 grupos (n=5-8): CDN (veículo + dieta normal - DN); CDH (veículo + dieta hipercolesterolêmica -DH); DD1 (DD 1 mg/kg + DH) e DD10 (DD 10 mg/kg + DH). Os animais foram tratados por via oral, uma vez ao dia durante 30 dias. Ao final do tratamento foram avaliados: peso, ingesta alimentar, lipídeos plasmáticos, marcadores de estresse oxidativo (peroxidação lipídica e SH-não protéico - NPSH), no fígado e cérebro, e tamanho da lesão aterosclerótica. O protocolo experimental foi aprovado pelo Comitê de Ética para o Uso de Animais da Universidade Federal de Santa Catarina (PP00226). **Resultados**: A dieta hipercolesterolêmica promoveu um acréscimo significativo nos níveis de lipídeos plasmáticos e o tratamento com o DD não foi capaz de alterar estes parâmetros. A hipercolesterolemia induziu um significativo aumento na lipoperoxidação, a qual não foi acompanhada pela modificação dos níveis de NPSH no cérebro dos animais, entretanto, o tratamento com DD foi capaz de prevenir o aumento da peroxidação lipídica (CDN, 20,69 ± 3,10; CDH, 34,69 ± ,66; DD1, 17,67 ± 2,40; DD10, 16,00 ± 1,97 nmol/g tecido). No fígado a hipercolesterolemia não induziu aumento na peroxidação lipídica, todavia diminuiu significativamente os níveis de NPSH. O tratamento com DD preveniu a redução nos níveis de NPSH de maneira significativa neste órgão (CDN, 622,20 ± 60,12; CDH, 347,95 ± 45,44; DD1, 674,06±38,12; DD10, 797,25± 58,21 nmol/g tecido). Os animais que receberam a menor dose de DD apresentaram redução no tamanho da lesão aterosclerótica na aorta em relação ao grupo CDH (CDN 30,83 ± 7,43; CDH, 199,26 ± 43,41; DD1, 89,00 ± 19,93; DD10 130,21 ± 17,39 x 10^3 μm^2). Discussão: O tratamento com DD foi capaz de promover um aumento das defesas antioxidantes hepáticas e reduzir os danos oxidativos aos lipídeos no cérebro de animais LDLr -/- submetidos à DH. Além disso, o tratamento com o composto foi capaz de reduzir a progressão da lesão aterosclerótica na aorta dos animais hipercolesterolêmicos. Estas propriedades apresentadas pelo DD sobre o processo aterosclerótico podem futuramente contribuir para o tratamento desta patologia. Apoio Financeiro: CNPq, Finep, Fapesc

06.008

Efeito do extrato de *Echinodorus grandiflorus* sobre a rarefação capilar: avaliação histológica e por microscopia intravital. Gomes F[1], Figueiredo MR[2], Benedito BN[1], Lessa MA[1], Tibiriçá E[1] [1]FIOCRUZ - Investigação Cardiovascular, [2]FIOCRUZ - Tecnologia em Fármacos / Produtos Naturais

Introdução: A hipertensão é um importante desafio para a saúde pública, e quando não tratada, predispõe a morbidade cardiovascular e a morte prematura. É sabido que a maior parte do aumento da resistência vascular na hipertensão, causadora de danos a órgãos alvos, é determinada no nível microvascular e evidências recentes sugerem que uma redução da densidade capilar pode contribuir significativamente para a elevação da resistência vascular e, conseqüentemente, da pressão arterial tanto em seres humanos como em animais de experimentação. O *Echinodorus grandiflorus* (EG), uma planta bem distribuída por todo o Brasil, vem sendo utilizada na medicina popular para tratamento de hipertensão e doenças inflamatórias. No entanto, não há estudos farmacológicos sobre os efeitos biológicos do extrato. **Objetivos**: Investigar os efeitos do EG sobre as alterações estruturais e funcionais da microcirculação da pele, do músculo esquelético, em ratos espontaneamente hipertensos (SHR). **Metodologia:** 50 animais SHR e Wistar-kyoto (WKY) com 14 semanas de vida foram utilizados. Após um período de adaptação, a pressão arterial e a frequência cardíaca dos ratos foram medidas de maneira não invasiva durante 4 semanas, 1 vez por semana. Todos os animais foram divididos em grupos de animais tratados durante 28 dias por gavagem com veículo (SHR, o grupo controle hipertenso e Wistar, o grupo controle normotenso), EG (doses 50mg/kg, 100mg/kg e 200mg/kg). Ao final do tratamento os animais foram submetidos a procedimentos anestésico-cirúrgicos para a realização da microscopia intravital e avaliação da microcirculação pela contagem de capilares em tempo real na pele da orelha e no músculo esquelético. As amostras foram cortadas e imersas em fixador para futuras análises. **Resultados:** O tratamento com EG reduziu a pressão arterial sistólica de todos os grupos. O tratamento com EG 100 e EG 200 reverteu completamente à rarefação capilar no músculo esquelético e na pele (316±60 e 353±93 capilares/mm^2 e 319±56 e 384±70 capilares/mm^2, $p<0,05$, respectivamente). **Conclusão**: O tratamento crônico com EG foi capaz de reduzir pressão sistólica e capaz de reverter completamente à rarefação capilar em animais espontaneamente hipertensos. **Apoio financeiro**: Fiocruz, PDTIS, CNPq.

06.009

Efeito da orquidectomia (ORQ) e da reposição hormonal com testoterona sobre as respostas de veias isoladas de rato à fenilefrina (Phe). Rossignoli PS[1], Pereira OCM[2], Chies AB[3] [1]IB-UNESP-Botucatu / FAMEMA - Farmacologia, [2]IB-UNESP-Botucatu - Farmacologia, [3]FAMEMA - Farmacologia

Introdução: O sistema cardiovascular tem sido considerado um importante alvo de ações androgênicas. Com efeito, dúvidas existem acerca dos mecanismos pelos quais a testosterona modifica a fisiologia cardiovascular, sobretudo em leitos venosos, um compartimento de capacitância que garante um adequado aporte sangüíneo ao coração. Assim, o objetivo do presente estudo foi investigar a influência da ORQ e da reposição com testosterona sobre as respostas vasomotoras de veias isoladas de rato à Phe. **Métodos:** Este trabalho foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Medicina de Marília (registro nº232/07). Foram utilizados ratos Wistar ♂ adultos (300-350g) controles (CONT) e orquidectomizados, seguidos (ORQ+T) ou não (ORQ) de reposição hormonal (propionato de testosterona, 10mg/kg, i.p., por 3 semanas, com intervalo de 5 dias entre as doses). Estes animais, ao final do tratamento, foram anestesiados com tribromoetanol (250mg/kg, i.p.) e exsanguinados**.** Anéis (4-5mm) de veias porta e cava foram isolados e montados em cubas contendo solução de Krebs-Henseleit, pH 7.4, gaseado com carbogênio (95%O_2 e 5%CO_2) e aquecido a 37°C. Modificações de tônus vascular em preparações incubadas com salina, L-NAME (10^{-4}M), L-NAME+indometacina (INDO;10^{-5}M), BQ-123 (10^{-6}M) e BQ-788 (10^{-6}M) foram registradas através de transdutores isométricos de tensão. Algumas preparações, antes dos estudos de reatividade vascular, tiveram seus endotélios removidos quimicamente pelo uso de solução de deoxicolato de sódio 0,75%. A partir das curvas concentração-resposta obtidas, calculou-se pD_2 ($-\log EC_{50}$) e resposta máxima em gramas (Rmax). Comparações (n de 6) foram feitas por meio de Análise de Variância de uma via (ANOVA) para 3 grupos ou teste *t* de Student para 2 grupos. Diferenças significativas quando $P<0,05$. A efetividade da ORQ e da reposição hormonal foi avaliada por pesagem de órgãos hormônio-dependentes e dosagem plasmática de testosterona. **Resultados:** A ORQ reduziu o peso das vesículas seminais (de 3,867±0,08g/kg animal para 0,427±0,02g/kg animal, $P<0,001$) e próstata (de 1,313±0,07g/kg animal para 0,251±0,02g/kg animal, $P<0,001$) e a concentração sérica de testosterona (de 200,4±52,67ng/dl para 9,87±4,70ng/dl, $P<0,001$), parâmetros estes revertidos pela reposição com testosterona. A ORQ aumentou a Rmax para Phe em veia porta isolada de rato (de 0,840±0,11g para 1,454±0,11g, $P<0,001$), mas não em veia cava. Este aumento de Rmax foi revertido pela reposição hormonal com testosterona. Além disso, o aumento de Rmax para Phe não foi suprimido na presença de L-NAME (de 1,559±0,17g para 2,045±0,11g, $P<0,05$) ou L-NAME+INDO (de 1,157±0,16g para 1,728±0,15g, $P<0,05$). Contudo, foi completamente suprimido na presença do BQ-123 e do BQ-788. A remoção endotelial também não suprimiu o observado aumento de Rmax para Phe em veia porta (de 0,258±0,04g para 0,622±0,13g, $P<0,05$). **Discussão:** A redução drástica dos níveis circulantes de testosterona induzida pela ORQ promoveu um aumento de resposta de veia porta de rato à Phe. Este aumento de resposta, possivelmente, envolveu produção sub-endotelial de endotelina que, por sua vez, atuou através da ativação tanto de receptores ET_A quanto ET_B. **Apoio Financeiro:** FAPESP - processo nº 07/53228-8

06.010

B1 and B2 kinin receptors regulate cardiovascular function. Lauton-Santos S[1], Capettini LSA[2], Fernandes VA[3], Castro CH[2], Rodrigues, ALP[4], Pesquero JL[2], Pesquero JB[5], Bader M[6], Almeida AP[2], Cruz JS[7] [1]UFS - Fisiologia, [2]UFMG-ICB - Fisiologia e Biofísica, [3]ISEAT-FHA,, [4]FUMEC-FCS, [5]UNIFESP - Biofísica, [6]Max-Delbrück - Molecular Medicine/Hipertension, [7]UFMG - Bioquímica e Imunologia

Introduction: Kinins have an important role in the control of cardiovascular system. Classically, there are two kinin receptors: B2 receptor (B2r) that is constitutively expressed and B1 receptor (B1r) that is induced by inflammatory stimulus. B2r are commonly associated to cardiovascular effects of bradykinin. **Aim:** We aimed evaluate the relative roles of both B1r and B2r on modulation in heart from mice. **Methods:** Were used C57Bl/6J mice (WT) as control and animals with genic deletion (knockout, KO) of B1r ($B1^{-/-}$), B2r ($B2^{-/-}$) and double KO mice to both B1r and B2r ($B1B2r^{-/-}$). All experiments were carried out according to Animal Research Ethical Committee of UFMG (protocol # 078/05 - CETEA). Cardiac function was analyzed by aortic retrograde perfusion in Langendorff apparatus. **Results and discussion:** Our results shown no significant difference (p=0,1429) between body weight/ heart weight in WT animals (6,65 ± 0,56 mg/g - n=5) when compared to $B1B2^{-/-}$ (7,92 ± 0,61 mg/g- n=3) $B1^{-/-}$ (6,81 ± 0,39 mg/g - n=8) and $B2^{-/-}$ (7,95 ± 0,50 mg/g- n=4). This shows an absence of cardiac hypertrophy in animals groups available, with age between 14 - 16 weeks. $B1^{-/-}$ (1,61±0,08g) and $B1B2^{-/-}$ (1,86 ± 0,03g) animals, but not $B2^{-/-}$ (2,59 ± 0,06g), presented significant reduction (p<0,0001) in systolic tension when compared to WT animals (2,58 ± 0,04g) suggesting exclusive participation of B1r in systolic modulation. There were no differences in diastolic tension in WT mice (0,35±0,005g) when compared to animals $B1B2^{-/-}$ (0,42 ± 0,009g), $B1^{-/-}$ (0,37 ± 0,02g) and $B2^{-/-}$ (0,41 ± 0,006g). Also, no statistic differences (p>0,05) were found analyzing heart rate in all groups WT (312 ± 3,2 bpm) when compared to animals $B1B2^{-/-}$ (260 ± 2,8 bpm), $B1^{-/-}$ (295 ±4,3 bpm) and $B2^{-/-}$ (296±3,2 bpm). On the other hand, coronary resistance was higher (p<0,05) in $B1^{-/-}$ (161,0±0,56 mmHg); $B2^{-/-}$ (163,2±1,7 mmHg) and $B1B2^{-/-}$ (174,7±0,46 mmHg) animals, when compared to WT animals (124±1,4mmHg), suggesting a vascular dysfunction induced by deficiency in kinin receptors. **Conclusion:** These data showed that both B1 and B2 kinin receptors regulate cardiovascular functions. **Financial support:** ISEAT/FHA, CNPq, UFS and FAPEMIG.

06.011

Routes and enzymes involved in the conversion of Ang II to Ang-(3-4) in renal basolateral membranes. Axelband F[1], Dias J[1], Miranda F[1], Ferrão F[1], Carmona AK[2], Barros, NM[2], Lara Morcillo LS[3], Vieyra A[1] [1]IBCCF-UFRJ, [2]UNIFESP - Biofísica, [3]UFRJ - Farmacologia Básica e Clínica

Introduction: We previously demonstrated that angiotensin II (Ang II) modulate the Ca2+-ATPase (PMCA) resident in the basolateral membranes of kidney proximal tubule cells (BLM) in a biphasic manner (Assunção-Miranda et al., 2005, Axelband et al., 2009). Picomolar concentrations inhibit the enzyme activity (Assunção-Miranda et al., 2005), whereas high Ang II concentration (5 × 10-7 M) led to the recovery of the Ca2+ pump (Axelband et al., 2009). This latter effect was attributed to Ang II hydrolysis to two metabolites, identified by HPLC as Ang-(3-4) and the aminoacid Tyr, providing Ang-(1-7) as an intermediate product (Axelband et al., 2009). Besides, Ang-(3-4) was confirmed as an active biologically peptide, potent reactivator of Ang II-inhibited PMCA (Axelband et al., 2009). The objective of this work was to identify the routes and enzymes involved in the conversion of Ang II to Ang-(3-4) in BLM. **Methods:** The activity of the angiotensin-converting enzyme (ACE), the angiotensin-converting enzyme 2 (ACE 2), aminopeptidases (AP) and neprilysin (NEP) were measured in BLM by spectrofluorometry using fluorescent substrates in the absence or presence of the enzymes inhibitors. The proteolysis of Ang II and Ang II-derived metabolites were also assayed in the presence of peptidases inhibitors and detected by HPLC. **Results:** There are detected ACE, NEP and AP activities, but not ACE 2 activity in BLM. Plummer's inhibitor (carboxypeptidase N, CPN, inhibitor) blocks the Ang II metabolization, but DX600 (ACE 2 inhibitor) allows its metabolization to Ang-(3-4) and Tyr. In the presence of losartan (ACE inhibitor) and using Ang-(1-7) as the initial peptide for hydrolysis, it is not possible to generate the two final metabolites, indicating that Ang-(1-5) must be formed and ACE is needed for it. Ang-(1-5) hydrolysis is partially blocked in the presence of Plummer's inhibitor. Furthermore, the Ang III incubation within the BLM is also able to generate Ang-(3-4) and Tyr and this route is partially inhibited by bestatin (AP inhibitor). The BLM incubation with Ang III in the presence of PCMB (carboxypeptidase inhibitor) doesn't modify the Ang(3-4) and Tyr formation. **Discussion:** CPN catalyzes the first step of Ang II conversion to Ang-(1-7). As Ang-(1-7) cannot be metabolized in the presence of losartan, Ang-(1-5) generation by ACE seems to be essential to lead the final metabolites. The participation of a CPN to convert Ang-(1-5) to Ang-(1-4) is shown to be the following step. Ang III could be another precursor for Ang-(3-4) and Tyr formation in BLM. This route is partially dependent of an AP activity to convert Ang III to Ang IV, but excludes Ang-(3-7) as an intermediate, once that PCMB does not alter the formation of Ang-(3-4) and Tyr. This results indicate that the main route for Ang II metabolization in BLM is Ang II - Ang-(1-7) - Ang-(1-5) - Ang-(1-4) - Ang-(3-4), having as an alternative route Ang II - Ang III - Ang IV - Ang-(3-4) + Ang-(5-8). **Financial Support:** FAPERJ, CNPq

06.012

Metabolism of angiotensins I and II in the perfusate and filtrate obtained from isolated rat kidney. Sivieri-Jr DO[1], Pereira HJV[2], Oliveira EB[2], Salgado MCO[1] [1]FMRP-USP - Pharmacology, [2]FMRP-USP - Biochemistry and Immunology

Introduction: We evaluated the angiotensin (Ang) I and II metabolism in vascular renal perfusate (VRP) and renal filtrate (RF) from isolated kidney of Wistar rats (n=4). **Methods**: The renal artery was cannulated with a polyethylene tube and the kidney was removed and perfused with Tyrode solution for 2 h at a constant flow rate of 4 mL/min in a water-jacketed organ bath maintained at 37°C. The VRP, 260 mL, was collected and concentrated 185-fold prior to use, and the RF, 1.4 mL, was collected simultaneously through a polyethylene cannula implanted in the ureter. Processing of Ang I and II was evaluated by incubating 20 nmol of either peptide for 40 min at 37ºC with samples of VRP or RF containing an amount of proteolytic activity corresponding to that released into these fluids during ~ 48 s of collection. 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 in this study 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: C**omparison of the ability of VRP and RF of generating various Ang fragments was achieved by determining the specific products formed during the Ang I cleavage reaction. The major products formed were (VRP vs. RF; nmol/min) Ang 3-10 (0.09 vs. 0.04) and Ang 1-7 (0.03 vs. 0.08). The presence of PHO decreased Ang 1-7 formation in the VRP-catalyzed cleavage of Ang I and abolished the Ang 1-7 formation in the reactions carried out with RF. BES increased the Ang 1-7 formation only in the VRP-catalyzed cleavage of Ang I and did not affect the Ang 3-10 generation. ORT decreased the generation of all fragments from Ang I in the reactions catalyzed by both fluids. Similarly, the Ang II degradation by proteolitic activities present in the VRP and RF was evaluated. The major products generated were (VRP vs. RF; nmol/min) Ang 4-8 (0.1 vs. 0.08) and Ang 5-8 (0.04 vs. 0.09). The presence of BES decreased Ang 5-8 and Ang 4-8 formation only in reactions carried out with RF. CHY decreased the Ang 4-8 formation only in the VRP-catalyzed cleavage of Ang II. ORT decreased the formation of all fragments from Ang II in the reactions catalyzed by proteolytic activity present in the VRP and RF. **Discussion**: The processing of Ang I and II effected by soluble peptidases of VRP and RF was similar concerning the total angiotensinolytic activity but was significantly distinct regarding the proteolytic specificities of the effector enzymes present in VRP and RF. It is noteworthy that the major proteolitic activities observed in both fluids belong to the metallopeptidase family of proteases. Although different aminopeptidases and endopeptidases are involved in the VRP and RF-catalyzed degradation of Ang, the neutral endopeptidase 24.11 seems to be the major proteolitic activity involved in the RF-catalyzed cleavage of Ang I. On the other hand, aminopeptidases play an important role in the VRP-catalyzed Ang I degradation. **Supported by**: FAPESP.

06.013

Role of reactive oxygen species and cyclooxygenase (COX) metabolites in ethanol-induced contraction and elevation in cytosolic calcium concentration in rat aorta.

Tirapelli CR[1], Yogi A[2], Callera GE[3], Hipólito UV[4], Silva CR[4], Touyz RM[3] [1]EERP-USP - Farmacologia, [2]USP - Farmacologia, [3]University of Ottawa - Kidney Research Center, [4]EERP-USP - Enfermagem Psiquiátrica e Ciências Humanas

Introduction: Ethanol can evoke direct contractile responses in different blood vessels. However, the molecular mechanisms and possible mediators underlying ethanol-induced contraction of vascular smooth muscle remain not completely understood. The present study has attempted to investigate the role played by reactive oxygen species (ROS) and cyclooxygenase metabolites in ethanol-induced contraction and elevation in intracellular Ca^{2+} ($[Ca^{2+}]i$). **Methods:** Vascular reactivity experiments, using standard muscle bath procedures, were used to evaluate ethanol-induced contraction in isolated rat aortic rings. Cultured vascular smooth muscle cells were used to detect ROS generation and ($[Ca^{2+}]i$) in response to ethanol. The protocols were approved by Comissão de Ética no Uso de Animais - USP Ribeirão Preto (Protocol number: 07.1.254.53.1). **Results**: Vascular reactivity experiments showed that ethanol (1-800 mM) induces contraction in endothelium intact (0.40 ± 0.04g, n=10) or denuded (0.81 ± 0.05g, n=9) rat aortic rings. Tiron (1 mM), a superoxide anion (O_2^-) scavenger and catalase (300 U/ml), a hydrogen peroxide (H_2O_2) scavenger reduced ethanol-induced contraction in both endothelium intact (0.18 ± 0.04g, n=4; 0.21 ± 0.05g, n=5, respectively) and denuded rings (0.49 ± 0.06g, n=5; 0.38 ± 0.03g, n=5 respectively) ($P<0.05$, ANOVA). Similarly, indomethacin (10 μM, a non selective COX inhibitor), SC560 (1 μM, a selective COX-1 inhibitor), AH6809 (10 μM, an antagonist of PGF_{2a} receptors) or SQ29584 (3 μM, an antagonist of PGH_2/TXA_2 receptors) inhibited ethanol-induced contraction. On the other hand, SC236 (0.1 nM, a selective COX-2 inhibitor) did not affect ethanol-induced contraction. In cultured vascular smooth muscle cells, ethanol (100 mM) induced O_2^- and H_2O_2 generation, which were detected by lucigenin chemiluminescence and Amplex red, respectively. Ethanol induced a transient increase in $[Ca^{2+}]i$, which was detected using FURA-2AM. This response was significantly inhibited in cells pre-exposed to tiron (1 mM) or indomethacin (10 mM). **Discussion**: These results support the importance of ROS and COX pathways to ethanol-associated vascular smooth muscle contraction and increase in $[Ca^{2+}]_i$. Although ethanol-induced vasoconstriction is clearly a complicated event, the approach taken in this study could help shed new light on the etiologies of hypertension, ischemia, and diverse vascular disease states associated with ethanol consumption.
Financial Support: FAPESP

06.014

Modulation of cardiac and renal ATPases in endothelial nitric oxide synthase gene knockout mice. Rezende DC[1], Caricati-Neto A[2], Jurkiewicz A[2], Pôças ESC[3], Noel F[1], Quintas LEM[1] [1]ICB-UFRJ - Farmacologia, [2]UNIFESP - Farmacologia, [3]IFRJ - Farmacologia

Introduction: eNOS polymorphisms have been associated, in humans, with some cardiovascular diseases. Importantly, NO has been shown to be an important endogenous cytoprotective scavenger of reactive oxygen species like superoxide anion. Here we evaluate the influence of the absence of eNOS on some important proteins from two organs that have crucial role on maintaining cardiovascular homeostasis: heart and kidney. Our aim was to assess enzymatic activity and protein expression of Na^+/K^+-ATPase (NKA) αisoforms and Ca^{2+}-ATPases (CaA) from mice knocked out for the endothelial nitric oxide synthase gene (eNOS/KO). **Methods:** All animal procedures were approved by the Ethics Committee (CEP/UNIFESP). Male 10-14-week-old eNOS/KO or wild-type (C57BL/6J) mice were weighed, anesthetized and their hearts and kidneys were excised. Organs were homogenized and passed through differential centrifugation to obtain particulate (plasmalemmal) fractions. To evaluate protein expression, samples ran on 5, 7.5 or 10% SDS-PAGE followed by immunoblotting with suitable antibodies. NKA activity was defined as the ATPase activity inhibited by ouabain, calculated as the difference between the activity in the absence (total) and in the presence of 1 mM ouabain. CaA activity was calculated as the difference between the ATPase activity in the presence (total) and in the absence of 10 µM Ca^{2+}. The free sulfydryl content was determined by the Ellman's method. This colorimetric method is based on the reaction of free sulfydryl groups with 0.1 mM 5,50-dithiobis-(2-nitrobenzoic acid), DTNB. Cystein (5-100 µM) was used as standard. **Results:** Myocardial NKA activity of eNOS/KO mice was 70% of wild-type values ($p<0.05$, n=3), while renal NKA activity of eNOS/KO mice was 86% ($p<0.05$, n=3). Immunoblot showed that protein expression of NKA α2 isoform, but not α1 or α3, was reduced (43% of wild-type, $p<0.05$, n=3) in eNOS/KO hearts. Likewise, no difference for the exclusive NKA α1 isoform density was observed in eNOS/KO kidneys. Myocardial CaA activity of eNOS/KO mice was similar of wild-type values, but SERCA density was significantly higher (290% of wild-type, $p<0.05$, n=3) in eNOS/KO. Assessing the content of free sulfydryl groups in heart and kidney preparations, a significant decrease of free sulfydryl groups in eNOS/KO mice was observed (77% for heart, 65% for kidney, $p<0.05$, n=3). **Discussion:** In eNOS/KO model, reduced local endogenous NO generation, caused by absence of eNOS may contribute to depressed NKA and CaA activity, when compared to their protein levels. Since the presence of reduced sulfydryl groups in the catalytic site of ATPases seems to be essential for its activity, our results indicate that redox unbalance in eNOS/KO mice could oxidize sulfydryl groups of the enzymes and thus depress their function. Besides, downregulation of the pressure-sensitive NKA α2 isoform in the heart may represent an adjustment to pressure overload (*digitalis-like* effect). Perhaps, alterations observed in the expression of SERCA may indicate an adaptation to compensate impaired myocardial Ca^{2+} homeostasis. **Financial Support:** FAPERJ, CAPES, CNPq, FAPESP

06.015

Chronic ethanol consumption decreases the relaxation induced by adrenomedullin and increases its expression in the isolated rat aorta. Hipólito UV[1], Tirapelli CR[1], Tirapelli D[2], Queiroz RHC[3] [1]EERP-USP - Farmacologia, [2]FMRP - Cirurgia e Anatomia, [3]FCFRP-USP - Toxicologia

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:** Ethical Animal Committee number (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. 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 (260 ± 10g) in control group and (266 ± 8g) in ethanol group. The treatment for 6 weeks reduced the body weight of the rats from ethanol group (499 ± 19g) when compared to control group (621 ± 28g) ($P<0.05$, Sudent's t test). Chronic ethanol consumption significantly decreased ADM-induced relaxation (31.9 ± 3.8%, n=7) in endothelium intact rings when compared to control group (43.4 ± 2.2%, n=4) ($P<0.05$, Sudent's t test). However, in endothelium denuded rings no differences were observed in control and ethanol treated rats. Acetylcholine and sodium nitroprusside-induced relaxation were not affected by ethanol consumption. 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.

06.016

Vermelho de rutênio reverte os relaxamentos produzidos pela acetilcolina em anéis de aorta torácica de coelho. Alves Filho FC[1], Silva JDP[2] [1]UFPI - Farmacologia e Bioquímica, [2]UFPI - Plantas Medicinais

Introdução: Em 1980, Furchgott e Zawadski obtiveram evidências experimentais de que o relaxamento de artérias isoladas pela acetilcolina (ACh) é dependente da presença das células endoteliais, e de que as mesmas são capazes de produzir e liberar substâncias que causam o relaxamento das células musculares (Furchgott R F, Nature **288**: 373, 1980). Desde então, uma multiplicidade de mecanismos foram aventados para explicar este efeito. Um evento essencial para produção e liberação de fatores relaxantes pela a ACh é o aumento da concentração intracelular de cálcio nas células endoteliais (Nilius B, Endoth. **10** (1): 5, 2003). Em nosso laboratório, acidentalmente, verificamos reversão da resposta vasodilatadora da ACh pelo vermelho de rutênio (VR) em anéis de aorta torácica de coelho, assim decidimos caracterizar melhor este efeito inibitório. **Métodos:** Os protocolos experimentais obedeceram às normas do Comitê de Ética em Pesquisa da UFPI (Protocolo Nº 03/2009). Anéis (3) de 3- 4 mm da aorta torácica, montados em forma de "corrente", foram suspensos em cuba para órgão isolado (10 ml; continuamente borbulhados com **AR**) contendo solução de Tyrode a 37ºC (com indometacina a 1 μM; previamente borbulhada com ar durante 12 hs), para registro das contrações isotônicas (6 g tensão, 8 vezes amplificado). Após 2 hs de estabilização induziu-se a contração dos anéis mediante a adição de fenilefrina (Fen, 0,3-1 μM); ACh (1 μM) ou nitroprussiato de sódio (NPS, 1 μM) foram adicionados no platô da contração. O VR foi adicionado antes ou após a adição de Fen (0,1-1,0 μM) e no platô das respostas relaxantes da ACh e NPS. **Resultados:** Os relaxamentos produzidos pela ACh (62,73 ± 3,95 % n = 3 da tensão desenvolvida pela Fen) foram revertidos pelo VR 10 μM (98,34 ± 7,36 % n = 3). A pré-incubação com VR antes da pré-contração com Fen também inibiu os relaxamentos provocados pela ACh (controle 48,99 ± 5,38 % versus 83,88 ± 2,28 % na presença de VR, n = 4); e a adição de VR após baixas concentrações de Fen (0,1-0,3 μM) produziu um contração significativa (63,18 ± 4,64 %, n = 4). O NPS (1 μM) induziu relaxamentos que não foram afetados nem pela pré-incubação nem pela adição do VR no seu platô de resposta. **Discussão:** Estes resultados apontam que mecanismos sensíveis ao VR são responsáveis por um efeito vasodilatador da ACh em aorta de coelho montada isotonicamente. Dentre os alvos já descritos do VR em vasos sanguíneos, podemos citar canais catiônicos endoteliais da família dos TRPVs (*Transient Receptor Potential Vanilloid*) e TRPA (*Transient Receptor Potential Ankyrin*). Recentemente alguns pesquisadores têm descrito resultados que sugerem o canal TRPV4 como molécula importante na liberação de fatores relaxantes endoteliais promovida pela ACh. Embora estes pesquisadores tenham verificado uma redução do efeito relaxante da ACh em artéria mesentérica de camundongos nocauteados para o gene que codifica o TRPV4, os mesmos não verificaram o efeito do VR na resposta da ACh (Zhang D X, Hypert. 53(3): 532, 2009). Assim nossos resultados corroboram para uma função de canais TRPs como moléculas sinalizadoras na resposta vasodilatadora a ACh, bem como sugerem sua participação na liberação "basal" de fatores relaxantes em aorta torácica de coelho. **Agradecimentos:** Dr. Paulo Cavalcanti e Dr. Gustavo Ballejo. **Apoio Financeiro:** UFPI

06.017

Impaired vascular responses to vasoactive drugs in rats exposed to HIGH SALT-intake. Crestani S[1], Marques MCA[1], da Silva-Santos JE[2] [1]UFPR - Farmacologia, [2]UFPA - Farmacologia Experimental e Pré-clínica

Introduction: High-salt intake is putatively associated with hypertension and cardiovascular risks. However, the structural and functional changes that may be induced in the cardiovascular system by high-salt exposition remain to be elucidated. In this work we evaluated the responses to vasoactive drugs in rats subjected to increased amounts of salt. **Methods:** Male Wistar rats (21 days old) were exposed to food containing NaCl at 2, 4 and 8% for 6 weeks. At the end of the sixth week the rats were anesthetized with ketamine/xylazine (100/20 mg/kg, i.m.), the femoral vein and carotid artery were isolated and heparinized polyethylene catheters were inserted for drug administration and mean arterial pressure (MAP) measurement, respectively. After the stabilization period (20 min) the animals were injected with acetylcholine (ACh, 1-30 nmol/kg), phenylephrine (PE, 1-30 nmol/kg), isoproterenol (ISO, 1-30 nmol/kg), or angiotensin I (AI, 1-30 pmol/kg) and the effects of these drugs in the MAP were recorded. All protocols were approved by the Institutional Ethics Committee of UFPR (authorization number 345). **Results**: We did not find changes in the basal MAP measured in groups NaCl 2, 4 and 8% (115 ± 5.7, 112.9 ± 2.1, and 114 ± 3.2 mmHg, respectively) when compared to control values (120 ± 3.9 mmHg). However, the hypotension induced by ACh and ISO was 20-40% smaller in animals subjected to NaCl 8%. For instance, ACh and ISO (both at 3 nmol/kg) reduced the MAP by 37.6 ± 2.5 and 93.1 ± 7.1 mmHg in the control group, and by 25.0 ± 0.9 and 61.2 ± 1.3 mmHg in the group NaCl 8% ($p < 0.05$; $n = 6$). On the other hand, the intravenous injection of AI (30 pmol/kg) and PE (30 nmol/kg) increased the MAP of NaCl 8% group by 79.3 ± 2.0 and 85.4 ± 1.0 mmHg, respectively, while the hypertensive effects seem in control animals for these same doses were 61.2 ± 7.7 and 46.2 ± 7.1 mmHg, respectively ($p < 0.05$; $n = 4$-6). **Discussion**: These data disclose that high-salt treated animals present both reduced responses to vasodilators and enhanced responses to vasoconstrictor drugs, suggesting that systemic exposition to high amounts of sodium may cause important changes in the cardiovascular contractile machinery *in vivo.* Understanding the relationship between chronic exposition to NaCl and the changes in vascular responsiveness may contribute for a better comprehension and management of hypertension. **Financial support:** CAPES and CNPq.

06.018

Increased leptin accounts for vascular hyporeactivity in a model of obesity induced by palatable diet. Silva JF[1], Mendes VC[1], Rezende BA[2], Capettini LSA[1], Pinho JF[2], Lima DC[1], Côrtes SF[3], Coimbra CC[1], Lemos VS[1] [1]UFMG - Fisiologia e Biofísica, [2]ICB-UFMG - Fisiologia e Farmacologia, [3]UFMG - Farmacologia

Introduction: Chronic consumption of a high-palatable diet induces obesity and vascular abnormalities. Previous work have shown that leptin increase eNOS expression in some vascular beds. The aim of this study is to investigate the role of leptin in vascular alterations in a model of obesity induced by palatable diet. **Methods:** Wistar rats (4 weeks) fed either standard laboratory chow throughout (controls) or a palatable diet (diet-fed) for 5 weeks. Vascular reactivity in aortic rings was assessed in an organ bath using isometric transducers. Nitric oxide (NO) was measured by fluorescence microscopy in aortas stimulated with acetylcholine. Leptin was measured by radioimmunoassay. All experimental procedures were approved by the animal ethics committee of the Federal University of Minas Gerais (protocol # 226/08). **Results:** Diet-fed rats (D) compared to control animals (C) had higher body weight (C= 295,6 ± 6,31; D=356,7 ± 7,27g) and abdominal fat (C=2,456±0,14; D=5,872±0,27 g $p<0,0001$). Diet-fed has improved relaxation to ACh ($p<0,01$). Furthermore, contractile response to phenylephrine was decreased in the aortas from obese animals ($p<0,001$). This contractile dysfunction was endothelium-dependent and reverted by the non-selective NOS inhibitor, L-NAME (300 mM) and by selective inhibition of eNOS but not iNOS. The basal and ACh-induced production of NO was increased in aortas from obese animals. Western blot showed increased levels of eNOS but not iNOS. Plasmatic leptin levels was increased in obese animals compared to controls (C=1,15 ± 0,016; D=1,997 ± 0,23 ng/ml $p<0,01$). Aortic rings from control animals pre-incubated with leptin 1mM for 6 hours also showed reduced contraction to phenylephrine ($p<0,01$) and increased relaxation to ACh similar to the obese animals vessels. **Conclusion:** These data suggest that the observed vascular hiporeactivity in obese animals are due to an increase in NO production, probably as a consequence of a rise in leptin levels.
Support: FAPEMIG/CNPq.

06.019

Cardiac KATP sensitive current is increased in diabetic mice. Rodrigues, ALP[1], Lauton-Santos S[2], Fernandes VA[3], Goméz, AM[4], Bénitah, JP[4], Pesquero JL[5], Cruz JS[6] - [1]FUMEC-FCS, [2] CCBS-UFS - Fisiologia, [3]ISEAT-FHA, [4]INSERM - Physiopathologie Cardiovasculaire, [5]UFMG - Fisiologia e Biofísica, [6]UFMG - Bioquímica e Imunologia

Introduction: Diabetes is a metabolic disorder that affects about 143 million people worldwide and is growing in developed countries. Among the diabetic population, 90% of the patients have type 2 diabetes, which is characterized by hyperglycemia, with a normal or high insulin blood levels. The development of type 2 diabetes is characterized by insulin resistance and may be caused by genetic and / or environmental factors. The atherosclerosis contributes importantly to the cardiovascular complications found in diabetic patients. Interestingly, cardiac changes may be found in diabetic patients even in the absence of vascular disease, suggesting the existence of diabetic cardiomyopathy. This cardiomyopathy is characterized by a reduction in diastolic compliance, contractility and the speed of cardiac relaxation. Cardiomyopathies are mainly derived from the disturbances induced in cardiac electrophysiology. These disorders can lead to a change in excitation-contraction coupling (ECC) or the mechanisms of cell repolarization. Changes found in both the activity of elements of the ECC and in K + currents in cardiomyocytes of diabetic mice could cause the decrease in contractility and explain the change of relaxation observed in type 2 diabetes. Several studies have shown the involvement of K^+ currents and Ca^{2+} signaling in different cardiac diseases and the putative involvement of kinins in the alleviation of cardio-deleterious effects, however with regard to diabetes type 2 there are only few studies addressing the mechanisms involved in cardiac dysfunction and cardioprotective alternatives. **Objective:** The aim this work was to evaluate the ATP sensitive potassium current in type 2 diabetic animals (db/db) and cardiac protein expression of these channels. **Methods:** Whole-cell voltage clamp was performed to measure ATP-sensitive K current (IKATP). The holding potencial was set at -80 mV and depolarizations from -40 mV to +50 mV in 10 mV steps were applied every 20 ms. Cardiac cells isolated from controls (n=5) and diabetic (db/db, n=8) hearts were processed to extract protein and submitted to gel electrophoresis (PAGE) and then subjected to western blot protocol. Specific antibody (anti-KIR 6.2 and anti-GAPDH from Santa Cruz Biotechnology and Affinity BioReagents, respectively) and a peroxidase conjugated secondary antibody (SIGMA, Sto Louis) were used. The signal was detected using ECL kit detection system (Amersham, IL, USA). The images generated were quantitatively analyzed for the protein levels with the use of KODAK software. All experiments were carried out according to European Union Council Directives (86/609/EEC) for the care of laboratory animals. **Results:** We found a significant increase (30%) in the ATP sensitive potassium current and also in the protein expression level of KATP channels (three times bigger in db/db animals when compared with control animals). **Conclusion:** Cardiac KATP sensitive current is increased in diabetic mice. **Support**: CAPES, CNPq, ISEAT-FHA and FAPEMIG

06.020

Testosterone induces aortic smooth muscle cells proliferation through P38 MAPK and NFKB. Chignalia AZ[1], Camargo LL[1], Yogi A[1], Lopes LR[1], Curi R[2], Fortes ZB[1], Carvalho MHC[1], Touyz RM[3], Tostes RCA[1] - [1]ICB-USP - Farmacologia, [2]ICB-USP - Fisiologia e Biofísica, [3]University of Ottawa - Kidney Research Centre

Introduction: Men have higher risk of developing atherosclerosis when compared to age-matched women. Different mechanisms are involved in atherosclerotic plaque formation (APF), including activation of p38 MAP kinase (p38) and nuclear factor kappa B (NFkB). However, androgen effects on APF remain unclear. Here, we investigated if testosterone modulates cellular events involved in atherosclerosis. **Methods:** Aortic rabbit vascular smooth muscle cells (AVSMC) were stimulated with testosterone 10^{-8}M and 10^{-7}M for 1 minute up to 24 hours. Activation of p38 was assessed by immunoblotting. Gene expression of collagen 3, fibronectin and biglycan was evaluated by real time PCR. AVSMC proliferation was investigated by MTT and wound assays. **Results:** Testosterone induced activation of p38 in a time and concentration-dependent manner. At 10^{-7}M testosterone induced a sustained activation of p38 whereas at 10^{-8}M the activation of p38 occurred in a transient manner (n=5, $p<0.05$). Non-permeable-testosterone was also capable of activating p38 (n=4, $p<0.05$). Flutamide (classical androgen receptor antagonist) did not block activation of p38 (n=4, $p>0.05$). Testosterone induced AVSMC proliferation after 24 hours of stimuli (n=5, $p<0.05$). AVSMC proliferation was inhibited by flutamide, SB203580 (p38 inhibitor) and sodium salicylate (NFkB inhibitor) (n=6, $p<0.05$). Testosterone induced only collagen 3 gene (n=4, $p<0.05$), having no effect on biglycan and fibronectin expression. **Discussion:** Our findings demonstrate that testosterone can induce cellular events that accompany APF including activation of signalling pathways, modulation of extracellular matrix components and augmentation of cell proliferation. Testosterone-induced p38 activation is a short-term, non-genomic effect, mediated by membrane-associated androgen receptor. AVSMC proliferation is a genomic effect, mediated by classical androgen receptor that depends on both p38 and NFkB activation. In conclusion, our results corroborate the concept that androgens are a risk factor to the development of cardiovascular diseases. Funding: FAPESP. License Number (Ethics Committee on Animal Experimentation):036.

06.021

Sodium nitroprusside and [Ru(terpy)(bdq)NO^+]$^{3+}$: aortic and venular effects. Paulo M[1], Bonaventura D[2], Araújo AV[3], Da Silva RS[1], Bendhack LM[1] [1]FCFRP-USP Physics and Chemistry, [2]UFMG - Pharmacology, [3]FMRP-USP Pharmacology

Introduction: Altered vascular tone, a characteristic feature of experimental and human hypertension, has been associated with impaired endothelium-dependent vasodilation due to reduced nitric oxide (NO) production. NO donors have been used for many years in the treatment of various clinical conditions, particularly coronary artery disease and hypertension. In this study we investigated the effects of NO donors, sodium nitroprusside (SNP) and [Ru(terpy)(bdq)NO^+]$^{3+}$ (Terpy), in thoracic aorta and inferior cava vein from normotensive (2K) and renal hypertensive rats (2K-1C). **Methods:** We have analyzed the maximal relaxation (Emax) and potency (pEC_{50}) of SNP and Terpy in rat aorta and cava vein. To avoid interference of endogenous NO, the experiments were performed on endothelium-denuded aortic rings and the cava vein preparations incubated with L-NAME (non-selective NOS inhibitor, for 30 min). Aortic rings were pre-contracted with EC_{50} of norepinephrine (100 nmol/L), and cava rings were pre-contracted with EC_{50} of endothelin-1 (30µmol/L). When the contractions reached a plateau, the NO donors were added cumulatively, in aortic rings (Terpy: 1 nmol/L - 300 µmol/L and SNP: 0.1 nmol/L - 0.3 µmol/L) and cava vein rings (Terpy: 10 pmol/L - 10 mmol/L and SNP: 10pmol/L - 0.1 mmol/L). All these procedures were in accordance with the guidelines of the Animal Ethics Committee, Ribeirão Preto Campus, University of São Paulo, Brazil (Protocol number: 08.1432.538). **Results and Discussion:** Vascular reactivity experiments showed that the Emax triggered by SNP was similar in aortic rings from 2K (102.2 ± 3.8%,n=6) and 2K-1C rats (95.4 ± 2.5%, n=7). Similarly, the Emax induced by Terpy was not different in rat aorta from 2K (104.7 ± 1.3%, n=6) and 2R-1C (101.0 ± 0.6%, n=7). However, the potency of both NO donors was lower ($p<0.001$) in aorta of 2K-1C as compared to 2K (SNP 2K: 8.22 ± 0.14 n=6; 2K-1C: 7.76 ± 0.17 n=7 and Terpy 2K: 6.77 ± 0.04 n=6; 2K-1C: 5.85 ± 0.04 n=7). In cava vein the potency of SNP was similar in 2K (pEC_{50}: 7.42 ± 0.13 n=6) and 2K-1C (pEC_{50}: 7.42 ± 0.26 n=7). Similarly that was observed for SNP, the potency of Terpy was not significantly different between cava vein from 2K (pEC_{50}: 8.40 ± 0.12 n=6) and 2K-1C rats (pEC_{50}: 8.30 ± 0.22 n=7). However, the Emax of both NO donors was lower ($p<0.001$) in cava vein from 2K-1C as compared to 2K rats (SNP 2K: 80.9 ± 2.4% n=6; 2K-1C: 60.1± 3.3% n=7 and Terpy 2K: 50.7 ± 3.2% n=6; 2K-1C: 40.6 ± 3.1% n=7).**Conclusion:** Taken together, our results demonstrated that Terpy and SNP induce aorta and vein relaxation from both 2K and 2K-1C rats. In aortic rings, but not in cava vein, the NO donors are less potent in 2K-1C than in 2K. In cava vein the maximum relaxation mediated by these NO donors are impaired in 2K-1C. Supported by FAPESP and CNPq.

06.022

Nitrate tolerance to sodium nitroprusside (SNP) involves NO-synthase uncoupling and reactive oxygen species (ROS) production. Bonaventura D[1], Lunardi CN[2], Rodrigues GJ[3], Bendhack LM[4] [1]ICB-UFMG - Farmacologia, [2]UnB, [3]FMRP USP - Farmacologia, [4]USP-FCFRP

Introduction: Organic nitrates, such as nitroglycerin, are commonly used in cardiovascular clinical. Though nitrovasodilators are considered as being safe and free of serious side effects, their clinical use is limited by reduction of efficacy upon long-term application, resulting in a complete loss of hemodynamic effects by continuous application. This phenomenon is called nitrate tolerance. This study aimed to verify if the inorganic nitrate SNP induces nitrate tolerance similar to nitroglycerin and which are the mechanisms involved. **Methods and results**: We have analyzed the maximal relaxation (Emax) and potency (pD_2) of SNP in control and tolerant rat aorta. Vascular reactivity experiments showed that previous exposition of intact rat aorta to SNP (EC_{50}: 1 nmol/L) for 15, 30, 45 and 60 minutes reduced the pD_2 values but not the Emax to SNP, suggesting the phenomenon of tolerance (pD_2 control: 8.92 ± 0.05, n=9; tolerant (15min): 8.59 ± 0.05, n=6; tolerant (30min): 8.64 ± 0.10, n=6; tolerant (45min): 8.64 ± 0.09, n=6; tolerant (60min): 8.65 ± 0.04, n=6). This effect was not observed in denuded rat aortic rings. The reduction in pD_2 induced by SNP tolerance was blocked by L-NAME, a non-selective NO-Synthase (NOS) inhibitor (pD_2 control+L-NAME: 8.54 ± 0.07, n=15; tolerant+L-NAME: 8.48 ± 0.05, n=16), by Tiron, a ROS scavenger (pD_2 control+Tiron: 8.77 ± 0.13, n=8; tolerant+Tiron: 8.82 ± 0.11, n=11) and by L-arginine, the NOS substrate (pD_2 control+L-ARG: 8.87 ± 0.03, n=9; tolerant+L-ARG: 8.85 ± 0.08, n=7). However, the effect of tolerance in SNP relaxation was not altered by tetrahydrobiopterin (BH_4, NOS cofactor) (pD_2control+BH_4: 8.82 ± 0.09, n=7; tolerant+BH_4: 8.42 ± 0.05, n=6), or indomethacin (INDO), a non-selective cyclooxigenase (COX) inhibitor (pD_2 control+INDO: 8.77 ± 0.08, n=9; tolerant+INDO: 8.45 ± 0.05, n=8). **Conclusion:** Taken together, our results demonstrated that *in vitro* development of SNP tolerance is not time of exposure dependent and it could be correlated to NOS uncoupling, due to reduction in L-ARG bioavailability and increase in ROS production. This phenomenon seems not to be due to COX activation, since indomethacin did not reverse the SNP tolerance. Supported by FAPESP, FAPEMIG and CNPq. Approved by CEUA n°05.1.260.53.0.

06.023

Cardiovascular hyporesponsiveness in sepsis is associated with G-protein receptor kinase (GRK) expression via a nitric oxide-dependent mechanism. Dal-Secco D[1], Olivon VC[2], Celes MRN[3], DalBó S[1], Bóf ER[1], Abreu, A[3], Rossi MA[3], de Oliveira AM[2], Cunha FQ[4], Assreuy J[1] [1]UFSC - Farmacologia, [2]USP-FCFRP, [3]USP - Patologia, [4]FMRP-USP

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. Therefore, in the present study, 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:** The experimental consisted of female C57Bl/6 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; 37ºC, 95% O_2 and 5% CO_2). After an equilibration period (60 min) the preparations were contracted with phenylephrine (Phe; 1□M), and the integrity of the endothelial layer was confirmed with acetylcholine (1□M). Aortic responsiveness was evaluated 6, 12 and 24 h after CLP surgery in the presence or absence of iNOS selective inhibitor (1400W; 10□M; 30min). GRK2 expression was analyzed on heart and aorta 6, 12 and 24 h after CLP from sham, septic and 1400W (1mg/kg)-treated septic mice by immunofluorescence analysis. The procedures have been approved by the animal ethics committees of UFSC (PP003/ Animal Use Ethics Committees-CEUA). **Results:** The vascular responsiveness to vasoconstrictor Phe was significantly reduced in aorta rings from septic mice evaluated 12 (57%) and 24 (78%) h after CLP. However, the 1400W incubation prevented this vascular hyporesponsiveness 12 h after CLP. Moreover, high expression of GRK2 was detected in aorta and heart of septic mice 12 (52%) and 24 (63%) h after CLP. Conversely, the treatment of septic mice with 1400W reduced the GRK2 high expression on aorta (75%) and heart (79%) of septic mice. Finally, the 1400W treatment enhanced significantly the survival rate of the septic mice (55%). **Discussion:** Our findings identify that NO, produced mainly from iNOS during sepsis, 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.

06.024

ACE inhibition aggravates the experimental acute chagasic cardiomyopathy in mice by bradykinin potentiation. Dalla Costa AP[1], Deckmann AC[2], Ramirez LE[3], Pereira AGA[4], Franchini KG[5], Dias da Silva VJ[6] - [1]FCM-UNICAMP, [2]UNICAMP - Clínica Médica, [3]UFTM - Ciências Biológicas, [4]IB-UNICAMP - Genética e Evolução, [5]FCM-UNICAMP - Clínica Médica, [6]UNICAMP - Fisiologia

Introduction: The development of the infection by *Trypanosoma cruzi* depends, crucially, of the amastigotes intracellular multiplication and the trypomastigotes invasion of new host cells, which is essential to the fulfilling of the evolutive cycle and survival of the parasite. In this aspect, it is known that the parasite uses the bradykinin B2 receptors to invade, for example, endothelial cells, *in vitro*. The aim of the present study was to evaluate *in vivo* the effects of increased levels of endogenous bradykinin, produced by the treatment with angiotensin-converting enzyme inhibitor, enalapril, upon the acute Chagas disease in rats. In order to measure an eventual effect of the reduction in the angiotensin II activity, another group of animals was also treated with losartan, a AT-1 receptor antagonist. **Methods**: Male Wistar rats (100-150gr.) were inoculated with $2x10^6$ tripomastigotes forms of Y strain of the *T. cruzi*. Following, the animals were divided in the groups: chagasic (CHG, n=10), chagasic-enalapril (CHG-EN, receiving enalapril, 0,2mg/ml/day in tap water, n=9) and chagasic-losartan (CHG-LOS, treated with losartan, 0,2mg/ml/day in tap water, n=10). No Inoculated and no treated animals formed the control group (CON, n=10). After the inoculation, parasitemia curve was evaluated during 20 days. At to the end of this period, electrocardiographic recordings (ECG) were performed and followed by histo-pathological studies and evaluation of the gene expression profile by means of cDNA microarray analysis. **Results**: It was observed that the parasitemia of the CHG-EN animals was significantly higher than that observed in CHG or CHG-LOS animals (56,1±15,7x103 versus 8,8±2,6x103 and 11,7±4,1x103 parasites/mL, respectively, measured in the ninth day after inoculation, $p<0,05$). Regarding to ECG parameters, it was observed in CHG-EN group an increase of the duration of the QRS complex (26,3±1,8ms versus. 20,9±1,5ms in the CHG group, $p<0,05$) and of corrected QT interval (111,2±6,9ms versus 97,4±3,2ms in the CHG group, $p<0,05$). In addition, relative cardiac weight of the CHG-EN animals was significantly higher in comparison with CHG group (4,7±0,2mg/g versus 3,6±0,1mg/g, respectively, $p<0,05$). Histo-pathological study revealed in all the chagasic animals, a myocarditis and a ganglionitis characterized by an inflammatory mononuclear infiltration, which was semi-quantitatively more intense in the CHG-EN group. In the evaluation of gene expression profile, it was observed that 11 genes are more expressed in the CHG-EN group in relation to the CHG group, while 13 genes were repressed in comparison to the CON group. Regarding to the treatment with losartan, it was verified only small modifications in the analyzed parameters. Therefore, the treatment with enalapril was able to worsen the parasitemia, the electrocardiographic parameters, the cardiac hypertrophy, the myocarditis and the ganglionitis, which is characteristic of the acute Chagas disease. **Conclusion**: All these results seem to suggest a possible role played by the endogenous bradykinin in the pathophysiology of the acute experimental Chagas disease in rats. Protocol 0345/2006, Ethics Committee of the use of animals/ CEUA/UFTM (Universidade Federal Triângulo Mineiro, MG, Brazil). Research support: CNPq and FAPEMIG.

06.025

Carvedilol reduces left ventricular dilation in postinfarction heart failure rats. Baldo MP[1], Zaniqueli D[1], Davel APC[2], Rossoni LV[2], Mill JG[1] - [1]UFES - Physiological Sciences, [2]USP- Physiology and Biophysics

Introduction: Extensive infarction leads to ventricular dilation and enhanced left ventricle end-diastolic pressure (LVEDP). Some works have shown important beneficial of beta-blocker therapy in ischemic dilated cardiomyopathy. Our aim was to evaluate the effects of carvedilol on left ventricular (LV) structure and function in rats after myocardial infarction (MI). **Methods.** Male wistar rats (8-9 wks) were infarcted by permanent left coronary artery occlusion. Control animals were submitted to false surgery (SO). Twenty-four hours later, animals received carvedilol (MI-CARV; 20mg/kg/day, gavage) or vehicle (MI-CONT; metilcelulose 0,5%) for 28 days. Animals were catheterized to evaluate left ventricular function and to obtain the pressure-volume curve by using a double lumen catheter and continuous saline infusion. Samples of each groups were selected to an exercise tolerance test of progressive intensity. Data are presented as mean±SEM. **Results.** MI-CONT animals presented increased LVEDP as compared to SO animals, and carvedilol reduced this parameter (SO=7±1, MI-CONT=20±2, MI-CARV=12±3mmHg; *P*<0,05). LV end-diastolic volume index was reduced by carvedilol treatment (SO=0,6±0,1, IM-CONT=2,2±0,2, IM-CARV=1,4±0,3ml/kg; *P*<0,05). The angular coeficient obtained by pressure-volume curve was reduced by MI, indicating a more dilated chamber, which was partially restored by carvedilol (SO=10,5±1,2, MI-CONT=4,4±0,6, MI-CARV=6,3±1 mmHg/ml.kg^{-1}; *P*<0,05). Moreover, LV developed pressure was reduced in MI-CONT group, and carvedilol increased significantly this parameter (SO=115±4, MI-CONT=84±3, MI-CARV=98±4mmHg; *P*<0,05). Maximal exercise tolerance was significantly better in carvedilol treated animals as compared to those received vehicle (MI-CONT=11,8±1, MI-CARV=17.3±1 min; *P*<0,05). **Discussion.** Here, we found that early carvedilol administration reduced LV dilation, improving cardiac function and aerobic performance in rats after MI. Thus, carvedilol may be an interesting choice to initiate postinfarction treatment, and to prevent heart failure progression. **Financial Support.** CNPq and FAPES

06.026

RHO-kinase participation in phenylephrine-induced contraction in contralateral carotid after balloon catheter injury. Pereira AC, Olivon VC, de Oliveira AM FCFRP-USP - Física e Química

Introduction: It was observed an increase in the contract response to phenylephrine (Phe) in contralateral carotids 4 days after balloon catheter injury[1]. Phe is an α_1-adrenergic agonist whose contractile response involves a variety of signaling molecules, such as Rho-kinase[2]. Reactive oxygen species (ROS) are able to modulate Rho-kinase activity and many others signaling pathways[3]. The aim of this work was to verify Rho-kinase and ROS participation in Phe-induced contraction in contralateral carotid arteries after balloon catheter injury. **Methods:** Male Wistar rats (80 days, 400-420g) underwent unilateral balloon catheter injury in left carotid artery. Reactivity studies were performed 4 days after injury in isolated control and contralateral right carotid rings (with and without endothelium). Concentration response curves to Phe (10^{-10}-10^{-5} mol/L) and to Y-27632 (Rho-kinase inhibitor, 10^{-10}-10^{-5} mol/L) were performed in absence and presence of Tiron, a superoxide scavenger (after 30 min. incubation). Concentration response curves to Y-27632 were examined after Phe (10^{-7} mol/L) precontraction. All procedures were in accordance with the standards and policies of the Animal Care Committee of this institution (06.1.1063.53.4). Parameters of maximum effect (E_{max}) and potency (pD_2) to Y-27632 and to Phe were analyzed. **Results:** Tiron reduced E_{max} to Phe in contralateral carotid artery to control values (see Table 1). E_{max} to Y-27632 was not altered in contralateral carotid, but it was observed reduction in Y-27632 potency (see Table 2) suggesting increase in Rho-kinase activity in contralateral carotid arteries. In presence of Tiron, Y-27632 potency in contralateral carotid was similar that observed in control (see Table 1).

Table 1: E_{max} values to Phe in absence and in presence of Tiron (10^{-3} mol/L).

	Phe	**Phe + Tiron**
Control	0.36 ± 0.03	0.35 ± 0.03
Contralateral	0.56 ± 0.04*	0.41 ± 0.04

Data are shown as tension (g), means ± s.e.mean. Anova and Newman-Keuls's post test, P<0.05. *Statistically significant differences from control group.

Table 2: E_{max} and pD_2 values to Y-27632 after Phe precontraction (10^{-7} mol/L) in absence and in presence of Tiron (10^{-3} mol/L).

	Y-27632		**Y-27632 + Tiron**	
	E_{max} (% Relaxation)	**pD_2**	**E_{max} (% Relaxation)**	**pD_2**
Control	105.37 ± 2.52	7.69 ± 0.10	105.37 ± 4.26	7.57 ± 0.53
Contralateral	112.80 ± 0.15	6.50 ± 0.12*	109.58 ± 4.91	7.07 ± 0.47

Data are shown as means ± s.e.mean. Anova and Newman-Keuls's post test, P<0.05. * Statistically significant differences from control group.

Conclusion: Results suggest that increase in Phe-induced contraction is related an increase in Rho-kinase activity in contralateral carotid artery, probably due to superoxide anions. [1]Accorsi-Mendonça, D.; et al. *Brit. J. Pharm*.142: 79. 2004. [2]Zhong, H.; et al. *Eur. J. Pharmacol*. 375: 261. 1999. [3]Knock, G.A.; et al. *Free Radic. Biol. Med.* 46: 633. 2009. **Financial Support:** Capes

06.027

The increase in PHE-induced contraction is not related to calcium mobilization: role of eNOS in contralateral carotid after balloon catheter injury.Pereira AC, Olivon VC, de Oliveira AM - FCFRP-USP - Física e Química - Farmacologia

Introduction: As previously demonstrated, there is an increase in phenylephrine (Phe) responsiveness on contralateral carotids 4 days after angioplasty[1]. The aim of this work was to verify calcium mobilization by Phe stimulation and the role of the NO pathway in this response in contralateral carotid artery. **Methods:** Male Wistar rats (80 days, 400-420g) underwent unilateral balloon catheter injury in the left carotid artery. Reactivity studies were performed 4 days after injury in isolated control and contralateral right carotid rings (with and without endothelium). The Phe-induced contraction (10^{-7}mol/L) in free-calcium Krebs solution was used to verify intracellular calcium mobilization. After successive Phe stimulations and store depletion, in same conditions, it was performed a concentration-response curve to $CaCl_2$ (0.05-2.50 mol/L) to analyze extracellular calcium mobilization by Phe. Experiments were conducted in presence and absence of the NO scavenger oxyhemoglobin (OxyHb), soluble guanylate cyclase inhibitor (ODQ), reactive oxygen species scavenger (Tiron), NOS inhibitor (L-NAME) and NOS isoforms specific inhibitors (L-NNA, 1400W and 7-NI). All procedures and protocols were approved by the University of Sao Paulo's Animal Care and Use Committee (06.1.1063.53.4). **Results:** Intracellular calcium mobilization in contralateral carotid was not altered. It was observed a reduction in Phe-induced contraction due to extracellular calcium mobilization in contralateral carotid artery (see Table 1). Similar results were observed in presence of 7-NI, 1400W, ODQ and OxyHb. In presence of Tiron, L-NAME and L-NNA this response in contralateral carotids was like control artery (see Table 1). **Table 1**: E_{max} values obtained in concentration-response curves to $CaCl_2$ in free-calcium Krebs solution after Phe stimulation (10^{-7}mol/L). Effect of L-NAME (10^{-4} mol/L), 7-NI (10^{-4} mol/L), 1400W (10^{-7} mol/L), L-NNA (10^{-4} mol/L), Tiron (10^{-3} mol/L), ODQ (10^{-6} mol/L) and OxyHb (10^{-5} mol/L).

		L-NAME	7-NI	1400W	L-NNA	Tiron	ODQ	OxyHb
Control	0.24±0.01	0.54±0.03	0.29±0.01	0.25±0.02	0.60±0.05	0.29±0.01	0.67±0.06	0.60±0.05
Contralateral	0.14±0.01	0.51±0.03	0.21±0.01*	0.16±0.02*	0.59±0.05	0.24±0.02	0.51±0.03*	0.45±0.04*

Data are shown as tension (g), means ± s.e.mean. Anova and Newman-Keuls's post test, $P<0.05$. *Statistically significant differences from control group.

DISCUSSION: The increase in Phe-induced contraction is not due to increase in calcium mobilization in contralateral carotid. Balloon catheter injury triggers reduction in extracellular calcium mobilization in contralateral artery after balloon catheter injury, probably due to oxidative stress and eNOS uncoupling. [1]Accorsi-Mendonça, D.; et al. *Brit. J. Pharm*.142: 79. 2004. **Financial Support**: Capes

06.028

In vivo oligodeoxynucleotides antisense knockdown of PI3K delta isoform, normalize L-type Ca^{2+} currents and vascular contractile response in type I diabetes. Pinho JF[1]; Medeiros MAA[2]; Rezende BA[1]; Capettini LSA[3]; Cortes SF[4]; Andrade SP[6]; Campos PP[1]; Cruz JS[5]; Lemos VS[3] [1]UFMG - Fisiologia e Farmacologia; [2]LTF-UFPB; [3]ICB-UFMG - Fisiologia e Biofísica; [4]UFMG - Farmacologia; [5]UFMG - Bioquímica e Imunologia

Introduction: Macro-and microvascular disease represent the principal causes of morbidity and mortality in patients with type I or type II diabetes. Vascular complications of diabetes are associated with abnormal vasoconstriction and Ca^{2+} handling by vascular smooth muscle cells (VSMCs) in which the alteration in L-type voltage-dependent Ca^{2+} channel (VDCC) currents may play an important role. In addition to be mediators of insulin action, phosphatidylinositol 3-kinases (PI3K) signaling has been shown to modulate voltage-dependent Ca^{2+} channels (VDCC) function. In this work, the involvement of the PI3K pathway in increased vascular contraction was investigated in type 1 diabetic mice aorta. **Methods:** Changes in isometric tension were recorded on myograph. Whole-cell Ca^{2+} currents in freshly dissociated mice aortic SMCs were measured using the patch-clamp technique. *In vivo* oligodeoxynucleotides antisense technique was used to knockdown PI3Kdelta isoform. All experimental procedures were approved by animal ethics committee of the Federal University of Minas Gerais(protocol number 068/07). **Results and Discussion:** Contractile responses to phenylephrine and KCl, were strongly enhanced in diabetic mice aorta independently on the presence of a functional endothelium. The magnitude of phenylephrine-induced Ca^{2+} currents through VDCC in isolated cells from diabetic mice, were also greatly increased. Western blot showed increased PI3Kdelta expression in aortas from diabetic animals. *In vivo* oligodeoxinucleotide antisense knockdown of PI3Kdelta normalized contractile responses to phenylephrine and KCl, as well as I_{Ca}, to the level found in controls. It is concluded that increased PI3Kdelta signaling in VSMCs from type 1 diabetic mouse leads to increased Ca^{2+} currents through VDCC that drives the increased vascular contractility in diabetes. Given that selective inhibition of PI3Kd isoform appears to be highly effective in blocking vasoconstriction in type 1 diabetes, PI3Kdelta could represent a novel target to expand the therapeutic strategy to treat vascular disease in diabetes. **Support:** FAPEMIG, CAPES and CNPq.

06.029

Toll-like receptors expression are increased in spontaneously hypertensive rats. Bomfim GF, Fortes ZB, Carvalho MHC USP - Farmacologia

Introduction: Significant progress in understanding the etiology of cardiovascular disease has come from recent recognition that chronic inflammation plays a key role in its development. The underlying mechanisms of the inflammatory response, however, are not fully understood (Foldes *et al*; *Rec Pat Inf Al Drug Disc*, p57, 2007). Tissues within the cardiovascular system are exposed to pathogens and danger signals (endogenous ligands), resulting in activation of pattern-recognition receptors (PRRs) including Toll like receptors (TLRs). TLR2 and TLR4 have been shown in experimental models of atherosclerosis, , heart failure, ischemic injury and septic cardiomyopathy, suggesting that TLR inhibition could have protective effects in cardiovascular diseases (Frantz *et al; Nat Clin Pract*, v4, p444, 2007). Hypertension, an important risk factor for cardiovascular disease, now considered as a chronic inflammatory condition with elevated proinflammatory cytokine blood levels and increased vascular COX-2 expression, has been associated with changes in vascular responses such as impairment of endothelium-dependent vasodilator responses or enhancement of vasoconstrictor responses to different agonists (Alvaréz *et al; J Pharm Exp Ther*, v321, p381, 2007). Based on the evidences mentioned above, it is tempting to speculate that TLRs, especially TLR2 and TLR4, might be involved in pathophysiology of hypertension. The purpose of this study was, thus, to investigate the presence of TLR2 and TLR4 in the aorta, mesenteric arterioles, heart and kidney of the Spontaneously Hypertensive Rats (SHR) in different hypertensive phases such as pre-hypertensive (5 weeks old) and hypertensive (10 and 15 weeks old) in comparison with aged-matched normotensive Wistar rats(WT). **Methods:** This study was approved by Ethics Committee ICB-USP with protocol number 98/04. The gene expression of TLR4 and TLR2 was determined by RT-PCR in aorta, mesenteric arterioles, heart and kidney isolated from Wistar and SHR at ages: 5 (pre-hypertensive phase), 10 and 15 weeks old (hypertensive phase). Blood Pressure (BP) was measured by indirect tail cuff method (plethysmography). **Results:** The mean of blood pressure in SHR aged 5 weeks old was 108.1±6,7mmHg similar to the BP in Wistar at all ages (118.3±8.8mmHg), while the SHR blood pressure aged 10 and 15 weeks old were 179,2 ± 11,4mmHg and 181.1±8,2mmHg, respectively. A gradual decrease in TLRs mRNA was observed in SHR aorta according to age [5 wk: TLR2(1,94±0,27), TLR4(1.7±0.31); 10 wk: TLR2(1.15±0.27)* TLR4(1.0±0.11)* and 15 wk: TLR2(0.76±0.02)* TLR4(0.53±0.13)* *p≤0.05 vs 5 wk], but not in WT rats, reaching lower levels than WT at 15 weeks old (TLR4: WT- 0,875±0,09* vs SHR 15 wk) . However, in mesenteric arterioles (1.1±0.25), heart (1.0±0.13) and kidney (1.51±0.34) isolated from SHR 15 weeks old, an increased TLR4 gene expression was observed when compared to 5 weeks old SHR (mesenteric: 0.46±0.11*; heart: 0,67±0,1* and kidney: 0,96±0,13* vs SHR 15 wk). The mRNA content of TLR2 was higher in kidney of SHR 15 weeks old (1.37±0.27) than SHR 5 weeks old (0.95±0.12)* and WT (0.95±0.24)*. **DISCUSSION**: It was observed that TLR2 and TLR4 gene expression in SHR correlates positively with increase in blood pressure. Nevertheless TLR2 and TLR4 seems to be involved in the pathophysiology of the hypertension. **Supported by**: CNPq and FAPESP

06.030

Angiotensin II-induced hypertrophy in aorta of rats: involvement of the B1-kinin receptor. Ceravolo GS[1], Akamine EH[1], Jordão MT[2], Costa, TJ[1], Tostes RCA[1], Fortes ZB[1], Chopard R[2], Carvalho MHC[1] [1]USP - Farmacologia, [2]USP - Anatomia

Introduction: Vascular injury is considered to be a primary event in the evolution of vascular disease. Of prime importance in the vascular injury is the pro-inflammatory effects of Angiotensin II (A II), wich leads to endothelial dysfunction and vascular remodeling. Objective: Because B1-kinin receptor (B1R) is expressed only in response to injury, as A II infusion (Ceravolo et al., Hypertension, 50:756, 2007), we hypothesize that it could play a critical role in the development of vascular disease caused by A II. Methods: All of the procedures used in this study were approved and performed in accordance with the guidelines of the ethics committee of the Institute of Biomedical Sciences-USP (n.98). Male Wistar rats were infused during 14 days with A II (400ng/kg/min-A II rats), saline (S rats) or A II (400ng/kg/min) plus B1R antagonist des-Arg9-Leu8-bradykinin (350 ng/kg/min, DAL rats), via osmotic minipumps subcutaneously implanted. The blood pressure (BP) was determined by the tail cuff method after implants. At day 14th the aorta was excised and RNA expression of B1R, interleukines (IL)-1 and IL-6 were determined by real time PCR. We also analised the phosphorylated and total expression of ERK1/2 by immunobloting (ERK 1/2 activity), reactive oxygen species (ROS) generation by dihydroethidine method and aorta cross-sectional area (CSA) in hematoxylin-stained sections. Results: A II (182±5.9*, n=11) and DAL (188±7.1mmHg*, n=10;) rats showed higher BP than S rats (118±2.2, n=8). The aorta B1R mRNA levels were 15-fold higher in A II than in S rats. The aorta of A II presented increased CSA (S: 453815±21955 vs AII: 652661±44824μm^2*), ROS generation (S: 31.5±1.6 vs A II:45.31±1.7*), ERK1/2 activity, IL-1 (S:0.95±0.2 vs AII:2.64±0.5*) and IL-6 (S:0.87±0.09 vs A II:1.92±0.2*) expression when compared with of S rats. The B1R antagonism reduces, but did not corrected, the ROS generation (39.1±1.3), CSA (504104±67195µm2), ERK1/2 activity and the IL-1 (1.37±0.2) and IL-6 (1.27±0.23) expression in aorta of A II rats. Discussion: Those data suggest that the B1R is involved in the vascular inflammation and hypertrophy induced by A II, indicating a new site of interaction between renin-angiotensin system and kallikrein-kinin system, and it providing an important contribution for a better understanding of the vascular pathophysiology in A II-dependent hypertension. *P<0,05 vs S rats. Financial support: FAPESP

06.031

The renal effects of *Bothrops pauloensis* venom in the rat perfused kidneys. Jorge RJB[1], Alves CD[2], Jorge ARC[3], Sousa DF[3], Barbosa JPC[2], Santos JVA[2], Silva Neto AG[3], Alves RS[3], Queiroz MGR[3], Evangelista JSAM[4], Toyama MH[5], Monteiro HSA[3] [1]UNIFOR - Enfermagem, [2]UNIFOR - Fisiologia e Farmacologia , [3]UFC - Farmacologia e Fisiologia, [3]UFC - Análises Clínicas, [4]UECE - Veterinária, [5]IB-UNICAMP

Introduction: In Brazil, ofidics accidents account for more than 20.000 cases reported annually to the Ministry of Health and the majority of these (90.5%) were caused by species of the genus Bothrops. Among them we can highlight the *Bothrops pauloensis* witch found in the southeast of the country. Its venom has many enzymes including proteinases, metalloproteinases, phospholipase A_2 (PLA_2) and bradykinin potentiating peptides that may contribute to their actions biological. **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 *B. pauloensis* venom (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 Ceará Animal Research Ethical Committee, license number of 68/08. **Results:** The venom of *B. pauloensis* (VBp) decrease PP and RVR at 90 and 120 minutes (PP_{30}=96,56±3,701; PP_{90}=58,93±6,651*;PP_{120}=57,32±7,96* mmHg/mLg^{-1}); (RVR_{30}=4,55±0,5071; RVR_{90}=2,88±0,4729*; RVR_{120}=2,96±0,5797* mmHg/mLg^{-1}) GFR at 60 minutes (GFR_{30}=0,640±0,0411; GFR_{60}=0,330±0,0254* mL/g.min^{-1}), as well as, increase the UF at 120 minutes (UF_{30}=0,1359±0,0129; UF_{120}=0,3015±0,0481* mL/g.min^{-1}). Was observed increase tubular transporte the percent of sodium (%TNa^+) and chloride (%TCl^-) at 60 and 120 minutes (%TNa^+_{30}=73,59±3,34; %TNa^+_{60}=51,60±5,84*; %TNa^+_{120}=24,66±5,058*; %TCl^-_{30}=71,73±3,38; %TCl^{-60}=50,17±6,37*; $_{VBp}$%TCl^{-120} =44,69±5,038%*), without alteration in potassium transport. **Discussion:** The venom of *B. pauloensis* showed similar effects to those of *B. moojeni* and *B. jararacussu*, suggesting renal tubular changes, but with a much more pronounced diuretic effect. **Financial Support:** CNPq; CAPES.

06.032

Effects of a conjugated equine estrogen replacement therapy on metabolic parameters and vascular reactivity in spontaneously hypertensive rats. Ceravolo GS[1], Filgueira FP[1], Lobato NS[1], Tostes RCA[1], Fortes ZB[1], Dantas APV[3], Carvalho MHC[1] [1]ICB-USP - Farmacologia, [3]Harvard Medical School

Introduction: Cardiovascular disease (CVD) is the leading cause of death among postmenopausal women in the world and it has been shown that metabolic state might contribute to CVD in this case. This circumstance has been partly attributed to the negative effects of estrogen deficiency on metabolism and cardiovascular system. However, until now the effects described for estrogen reposition is controversial. **Objective:** We hypothesized that conjugated equine estrogen (CEE) improves vascular function and metabolic state in ovariectomized SHR. **Methods**: All of the procedures used in this study were in accordance with the guidelines of the ethics committee of the Institute of Biomedical Sciences-USP (n.98). Concentration-effect curve to noradrenaline (NE:10^{-9}-10^{-5}M), was performed in aortic rings with (E+) or without (E-) endothelium isolated from SHR into physiological estrous (Sham), OVX and OVX treated with CEE (OVX+CCE-0.0625mg/day, women´s equivalent dose). To analize the role of nitric oxide in the vascular effect of CEE, a concentration-effect curve to L-NAME was built in aortic rings pre-contracted with NE ($3x10^{-8}$M). The oxidative profile was evaluated by determination of reactive oxygen species (ROS) generation in aorta (dihydroethydine method), plasmatic lipid peroxidation (thiobarbituric acid) and total antioxidant status. The metabolic status was determined by the Lee index (obesity), HOMA index (insulin resistance) and total cholesterol (TC). The blood pressure was determined by indirect tail cuff method. **Results:** We observed that maximal response (Rmax) to NE in aorta rings E+ isolated from OVX+CCE was reduced in comparison of either OVX or Sham. The Rmax to NE in rings E- was similar between groups. The OVX-CEE also increased the response to L-NAME when compared with OVX rats. An increase in aorta ROS generation, in plasma lipid peroxidation and in oxidative status were observed in OVX in comparison with Sham. Treatment with CEE reduced the OVX oxidative profile.The OVX presents obesity, increased insulin resistance and TC and all those parameters were prevented by CEE treatment. **Discussion:** Our results show that OVX-SHR display abnormalities similar to those found in metabolic syndrome. CEE treatment improves endothelial function, oxidative profile and shows favorable metabolic effects. In conclusion, it might be suggested that CEE has a protective effect in the cardiovascular system in hypertensive states. Supported: FAPESP

06.033

Neuronal nitric oxide synthase-derived hydrogen peroxide is a new major endothelium-dependent relaxing factor. Capettini LSA[1], Côrtes FS[2], Gomes MA[3], Silva GAB[4], Pesquero JL[1], Teixeira MM[5], Lemos VS[1] [1]ICB-UFMG - Fisiologia e Biofísica, [2]UFMG - Farmacologia, [3]UFMG - Parasitologia, [4]UFMG - Morfologia, [5]UFMG - Bioquímica e Imunologia,

Introduction: Endothelium-dependent vasorelaxation in large vessels is mainly attributed to L-NAME-sensitive eNOS-derived NO production. Endothelium-derived hyperpolarizing factor (EDHF) is the component of endothelium-dependent relaxations that resists full blockade of NO synthases (NOS) and cyclooxygenases. H_2O_2 has been proposed as an EDHF in resistance vessels. However, a role for H_2O_2 in aorta, where NO is supposed to be the unique endothelium-derived relaxant factor, was never reported. In this work we propose that in mice aorta neuronal (n)NOS-derived H_2O_2 accounts for a large proportion of endothelium-dependent ACh-induced relaxation. **Methods:** We used 12 week old C57Bl/6J mice (protocol number 26/2007). Changes in isometric tension were recorded on myograph. NOS expression and localization were evaluated by Western blot and immunohistochemistry. NO and H_2O_2 production were measured by fluorescence microscopy, chemiluminescence and using carbon microsensors. *In vivo* anti-sense oligodeoxynucleotides (AS-ODN) technique was used to knockdown nNOS and eNOS. **Results and Discussion:** In mice aorta rings, ACh-induced relaxation (Emax=97.36±2.31%; pEC_{50}=7.38±0.22) was inhibited by L-NAME (300μM), and L-NNA (100μM), two nonselective inhibitors of NOS, and attenuated by selective inhibition of nNOS with 1μM L-Arg^{NO2}-L-Dbu-NH_2 2TFA (L-Arg^{NO2}-L-Dbu; E_{max}=37.74±3.11%; p<0.001) and 100μM 1-(2-trifluoromethylphehyl) imidazole (TRIM; E_{max}=49.27±5.88%; p<0.001). The relaxation induced by ACh was associated with enhanced H_2O_2 production in endothelial cells that was prevented by the addition of L-NAME, L-NNA, L-Arg^{NO2}-L-Dbu, TRIM, and removal of the endothelium. The addition of catalase (2400IU/mL), an enzyme that degrades H_2O_2, reduced ACh-dependent relaxation (E_{max}=56.36±12.11%; p<0.001) and abolished ACh-induced H_2O_2 production. RT-PCR experiments showed the presence of mRNA for eNOS and nNOS but not inducible NOS in mice aorta. The constitutive expression of nNOS was confirmed by Western blot analysis in endothelium-containing vessels but not in endothelium-denuded vessels. Immunohistochemistry data confirmed the localization of nNOS in the vascular endothelium. Antisense knockdown of nNOS decreased protein expression (~83%), as well as ACh-dependent relaxation (E_{max}=57.71±2.14%; pEC_{50}=6.5±0.08; p<0.001) and ACh-induced H_2O_2 production, while MM-ODN was without effect. Antisense knockdown of eNOS decreased protein expression (~75%) and ACh-induced relaxation (E_{max}=64.03±2.98%; pEC_{50}=5.99±0.14; p<0.001), but not H_2O_2 production. Residual relaxation in eNOS knockdown mouse aorta was further inhibited by the selective inhibition of nNOS with L-Arg^{NO2}-L-Dbu. In conclusion, these results show that nNOS is constitutively expressed in the endothelium of mouse aorta and that nNOS-derived H_2O_2 is a new major endothelium-dependent relaxing factor. Hence, in the mouse aorta, the effects of nonselective NOS inhibitors cannot be solely ascribed to NO release and action without considering the co-participation of H_2O_2 in mediating vasodilatation. **Financial support:** CAPES; FAPEMIG, CNPq.

06.034

Reduction of nNOS-derived H_2O_2 is involved in endothelial dysfunction in a murine model of atherosclerosis. Capettini LSA[1], Côrtes FS[2], Alvarez-Leite JI[3], Lemos VS[4]
[1]ICB-UFMG - Fisiologia e Biofísica, [2]UFMG - Farmacologia, [3]UFMG - Bioquímica e Imunologia, [4]UFMG - Fisiologia e Biofísica

Introduction: Decreased eNOS-derived NO bioavailability is wildly recognized as a key event in endothelium dysfunction related to atherosclerosis. Previous work from our group demonstrated that nNOS-derived hydrogen peroxide (H_2O_2) is an important vasorelaxant factor in the mouse aorta. **Aim:** In this work we aimed to investigate the role of nNOS-derived H_2O_2 in the impaired vasodilation elicited by acetylcholine in aortas from apolipoprotein knockout (apoE$^{-/-}$) mice. **Methods:** We used thoracic aorta from 12 week old C57Bl/6J and apoE$^{-/-}$ mice (protocol number 26/2007). Changes in isometric tension were recorded on myograph simultaneously with NO measurements by mean of carbon microsensors. NOS expression and localization were evaluated by Western blot and immunofluorescence. H_2O_2 production was measured by fluorescence microscopy and chemiluminescence. *In vivo* anti-sense oligodeoxynucleotides (AS-ODN) technique was used to knockdown nNOS and eNOS. **Results and discussion:** ApoE$^{-/-}$ mice showed accentuated reduction in ACh-induced vasodilation (E_{max}=97.4±2.3% versus 50.4±3.8% to control and apoE$^{-/-}$, respectively), which was paralleled by a decrease in NO and H_2O_2 production. Previous work from our laboratory has shown that nNOS-derived H_2O_2 participates in endothelium-induced vasorelaxation in mouse aorta. Accordingly, in control animals ACh-induced relaxation (E_{max}=97.36±2.31%; pEC_{50}=7.38±0.22) was inhibited by half after selective inhibition of nNOS with L-Arg^{NO2}-L-Dbu-NH_2 2TFA (1μM; E_{max}=37.74±3.11%; $p<0.001$) and TRIM (100μM; E_{max}=49.27±5.88%; $p<0.001$) or selective knockdown of nNOS and by the use of catalase. Interestingly, in apoE$^{-/-}$ mice aorta, inhibition of nNOS with L-Arg^{NO2}-L-Dbu (1μM), knockdown of nNOS and catalase, minimally altered the ACh-induced relaxation or H_2O_2 production, indicating that in this model of atherosclerosis, n-NOS-derived relaxation and H_2O_2 production is impaired. Corroborating with this conclusion, ACh-induced phosphorilation levels of nNOS, as assessed by Western blot analysis, was also diminished in apoE$^{-/-}$ mice aorta suggesting a decreased function of this enzyme. Our immunofluorescence data show localization of nNOS restricted to endothelial cells in control animals, while in apoE$^{-/-}$ aorta nNOS appeared mainly in neointimal formation and smooth muscle cells layer. **Conclusion**: Together, our data clearly show for the first time that endothelial nNOS-derived H_2O_2 formation and vasorelaxation is impaired in apoE$^{-/-}$ mouse aorta and certainly contribute to the endothelial dysfunction in atherosclerosis. **Financial support:** CAPES; FAPEMIG, CNPq.

06.035

Efeito inotrópico positivo de frações de uma mistura esteroidal isolada das folhas de *Physalis angulata.* Gomes VM[1], Oliveira HCS[1], VERAS, M. L[2], Pessoa ODL[2], Fonteles MC[3], Santos CF[4], Nascimento NRF[5] [1]UFC - Ciências Fisiológicas, [2]UFC - Química Orgânica e Inorgânica, [3]Universidade Presbiteriana Mackenzie - Fisiologia e Farmacologia, [4]UFC - Medicina, [5]UFC - Medicina Veterinária

Atualmente, a insuficiência cardíaca é uma das principais doenças que atinge o sistema cardiovascular e está geralmente associada a patologias como diabetes e hipertensão. A terapêutica atual para o tratamento da insuficiência cardíaca ainda se mostra insuficientemente segura farmacologicamente devido aos mecanismos de ação intrinsecamente baseados na sobrecarga de cálcio. Busca-se então a prospecção de substâncias com atividade cardiotônica visando uma melhora na terapêutica da insuficiência cardíaca com maior eficácia e segurança. Uma mistura obtida por cromatografia, denominada PHY, oriunda das folhas de *Physalis angulata*, demonstrou previamente efeito inotrópico positivo. As frações isoladas de PHY, chamadas F9 e F13 foram estudadas nas contrações de cobaio atriais e ventriculares comparativamente aos digitálicos. Cobaias *Cavia porcellus* machos, foram sacrificados por deslocamento cervical, e em seguida o átrio direito e esquerdo e tiras de ventrículo foram retirados para imersão em solução nutritiva de Krebs-Henseleit, a 37°C e aerados com mistura carbogênica(95% O_2 e 5 % CO_2). Os tecidos foram montados em banhos de órgãos para registro isométrico acoplado a polígrafo de quatro canais para avaliação de parâmetros como frequência cardíaca das contrações espontâneas de átrio direito, tempo para resposta máxima (Tmax), tempo para alcançar 80% do relaxamento (TR80) em átrio esquerdo e ventrículo. Curvas dose-resposta de PHY, F13, F9 (0,1 a 100 µg/ml), DMSO (isovolumetricamente) e Ouabaína (0,88-62 µg/ml) foram realizadas nas preparações citadas sendo as respostas foram medidas 5 minutos após adição de cada concentração.Em átrio esquerdo de cobaia F9 promoveu aumento concentração-dependente no inotropismo (377,57 ± 24,73 %; n= 5) enquanto que F13 alterou negativamente o inotropismo (F13 10 µg/mL ; - 72,88 ± 7,66 %;n=5) sem alterar significativamente a frequência cardíaca em átrio direito. Não houve alterações com significância em relação ao tempo para atingir a resposta máx (Tmax) para PHY, F9 e F13 quando comparados ao controle.Porém, F9 diminuiu o tempo para atingir 80% do relaxamento quando comparado à PHY(PHY; Δ 13,80 ± 4,84 % vs. F9 100 µg/mL; Δ - 17,77 ± 9,36 %;n=5). As frações F9 e F13 apresentam efeitos inotrópicos opostos, sendo F9 inotrópico positivo e F13 inotrópico negativo.A fração F9 é, portanto mais potente e eficaz do que a mistura PHY.Concluímos então que a fração F9 deve ser considerada isoladamente para estudos posteriores. O número da Licença de Autorização do Comitê de Ética em uso Animal é 08627944-0. Apoio Financeiro: CNPq

06.036

Nitric oxide alterations in kinin B_1 receptor knockout mice. Loiola RA[1], Reis F[1], Hilzendeger AM[1], Guimarães AO[1], Kawamoto EM[2], Scavone C[2], Abdalla DSP[2], Bader M[3], Pesquero JB[1], Fernandes L[6] [1]UNIFESP - Biofísica, [2]ICB-USP - Farmacologia, [3]Max-Delbrück - Molecular Medicine - Hypertension, [6]UNIFESP - Ciências Biológicas

Introduction: Kinins play an important role in several biological functions, including inflammation and cardiovascular homeostasis. While B_2 receptors are constitutively expressed and mediate most of the effects assigned to kinins, B_1 receptors are weakly detectable under physiological conditions, but rapidly induced by inflammatory stimuli. In the vascular system, B_1 and B_2 activation by specific agonists (des-arg^9-bradykinin and bradykinin, respectively) results in nitric oxide (NO)-mediated vasodilation. Mice with a targeted B_1 receptor deletion ($B_1^{-/-}$) are healthy, fertile and normotensive; however, little is known about the role of NO on the cardiovascular system of these animals. The present study evaluated the NO-mediated vascular responses, NO vascular and circulating levels and NOSintase (NOS) activity and expression in $B_1^{-/-}$. **Methods:** Experiments were performed in male C57Bl/6 wild type (WT) and $B_1^{-/-}$ mice, aged 10 - 14 weeks (n=5-7). Procedures were approved by the Ethics Committee of UNIFESP (0928/05). The arterial mesenteric bed was isolated and perfused with Krebs solution at a constant rate of 2 mL/min using a peristaltic pump. Vasoactive agents were applied *in bolus* and vascular responses were evaluated as changes in the perfusion pressure (mmHg) measured by a data acquisition system (Power Lab 8/S). Acetylcholine (ACh), an endothelium-dependent vasodilator, and Sodium Nitroprusside (SNP), an endothelium-independet vasodilator, were tested (0.1, 1 and 10 nmol) in preparations pre-contracted with noradrenaline [NA, 10 µM]. NO plasmatic levels were evaluated by NO derivatives nitrate and nitrite, using a NO analyzer (NOA^{TM280}). Tissue NO production was determined in transverse mesenteric arteriole sections incubated with 4,5 diaminofluorescein diacetate (DAF 2DA), a NO-sensitive fluorescent dye, and digital images were quantified through the Image Plus® [arbitrary units (a.u.)]. In the mesenteric vessels, NOS activity was evaluated by biochemical conversion of L-[3H] arginine to L-[3H] citrulline and the genetic expression of NOS isoforms (nNOS, iNOS and eNOS) was analyzed by Real Time PCR. **Results:** ACh-vasodilation was significantly reduced in $B_1^{-/-}$ in comparison to WT, whereas no differences were observed for SNP responses (Table 1). $B_1^{-/-}$ presented decreased plasma (Wt 141.9±17.3 µM *vs* $B_1^{-/-}$ 49.6±10.5 µM*) and tissue NO (Wt 0.523±0.09 a.u *vs* $B_1^{-/-}$ 0.167±0.03 a.u.*). NOS activity was increased in $B_1^{-/-}$ vessels (WT 0.45±0.02 pmol/mg.min *vs* $B_1^{-/-}$ 1.73±0.65 pmol/mg.min*), whereas no differences were observed in mRNA levels for eNOS, iNOS and nNOS between groups.

Table 1: Vasodilation induced by ACh and SNP in mesenteric arterioles of WT and $B_1^{-/-}$

	ACh (nmol)			SNP (nmol)		
	0.1	1	10	0.1	1	10
WT	6.3±0.6	12.6±1.5	14.7±1.3	29.3±1.3	50.5±1.3	58.4±2.2
$B_1^{-/-}$	1.5±0.7*	2.5±1.2*	4.9±1.1*	32.6±3.3	49.1±2.4	53.7±2.5

Results (mean ± sem) are expressed as % of contraction to noradrenaline [10 µM]. *P<.05 *vs* WT

Discussion: B_1 receptor deletion in mice induces important alterations in the vascular reactivity and NO metabolism. The severe impairment in the endothelial-mediated vasodilation accompanied by decreased NO plasma and tissue levels, despite the augmented NOS activity, may strongly suggest the exacerbation of NO inactivation in this animal model. Otherwise, reduced NO bioavailability in $B_1^{-/-}$ might be due to a deficient production of this molecule, probably by decreased concentrations of L-arginine and/or co-factors employed by NOS. **Financial Support:** FAPESP (07/59039-2) and CNPq

06.037

Functional alterations in detrusor smooth muscle of renovascular and spontaneous hypertensive rats. Ramos-Filho, ACS[1], Rojas-Moscoso, J. A.[1], Monica FZT[1], Bau FR[1], Antunes E[1] [1]FCM-UNIICAMP Pharmacology -

Introduction: Arterial hypertension has been associated with an overactivity bladder resulting in increased urinary frequency and non-voiding detrusor contractions, but little is known about the mechanisms underlying such alterations. This study aimed to investigate the contractile and relaxing responses in isolated detrusor smooth muscle (DSM) of spontaneous hypertensive (SHR) and renovascular hypertensive rats (2 kidney, 1 clip model; 2K-1C). **Methods:** The experimental procedures were approved by the Animal Care and Use Committee of the State University of Campinas (UNICAMP-protocol n°1683-1). Male rats were stunned by inhalation of CO_2, euthanized by decapitation, and exsanguinated. The bladder was quickly removed and placed in Krebs-Henseleit buffer (95%O_2/5%CO_2, 37°C). DSM was mounted in 10-ml organ bath, and connected to force displacement transducers at 10 mN tension. Changes in isometric force were measured using transducers and recorded in a MacLab data acquisition system. Relaxant responses to sodium nitroprusside (SNP; 0.001-100 μM) and BAY 41-2272 (NO-independent stimulator of soluble guanylyl cyclase stimulator; 0.001-100 μM), as well as contractile responses to muscarinic agonist carbacol (0.001 a 100 μM) were performed. **Results and discussion:** In SHR group, a significant decrease ($P<0.001$) in the maximal DSM relaxing responses for SNP (47.9±3.2%) and BAY 41-2272 (75.7±6.9%) in comparison with normotensive Wistar Kyoto rats (73.2±6.9% and 84.7±7.1%, respectively) was observed. In 2K-1C group, no differences in DSM relaxing responses for SNP and BAY 41-2272 were found. The carbachol-induced DSM contractions in 2K-1C were higher compared with sham-operated rats (208.4±26.6 and 145.8±12.5 mN, respectively; $P<0.001$), whereas in SHR group no differences in were found. Our data show that functional alterations in DSM of hypertensive rats reflects lower relaxant activity and/or higher contractile activity depending of the arterial hypertension model employed. **Financial support:** CNPQ

06.038

Doxycycline reduces mortality in a rat model of acute pulmonary embolism. Cau SBA, Barato RC, Uzuelli JA, Tanus-Santos JE FMRP-USP - Farmacologia

Introduction: Inflammatory response contributes to the pathophysiology of acute pulmonary embolism (APE). Some inflammatory mediators such as matrix metalloproteinases (MMP) were associated with the cardiovascular damage caused by APE. We investigate the effects of doxycycline, a MMPs inhibitor, on APE-induced 24-hr mortality rate and inflammatory response in lungs of 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 treated as follows (4 experimental groups): intraperitoneal (I.P.) vehicle administration 30 minutes before saline intravenous administration into the tail vein (Vehicle + Control group); doxycycline (30 mg/kg; I.P.) administration 30 minutes before saline intravenous administration (Doxy + Control group); vehicle administration (I.P.) 30 minutes before intravenous administration of a suspension of microspheres (21 mg/kg) into the tail vein (Vehicle + APE group); and doxycycline (30 mg/kg; I.P.) administration 30 minutes before intravenous administration of a suspension of microspheres (Doxy + APE group). All groups were followed up for 24 hrs. After 24 hrs, the surviving animals were anesthetized with urethane (1 g/kg; I.P.) and bronchoalveolar lavage fluid (BAL) was collected. Perfused lungs were saved for biochemical dosages. **Results and discussions:** 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; $p<0.05$). The myeloperoxidase activity (MPO) was measured as a marker of neutrophils accumulation. APE increased neutrophils in the lungs of rats from both *Vehicle + APE* ($1.51x10^6 \pm 0.15x10^6$ to $2.63x10^6 \pm 0.20x10^6$ neutrophils /100 mg of lung; $p<0.05$) and *Doxy + APE* group (to $2.58x10^6 \pm 0.19x10^6$ neutrophils /100 mg of lung; $p<0.05$). The APE-induced BAL alterations in total proteins and cells were determined. Total proteins were increased in *Vehicle + APE* (to 489 ± 60 µg/ml; $p<0.05$) and *Doxy + APE* group (to 497 ± 63 µg/ml; $p<0.05$) compared with *Vehicle + Control* group (191 ± 16 µg/ml). APE was associated with non significant increases in total cell count in BAL. However, the percentage of neutrophils increased in *Vehicle + APE* group (3.9 ± 0.9 to 33.5 ± 5.0 % of neutrophils; $p<0.05$), and treatment with doxycicline did not attenuated the neutrophil exudation to alveolus in *Doxy + APE* group (to 32.8 ± 5.6 % of neutrophils; $p<0.05$). Gelatin zymography of BAL was performed. Compared with nonembolized animals, BAL from embolized rats had higher levels of both 72 KDa- (0.71 ± 0.08 and 3.03 ± 0.05 arbitrary units, respectively; $p<0.05$) and 92 KDa-MMPs (0.30 ± 0.16 and 1.21 ± 0.28 arbitrary units, respectively; $p<0.05$). Moreover, treatment with doxycycline attenuated the 92 KDa-MMP increases (to 0.85 ± 0.23; n.s.). None of the evaluated parameters was significantly altered in *Doxy + Control* group. Our results suggest that pretreatment with doxycycline increases 24-hr survival rate, however with minimal effects on APE-induced lung inflammatory response. **Acknowledgments:** FAPESP, CAPES and CNPq.

06.039

Avaliação da atividade cardiovascular do extrato bruto de *Lycnhophora trichocarpha* em ratos wistar. Souza ACM[1], De Paula DCC[1], Guimarães HN[2], Saúde-Guimarães DA[1], Grabe-Guimarães A[1] [1]UFOP - Farmácia, [2]UFMG - Engenharia Elétrica

Introdução: Várias espécies de *Lychnophoras*, popularmente conhecidas como "arnicas", são plantas nativas do Brasil, utilizadas pela população como analgésicos e antiinflamatórios. O uso indiscriminado das diversas espécies de *Lychnophoras*, na forma de extratos etanólicos, leva á necessidade de investigações que avaliam a sua segurança terapêutica. O presente trabalho tem como objetivo caracterizar, em ratos Wistar, a atividade cardiovascular *in vivo* do extrato bruto de *Lychnophora trichocarpha* Spreng. **Métodos:** Foram utilizados ratos Wistar machos, seguindo devidamente o comitê de ética 2009/11, divididos em 5 grupos que receberam extrato etanólico de *L. trichocarpha* 1,5 g/kg (100 mg/ml) solubilizado em capriol:água (6,5:3,5) por via oral em dose única (n=30): 1) Controle: veículo; 2) Somente extrato**;** 3) Extrato + atenolol (5 mg/kg); 4) Extrato + captopril (10 mg/kg); 5) Extrato +Prazosin (1 mg/kg). Cento e cinquenta minutos após a administração do extrato, os animais foram anestesiados pelo tiopental (60 mg/kg), e tiveram cateteres implantados na artéria femoral para registro da PA e na veia femoral para administração dos fármacos. Eletrodos foram introduzidos no tecido subcutâneo para registro do ECG na derivação DII. Para os grupos 1 e 2, os registros foram obtidos a partir de 3,5 h após administração do extrato. Os outros grupos receberam 4 h após a administração do extrato. **Resultados:** A avaliação dos parâmetros cardiovasculares mostrou que o extrato de *L. trichocarpha* induziu à hipertensão arterial, sendo o valor máximo para PAS de 197 mmHg no tempo 3 h e 55 min e PAD de 149 mmHg em 4 h após a administração. Foi observada também a presença de taquicardia durante todo o tempo analisado, sendo o máximo de 442 bpm. O prazosin reduziu significativamente a PAS e PAD, sendo o máximo de redução 34,25% e 39,11% respectivamente. O atenolol reduziu significativamente a FC sendo o máximo de 34,25%. O captopril não alterou significativamente a hipertensão arterial induzida pelo extrato. **Conclusões:** Os resultados sugerem que o extrato bruto de *Lychnophora trichocarpha* apresenta atividade hipertensiva, por isso seu uso por via oral deve ser evitado. O provável mecanismo parece ocorrer pela hiperatividade do sistema nervoso simpático periférico ao nível vascular. **Apoio financeiro:** FAPEMIG, UFOP , UFMG e CNPQ

06.040

Focal adhesion kinase regulates load-induced PGC-1a expression and mitochondrial biogenesis in mice left ventricle. TornatoreTF[1], Clemente CFMZ[1], Judice CC[1], Rocco SA[1], Theizen TH[1], Macedo AHP[1], Dalla Costa AP[2], de Lima J[1], Oliveira MV[1], Franchini KG[1] [1]UNICAMP - Clínica Médica, [2]FCM-UNICAMP

Aims: Mechanical stress invokes mitochondrial biogenesis and shifts of the substrate metabolism of myocardium. These processes are mostly drive by NRF-1 and Tfam transcription factors, which are coordinately regulated by transcriptional co-activator PGC-1a. The molecular mechanisms involved in the activation of PGC-1a by the mechanical stimuli are not known. Herein, we examined whether signaling mediated by FAK (Focal Adhesion Kinase) plays a role in the activation of PGC-1a and mitochondrial biogenesis induced by pressure overload in mice left ventricle (LV). **Methods:** Knockdown of FAK in mice LV was obtained by administering siRNA targeted to FAK ($siRNA^{FAK}$) through the jugular vein. Pressure overload of LV was induced by transverse aortic constriction (TAC). The levels of FAK and ANP transcripts were obtained by quantitative RT-PCR (qRT-PCR). Western blotting was used to detect the protein levels of FAK, phosphoFAK-Tyr397, PGC-1a and NFR-1. Mitochondrial biogenesis was assessed by the mtDNA/nDNA ratio obtained by qRT-PCR of D-Loop and 18S genes. Myocardial AMP and ATP levels were obtained by a chromatographic method. **Results:** $siRNA^{FAK}$ reduced myocardial FAK transcript and protein by ~50, 25 and 20% after 1, 7 and 15 days of TAC, respectively. FAK depletion markedly attenuated the hypertrophic growth and the increases of myocardial ANP transcript in TAC mice, in respect to TAC mice treated with $siRNA_{GFP}$. TAC enhanced the myocardial PGC1-a, and NRF-1 and FAK knockdown attenuated this increase. Pressure overload lasting 7 and 15 days markedly increased the mtDNA/ nDNA ratio in the LV. Depletion of FAK attenuated the rises in the mtDNA/ nDNA ratio induced by aortic banding. The AMP/ATP ratio was show to remain unchanged in the myocardium of 1 day but it was progressively reduced in 7 and 15 day TAC in respect to SO mice. **Conclusion:** These findings demonstrate that signaling mediated by FAK controls mitochondrial biogenesis in the myocardium in response to pressure overload by regulating the expression of PGC-1a and NRF-1. These are novel evidences that FAK may be involved in the pathophysiology of cardiac hypertrophy and failure by regulating myocardial energy metabolism.

06.041

Nanoparticles of gold potentiated vasodilatation induced by nitric oxide donors in endothelium intact rat aortic rings. Silva BR[1], Lunardi CN[2], Da Silva RS[2], Bendhack LM[2] [1]FMRP-USP - Farmacologia, [2]FCFRP-USP - Física e Química

Introduction: Nitric Oxide (NO) plays important role in the control of the vascular tone. NO can be produced in the endothelial cells (EC) or released from NO donors in vascular smooth muscle cells (VSMC). Activation of endothelial muscarinic receptors induces NO-synthase activation and NO production. NO activates the soluble guanylyl-cyclase (sGC) and G-kinase protein, which decreases the cytosolic calcium concentration ($[Ca^{2+}]c$) in the VSMC. Gold Nanoparticles (AuNPs) have been synthesized and functionalized to NO donor ruthenium complex to modify the NO release. This study aimed to investigate whether the functionalization of (AuNPs) to Cis-$[Ru(bpy)_2(NO)(4PySH)].(PF6)_3$ (Ru-4PySH) - forming AuNPs-$\{Ru\text{-}4PySH\}_n$ - potentiates the relaxation induced by this NO donor (Ru-4PySH) and to study their effects on sGC activation, $[Ca^{2+}]c$, and the contribution of the EC to the vascular relaxation induced by the NO donors. **Methods:** Cumulative concentration-effect curves for the AuNPs, Ru-4PySH and AuNPs-$\{Ru\text{-}4PySH\}_n$ were constructed in rat aortic rings pre-contracted with 100nM phenylephrine in the presence or in the absence of the selective sGC inhibitor (ODQ, 1µM) in denuded endothelium rat aortic ring. Concentration-effect curves for the NO donors were also constructed in intact endothelium aortic rings in the presence or in the absence of NO-synthase inhibitor (L-NAME, 100 µM) or muscarinic antagonist (Atropine 0.5 µM). $[Ca^{2+}]c$ was measured in VSMC with fluorescent probe FLUO-3AM (10 mM) by using a fluorescence Olympus microscope. **Results:** The compounds Ru-4PySH and AuNPs-$\{Ru\text{-}4PySH\}_n$ released NO as detected by amperometry with a selective NO sensor**.** Both compounds induced dependent-concentration vasodilatation in denuded endothelium aortic rings: Ru-4pySH (Emax: 104.6±0.9%; pD_2: 6.22±0.13; n=10; P<0.05) that was similar to AuNPs-$\{Ru\text{-}4PySH\}_n$ (Emax: 102.2±2.2%; pD_2: 6.34±0.07 n=10; P<0.05), whereas AuNPs almost did not induce vascular relaxation (Emax: 8.9±4.6%; n=4, P<0.05). ODQ reduced the relaxation to both compounds Ru-4PySH (Emax: 6.2±3.3%; n=3; P<0.05) and AuNPs-$\{Ru\text{-}4PySH\}_n$ (Emax: 25.4±8.0%; n=7; P<0.05). In intact endothelium aortic rings, the relaxation was potentiated to AuNPs-$\{Ru\text{-}4PySH\}_n$ (pD_2: 6.82±0.18; n=5; P<0.05), and this response was inhibited by L-NAME (pD_2: 6.38±0.03; n=5; P<0.05) or by the antagonist Atropine (pD_2:6.09±0.05; n=4; P<0.05). The complex AuNPs-$\{Ru\text{-}4PySH\}_n$ (10 mM) decreased $[Ca^{2+}]_c$ in VSMC (%ΔFI= -13.4±1.3%, n=4, P<0.05), which effect was greater than that obtained for Ru-4Py-SH (10 mM) (%ΔFI= -5.84±0.54%, n=3, P<0.05). **Discussion:** Our results demonstrate that the relaxation induced by the new NO donors Ru-4PySH and the form AuNPs-$\{Ru\text{-}4PySH\}_n$ involves the activation of sGC and reduction of $[Ca^{2+}]_c$. The complex AuNPs-$\{Ru\text{-}4PySH\}_n$ had greater effect than Ru-4Py-SH in reducing $[Ca^{2+}]c$. The functionalization of the Gold Nanoparticles potentiated the vasodilatation induced by the compound Ru-4PySH in intact endothelium aortic rings, probably due the mechanisms that seem to involve endothelial muscarinic receptor and endothelial NO-synthase activation with consequent NO production. Comitê de Ética FMRP-USP (No. 07.1.608.53.8). **Supported by** FAPESP, CAPES and CNPq.

06.042

The agonist of angiotensin-(1-7) receptor, AVE 0091, improves inflammatory response following renal ischemia and reperfusion. Barroso LC[1], Silveira KD[2], Lima CX[3], Borges VO[4], Santos RAS[2], Simões e Silva AC[5], Souza DG[6], Teixeira MM[1] [1]UFMG - Bioquímica e Imunologia, [2]UFMG - Fisiologia e Biofísica, [3]UFMG - Fisiologia e Farmacologia, [4]UFMG - Bioquímica, [5]UFMG - Pediatria, [6]UFMG - Microbiologia

Introduction: The process of renal ischemia is responsible for important damages to renal function. The reperfusion of tissue is the main factor of damages to the target organ, leading to the recruitment of inflammatory cells and production of mediators to the tissue. This work evaluated the renal response to treatment with the agonist of angiotensin-(1-7) receptor, AVE 0991, after induction of renal ischemia reperfusion. **Methods:** Male C57BL/6 mice were divided in the following groups: animals submitted to bilateral ischemia (30 min) and reperfusion (24 hours) of renal pedicle, treated with different concentrations of AVE (0.03 mg/kg, 0.3 mg/kg and 3.0 mg/kg of weight); animals submitted to IR, treated with vehicle received NaCl 0.9%; control group, consisted of animals submitted to the same surgical traumas, except for the renal ischemia. The animals were sacrificed, under anesthesia, for plasma and renal tissue collecting. The renal parameters (plasmatic and urinary creatinin, urinary flow, osmolality and creatinine clearance) were evaluated, as well the inflammatory parameters (migration of neutrophils and cytokine levels in renal tissues - TNF-a and CXCL1/KC - by mieloperoxidase activity (MPO) and ELISA, respectively). Data were expressed as mean ± standard error and analyzed by ANOVA followed by Student Newman Keuls post test. The differences were considered significant when $p<0.05$. **Results:** The process of ischemia/reperfusion caused a decrease of renal function characterized by an increase in plasma creatinin levels and by polyuria presence. Increase on neutrophils number and CXCL1/KC levels were also observed. Animals that received AVE 0991 showed reduction in neutrophils migration to tissue, as well a reduction of renal KC levels and plasma creatinin in a dose-dependent manner. However AVE 0991 treatment did not improve renal function after IR. **Discussion:** The agonist of Angiotensin-(1-7) receptor, AVE 0991, showed an important anti-inflammatory effect in ischemic renal tissue. **Financial Support**: CAPES, CNPq and FAPEMIG. Licença de autorização do comitê de ética animal: 263/2008.

06.043

Characterization of human kidney Na^+,K^+-ATPase inhibition by the combination of cardiac glycosides. Touza N[1], Pôças ESC[1], Quintas LEM[1], Cunha-Filho GS[2], Santos ML[2], Noel F[1] [1]ICB-UFRJ - Farmacologia Bioquímica e Molecular, [2]IQ-UNB - Isolamento e Transformação de Moléculas Orgânicas

Introduction: We recently showed that ouabain (a cardiac glycoside) and 8-metoxy-3,9-dihydroxy coumestan (a non-steroidal inhibitor of Na^+,K^+-ATPase) have a synergistic effect on Na^+,K^+-ATPase (Pôças et al., Life Sci 81:1199, 2007). Since recent studies reveal the presence of endogenous cardiac glycosides in humans, the objective of present work was to verify if such synergism could occur between some of these putative endogenous glycosides and/or between such glycosides and digoxin, clinically used for the treatment of cardiac failure. **Methods**: We studied the level of inhibition of Na^+,K^+-ATPase obtained from human kidney (CEP/HUCFF-UFRJ, protocol Nr. 038/08) in the presence of different combinations of bufadienolides (marinobufagin and telocinobufagin, isolated from the venom of Brazilian toad *Rhinella schneideri*) and cardenolides (ouabain and digoxin, commercially available), comparing the theoretical (composite additive) and experimental (mixture) inhibition curves. For the construction of the (experimental) combination curves, we used increasing concentrations of the mixtures Drug1:Drug2 in a fixed ratio for determining the IC_{50} value by non-linear regression analysis of the data (Prism software). Our second analysis approach was based on the classical construction of an isobologram, a graph of equally effective dose pairs (isoboles) for a single effect level. The colorimetric method of Fiske and Subbarow was used for quantification of the inorganic phosphate released from the ATP hydrolysis. **Results**: With all combinations tested (marinobufagin:ouabain, marinobufagin:digoxin, telocinobufagin:digoxin and ouabain:digoxin), the experimental (mixture) curve was superimposed on the theoretical additive curve indicating that the combination resulted in simple additivity and not synergism or antagonism, as also confirmed through the isobologram analysis. **Discussion**: Our results indicate that a simple additive effect is to be expected with respect to Na^+,K^+-ATPase inhibition in pathophysiological conditions where endogenous cardiac glycosides are released in the circulation, as reported in chronic renal failure, cardiac congestive failure or pre-eclampsia (Bagrov et al., Pharmacol Rev 61:9, 2009). The same additive effect could occur between such endogenous cardiac glycosides and digoxin, in the case of treatment of cardiac failure with this cardenolide. **Financial Support**: FAPERJ, CNPq

06.044

Characterization of erectile dysfunction in obese mice. Silva FH[1], Flores Toque HA[1], Claudino MA[1], Calixto MC[1], Lintomen L[1], Saad MJA[2], De Nucci G[1], Antunes E[1]
[1]UNICAMP - Pharmacology, [2]UNICAMP - Clínica Médica

Introduction: Corpus cavernosum (CC) smooth muscle tone is mainly regulated by nitric oxide (NO) released in the nitrergic nerve terminals and endothelial cells. NO activates soluble guanylyl cyclase (sGC) enzyme, resulting in an increased intracellular cyclic guanosine monophosphate (cGMP) level, which leads to relaxation of smooth muscle in the corpus cavernosum and penile erection. Several studies have shown a strong association between erectile dysfunction (ED) and obesity. The obesity causes impaired in the CC relaxations *in vitro* and *in vivo*, possibly by changes in the normal balance of vasoconstriction mechanisms and reduction in the NO bioavailability in erectile tissue, but little is known about the physiopathology connecting ED with obesity. Thus, the aim of work was to characterize the CC relaxing and contractile mechanisms in a murine model of obesity. **Methods:** The experimental protocols were approved the Animal Ethical Committee of UNICAMP. C57/BL6 mice were used in all experiments. Animals were divided into two groups: (a) Control: received a standard chow diet (carbohydrate 70%; protein 20%; fat: 10%) and (b) Obesity: received a high-fat diet (carbohydrate 29%; protein 16%; fat: 55%). After 10 week-diet, lipid profile and glucose levels were evaluated. Concentration-response curves to the relaxing agents acethylcholine (ACh) and the Rho-kinase inhibitor Y 27632, as well to the contracting agent phenylephrine (PE) were obtained in mice CC. Values of potency (pEC_{50}) and maximal responses (E_{max}) were calculated. The contractile and relaxant responses induced by electrical-field stimulator (EFS) were also obtained in all groups. **Results:** High-fat diet mice exhibited a 44% and 400% increase in body weight and epididimal fat, when compared with control group ($P<0.05$). Obese mice also showed increased plasma levels of total cholesterol and LDL ($P<0.05$). Relaxing responses induced by EFS (nitrergic relaxations) in high-fat fed mice were significantly reduced in all frequency studied. Obese mice also showed a significant reduction of ACh-induced relaxation (pEC_{50}: 6.81±0.06 and Emax: 54±6%) compared with control animals (7.38±0.04 and 80±3%, respectively). High-fat diet did not change the E_{max} values for Y-27632 when compared with control group (95 ± 13% and 110 ± 5%, respectively). The phenylephrine-induced contractile responses were significantly higher in obese mice compared with control group (E_{max}: 127±6% and 101±7%; $p<0.05$). The EFS-induced contractile responses remained unaltered in all investigated groups. In presence of Y-27632, EFS (32 Hz)-induced contractile responses in obese mice were significantly lower (53±3%; $P<0.05$) compared with control group (84 ± 8%). **Conclusion:** Our findings showed that high-fat diet mice exhibits impaired nitrergic- and endothelium-dependent relaxations in corpus cavernosum of obese mice, along with increased contractile response with participation of Rho-kinase signaling pathway. The murine model of obesity used is suitable to further understand the mechanisms underlying the erectile dysfunction in obese individuals. **Financial Support:** FAPESP and CAPES.

06.045

Impaired cardiac left ventricular hypertrophy and neointimal hiperplasy are not related to an additional blockade of the RAS and reduction in arterial pressure of 2 kidney-1 clip hypertensive rats. Correa JWN[1], Prado CM[2], Rossi MA[2], Bendhack LM[3] [1]FMRP-USP - Farmacologia, [2]FMRP-USP - Patologia, [3]FCFRP-USP

Introduction: The main pathophysiological effector induced by the activation of the renin-angiotensin system (RAS) is angiotensin II, which can be inhibited by ACE inhibitor (enalapril) or by the AT_1 receptor antagonist (losartan). This study aimed to evaluate the effects of the treatment of renal hypertensive rats and normotensive rats with losartan and enalapril, alone or in combination, on systolic arterial pressure (SAP), left ventricular hypertrophy and fibrosis and aortic remodeling. **Methods:** Rats were submitted to the surgery to induce renal hypertension (2K-1C) and control rats were sham-operated (2K). Six weeks after the surgeries, they were treated by gavage with losartan (3mg/kg/d), enalapril (2mg/kg/d), losartan+enalapril (2 and 3mg/kg/d, respectively) or saline for 14 days. The SAP was measured every 3 days during the period of treatment. Then, hearts and aortas were removed and morphometry was analyzed. **Results:** Six weeks after the surgeries, SAP of 2K-1C (208.8±3.3mmHg, n=44) was higher than 2K (112.6±1.5mmHg, n=48, P<0.0001). All the treatments did not alter SAP in 2K rats. The treatment with losartan and enalapril decreased SAP of 2K-1C to 184.4±11.9mmHg (n=9, P<0.05) and 177.5±8.7mmHg (n=6, P<0.05, respectively. The combination of losartan and enalapril produced additional reduction of SAP in 2K-1C rats (152.8±9.2mmHg, n=7, P<0.05). The cardiac hypertrophy index (HW/BWx1000) was higher in 2K-1C (4.55±0.04, n=4) than in 2K rats (3.05±0.14, n=3, P<0.05). No significant effect was observed after the treatments. Cardiomyocites diameter of 2K-1C (12.17±0.29µm, n=6) was higher than 2K (11.14±0.15µm, n=6, P<0.005), that were not modified by the treatments. On the other hand, cardiomyocite hypertrophy of 2K-1C rats was reversed by all the treatments. The treatments reduced collagen deposition in 2K from 1.28±0.09% to 0.75±0.05% (P<0.05). It was higher in 2K-1C rats (1.77±0.16%, n=7, P<0.05). Losartan reversed the fibrosis induced by hypertension (1.12±0.14%, n=5, P<0.05). Enalapril alone (0.81±0.11%, n=6, P<0.05) or in combination with losartan, additionally reduced the left ventricular collagen content to 0.86±0.11% (n=6, P<0.05). It was observed a medial thickening in 2K-1C aortas (166.3±6.9µm, n=6, P<0.0001) as compared to 2K aortas (108.8±3.6µm, n=6). All the treatments similarly reduced the medial thickening in approximately 12%. Intimal hipertrophy induced by hypertension (2K-1C: 15.56±0.89 µm, n=7, P<0.0001) vs. (2K: 8.24±0.80 µm, n=6) was reversed by enalapril (9.52±0.45µm, n=5, P<0.0001) or by the combination of enalapril and losartan (8.17±0.53µm, n=6, P<0.0001). Losartan alone did not reduce intimal thickening (13.13±0.85 µm, n=6). Treatments did not alter the medial and intimal thickening in 2K rats. **Discussion**: RAS dual blockade induces additional hypotensive effect in the SAP of 2K-1C rats as compared to monotherapy but the combination of losartan and enalapril does not have additional effect to the treatment with enalapril alone in relation to the impaired left ventricular hypertrophy and fibrosis and intimal thickening. **Protocol CEUA-USP-RP nº07.1.607.53.1. Supported by** FAPESP and CNPq**.**

06.046

Reactive oxygen species participation on angiotensin II-induced vascular hyper-reactivity in contralateral artery after balloon catheter injury. Olivon VC[1], Gomes MS[1], Pereira AC[1], Ramalho L[2], de Oliveira AM[1] [1]FCFRP-USP - Física e Química, [2]USP - Patologia

Introduction: Balloon catheter injury induced increased Ang II-contraction in contralateral carotid arteries when compared to control 15 days after injury. The injury increased substance P (SP) levels, at 1 day after balloon catheter injury. It is well kwon that SP could induce generation of reactive species of oxygen (ROS). Among the numerous signaling molecules involved in Ang II-induced vascular actions, ROS appear to be critical. Vascular ROS are produced in endothelial and VSMCs derived predominantly from vascular NAD(P)H oxidase. Vascular NAD(P)H oxidase comprises at least four components: cell membrane-associated p22phox, gp91phox and cytosolic subunits, p47phox and p67phox. **Aim**: Based in this information, the aim of this study was investigated ROS modulation in Ang II-induced vascular hyper-reactivity in contralateral artery after balloon catheter injury. **Methods:** Vascular reactivity experiments to Ang II were realized with endothelium-intact and endothelium-denuded rats carotid artery rings, 15 days after the injury. To analyze ROS production, pre-incubation with 4,5-Dihydroxy-1,3-bezenedisulfonic acid disodium salt monohydrate (Tiron, ROS scavanger, 10 mmol/L, 30 min.). To study ROS formation was realized DHE fluorescence and imunohistochimistry to $p22^{phox}$. All procedures were in accordance with the standards and policies of the Animal Care Committee of this institution (06.1.1019.53.5). **Results:** Ang II maximum effect (E_{max}) was increased after balloon catheter injury in contralateral (0.38 ±0.02 g) when compared to control (0.29±0.02g), but the potency (pD_2) was not different (Control: 9.08 ±0.10 g; Contralateral: 9.15 ±0.10 g). In the presence of Tiron, E_{max} to Ang II in control with endothelium was not different, but in contralateral with endothelium there was reduction in E_{max} (0.24 ±0.02 g) and pD_2 (8.61 ±0.10 g) values. DHE fluorescence and $p22^{phox}$ expression were increased in contralateral. **Conclusion**: The reactive oxygen species participated in vascular hyper-reactivity to Ang II in contralateral after balloon catheter injury. **Financial Support**: FAPESP and CNPq

06.047

The role of nitric oxide pathway in the increased angiotensin II-induced contraction in contralateral artery after balloon catheter injury. Olivon VC[1], Gomes MS[1], Evora PRB[2], Ramalho L[3], de Oliveira AM[1] [1]FCFRP-USP - Física e Química, [2]FMRP - Cirurgia e Anatomia, [3]USP - Patologia

Introduction: The adverse functional effects of balloon angioplasty include simple procedure failure, compromise of vessel lumen and restenosis. Balloon injury promoted Angiotensin II (Ang II) vascular hyper-reactivity in contralateral artery. Basal release of Nitric Oxide (NO) from the endothelium reduces the contractile effect of Ang II and many other vasoconstrictors. There is, however, a more profound inter-relationship between NO and Ang II. NO is emerging as a regulating factor of the rennin-angiotensin system at different levels, whereas Ang II seems also to have a regulatory function on NO generation. **AIM**: The aim of this study was analyzed the role of the NO pathway on Ang II vascular hyper-reactivity in contralateral artery after balloon catheter injury. **Methods:** Vascular reactivity experiments to Ang II were realized with endothelium-intact and endothelium-denuded rats carotid artery rings, 15 days after the injury. To investigate the nitric oxide pathway, pre-incubation with N^G-nitro-L-arginine-methyl-ester (L-NAME, non selective nitric oxide inhibitor, 100 µmol/L, 30 min.), 7-Nitroidazole (7-Ni, selective inhibitor nNOS, 100 µmol/L, 30 min.), N^G-nitro-L-Arginine (L-NNA, selective inhibitor eNOS, 100 µmol/L, 30 min.) or N-[3-(aminomethyl)benzyl] acetamina (1400W, selective inhibitor iNOS, 10 µmol/L, 30 min.). Imunohistochimistry was realized to eNOS, iNOS and nNOS. Nitrate/nitrite tecidual was measured. All procedures were in accordance with the standards and policies of the Animal Care Committee of this institution (06.1.1019.53.5). **Results:** Ang II maximum effect (E_{max}) was increased after balloon catheter injury in contralateral when compared to control, but the potency (pD_2) was not different. The L-NAME induced increased in Ang II E_{max} in control artery. However, on contralateral artery in presence of the L-NAME the E_{max} value was not different, but the pD_2 was reduced. The same results were obtained in the presence of 7-NI and 1400W inhibitors. The L-NNA evoked an increased in E_{max} value on contralateral artery. The expression of nNOS, eNOS, iNOS and nitrate/nitrite tecidual was reduced.

Groups	E_{max}		pD_2	
Agonist	Control	Contralateral	Control	Contralateral
Ang II (E+)	0.28±0.01g	0.38±0.01g*	9.24±0.13g	9.20±0.13g
Ang II (E-)	0.36±0.01g*	0.34±0.02g	8.91±0.11g	9.11±0.11g
Ang II(E+)+L-NAME	0.52±0.05g*	0.43±0.03g*	8.73±0.11g	8.07±0.10g#
Ang II(E-)+L-NAME	0.67±0.05g*	0.55±0.04g*#	8.79±0.09g	7.83±0.16g#
Ang II(E+)+7-NI	0.34±0.04g*	0.39±0.04g	7.74±0.19g	8.36±0.17g#
Ang II(E+)+L-NNA	0.38±0.04g*	0.49±0.05g*#	8.40±0.10g	8.32±0.10g#
Ang II(E+)+1400W	0.39±0.04g*	0.42±0.04g	8.13±0.51g	8.45±0.16g#

Data are shown as means ± s.e.mean. Anova and Newman-Keuls's post test, $P<0.05$. *Statistically significant differences from control group and # from contralateral group.

Conclusion: The reduction in NO formation/biodisponibility could contribute for hyper-reactivity to Ang II in contralateral artery after balloon catheter injury. **Financial Support**: FAPESP and CNPq

06.048

FAK depletion impairs cellular proliferation/differentiation and MMP-2 activation in cardiac fibroblast by mechanical stress through defective mobilization OF mTOR complex. Dalla Costa AP[1], Clemente CFMZ[1], Cardoso AB[3], Carvalho HF[3], Carvalheira JBC[2], Franchini KG[2] [1]FCM-UNICAMP, [2]UNICAMP - Clínica Médica, [3]UNICAMP - Biologia Celular

Introduction: Myocardial fibrosis that occurs in response to hemodynamic overload and cell injury depends on activation of cardiac fibroblasts. This is a coordinated process that involves proliferation and phenotypic transition of quiescent fibroblasts to contractile and secretory myofibroblasts. Here, we investigate whether signaling mediated by FAK plays a role in the activation of cardiac fibroblasts in response to mechanical stress. The purpose of this study was to evaluate the role of FAK in controlling the MMP 2 and 9 expression and activity in rat cardiac fibroblast. **Methods***:* The study was performed in culture of neonatal rat cardiac fibroblasts at 3rd passage and 80% of confluence (CF-P3/80), grown on silicon plates and then subjected to cyclic stretch (10%) for up to 8 hours, except the controls. The FAK expression and activity were assessed for imunoblottings through with specific antibodies to FAK or phospho - FAK Tyr397, respectively. The proliferation (anti-BrDU and anti-Ki-67 nuclear labeling), differentiation to myofibroblasts (expression and staining of alpha-smooth muscle actin - α-SMA) The expression of MMP 2 and 9 was evaluated with specific antibodies in imunoblottings and activity through zimography both in the supernatant culture. Furthermore, FCRNs depleted of FAK was defective in AKT Ser473, TSC-2 Thr1462, and S6 kinase Thr389 phosphorylation in response to cyclic stretch. The activation of CF-P3/80 invoked by cyclic stretch was prevented by pre-treatment with the mTOR complex inhibitor rapamycin (20 nM) and activator leucine (4 mM). These findings demonstrate that FAK signaling plays a critical role in mediating the activation of cardiac fibroblasts invoked by mechanical stress possibly by coordinating the downstream mTOR signaling pathway. **Results**: We show that cyclic stretch of CF-P3/80 (biaxial, 1 Hz, 5-15% above initial length, 10min to 8hs) was paralleled by increases in FAK phosphorylation at Tyr397. Cyclic stretch lasting for 4hs enhanced CF-P3/80 proliferation (anti-BrDU and anti-Ki-67 nuclear labeling), differentiation to myofibroblasts (expression of α-SMA) and the activity of MMP-2 (matrix metalloproteinase-2; ratio *Active*MMP-2:*Pro*MMP-2) in the culture medium. Depletion of FAK by specific small interfering RNA suppressed all three aspects of CF-P3/80 activation invoked by cyclic stretch. Additionally, CF-P3/80 depleted of FAK were defective in AKT Ser473, TSC-2 Thr1462, and S6 kinase Thr389 phosphorylation in response to cyclic stretch. The activation of CF-P3/80 invoked by cyclic stretch was prevented by pre-treatment with the mTOR complex inhibitor rapamycin, whereas stimulation of mTOR complex with leucine suppressed the inhibitory influence of FAK depletion on CF-P3/80 proliferation and differentiation induced by cyclic stretch. **Conclusion***:* These findings demonstrate that FAK signaling plays a critical role in mediating the activation of cardiac fibroblasts invoked by mechanical stress through the regulation of mTOR complex activity. These considerations have important implications to our understanding of the ECM remodeling process in damaged and failing myocardium. Furthermore, the identification of FAK and mTOR complex as participants of the signaling route by which mechanical stress influences cardiac fibroblast activation may provide novel opportunities for pharmacological intervention of cardiac remodeling. Animal Experimentation Ethics Committee of the Biology Institute/ State University of Campinas (UNICAMP-IB-CEEA), according to Protocol No 1643-1. Research support: CNPq

06.049

Participation of superoxide derived from cyclooxygenase 2 on alteration of reactivity to phenilephrine in rat contralateral carotid following arterial balloon injury. Pernomian L[1], Gomes MS[2], de Oliveira AM[2] [1]FMRP-USP - Farmacologia, [2]FCFRP-USP - Física e Química

Introduction: Balloon catheter injury increases phenylephrine (Phe)-induced contraction in contralateral carotid (CL), 4 days after surgery. The inhibition of cyclooxygenase 2 (COX-2) decreased Phe-induced contraction in CL, suggesting the participation of COX-2 products in this response (Accorsi-Mendonça *et al*., 2004). COX-2 is able to produce superoxide anion (O_2^-), suggesting the participation of O_2^- derived from COX-2 in alteration of reactivity to Phe in CL. **Methods:** This work was approved by Ethical Commission of the College of Medicine of Ribeirão Preto of the University of São Paulo (nº121/2007). Reactivity studies were conducted in common carotid arteries of control adult male Wistar rats (CO), and in CL carotid arteries from rats underwent to the balloon injury, 4 days after surgery. Phe responses was studied by cumulative concentration-response curves (10^{-10} - 10^{-5} mol/L for Phe-induced contraction and 10^{-15} - 10^{-10} mol/L for Phe-induced relaxation) in endothelium-intact rings. The participation of endothelium in these responses was studied by endothelial remove. The participation of COX products in these responses was studied by addiction of indomethacin (non-selective COX inhibitor, 10^{-5} mol/L), SC560 (selective inhibitor of COX-1, 10^{-9} mol/L) or SC236 (selective inhibitor of COX-2, 10^{-10} mol/L). The participation of superoxide (O_2^-) on responses to Phe was assessed by addition of tempol (selective scavenger of O_2^-, 10^{-3} mol/L). The generation of O_2^- was measured by flow cytometry in endothelial cells from CO and CL arteries. The participation of COX-2 in generation of O_2^- in CL arteries was studied by addition of SC236 (10^{-10} mol/L) in endothelial cells suspension during flow cytometry. **Results:** Phe-induced contraction was increased after balloon injury in endothelium-intact CL (Emax = 0,54 ± 0,01 g, *n*=5) when compared to endothelium-intact CO (Emax = 0,38 ± 0,01g, *n*=5). Phe-induced relaxation was abolished in endothelium-intact CL (Emax = 2,13 $\pm$ 0,98%) when compared to endothelium-intact CO (Emax = 40,06 $\pm$ 1,23%, *n*=5). The removal of endothelium normalized Phe-induced contraction in CL arteries (Emax = 0,35 $\pm$ 0,03g, *n*=5). Indomethacin and SC236 normalized Phe-induced contraction (Emax = 0,36 ± 0,01g, *n*=5, and Emax = 0,34 ± 0,02g, *n*=5, respectively) and relaxation (Emax = 37,06 ± 1,11%, *n*=5, and Emax = 39,27 ± 1,15%, *n*=5, respectively) in CL arteries. Tempol also normalized Phe-induced contraction (Emax = 0,42 ± 0,01g, *n*=5) and relaxation (Emax = 39,91 ± 1,30%, *n*=5) in CL arteries. There was an increase in O_2^- production in endothelial cells from CL arteries (FI = 29.496,4 $\pm$ 1.204,6U, *n*=5) in relation to CO ones (FI = 16.874 $\pm$ 1.374,6U, *n*=5). SC236 normalized O_2^- production in endothelial cells from CL arteries (FI = 15.774,2 $\pm$ 902,1U, *n*=5). **Conclusions:** Balloon catheter injury increased Phe-induced contraction, abolished Phe-induced relaxation, and increased endothelial generation of O_2^- in CL 4 days after surgery. O_2^- derived from endothelial COX-2 is responsible for these functional alterations. **Reference:** Accorsi-Mendonça D, *et al.* The balloon catheter induces an increase in contralateral carotid artery reactivity to angiotensin II and phenylephrine. *Br J Pharmacol*, 142, 79-88, 2004.
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06.050

Phenilephrine-induced relaxation in rat carotid is mediated by endothelial α_{1D}-adrenoceptors different from muscular α_{1D}-adrenoceptors. Pernomian L[1], Gomes MS[2], de Oliveira AM[2] [1]FMRP-USP - Farmacologia, [2]FCFRP-USP - Física e Química

Introduction: α_1-adrenoceptor agonist phenylephrine (Phe) reduced perfusion pressure in rat mesenteric bed, suggesting a vasorelaxant response mediated by these receptors (Filippi *et al.*, 2001). **Methods:** This work was approved by Ethical Commission of the College of Medicine of Ribeirão Preto of the University of São Paulo (nº121/2007). Reactivity studies were conducted in rings from common carotid arteries of adult male Wistar rats. Phe-induced relaxation was studied by cumulative concentration-response curves for the agonist (10^{-15} - 10^{-10} mol/L), over pre-contraction with prostaglandin $F_{2\alpha}$ (10^{-5} mol/L), in presence of WB 4101 (selective α_{1A}-adrenergic antagonist, 10^{-10} mol/L). The characterization of Phe-induced relaxation receptors was performed from concentration-response curves for Phe (10^{-15} - 10^{-10} mol/L) in absence and presence of endothelium, prazosin (Pz, selective α_1-adrenergic antagonist, 10^{-9} mol/L), BMY 7378 (selective α_{1D}-adrenergic antagonist, 10^{-8} mol/L), yohimbine (selective α_2-adrenergic antagonist, 10^{-5} mol/L) and propranolol (non-selective β-adrenergic antagonist, 10^{-6} mol/L). The participation of endothelium and gap-junctions in Phe-induced relaxation was characterized by endothelial remove and the addition of β-glycyrrhetinic acid (β-GA, selective gap-junctions blocker, 10^{-5}mol/L), respectively. Phe-induced contraction was studied by cumulative concentration-response curves for the agonist (10^{-10} - 10^{-5} mol/L) in endothelium-intact rings. The antagonism on α_{1D}-adrenoceptors and the potency of the antagonists on these receptors, were characterized by Schild's analysis of data obtained from reactivity studies with crescent concentrations of Pz ($3x10^{-11}$ - 10^{-9}mol/L) and BMY ($3x10^{-10}$ - 10^{-8}mol/L) in Phe-induced contraction and relaxation. **Results:** Phe induced relaxation in endothelium-intact rat carotid (Emax = 40,06 ± 1,23%, pD_2= 12,03 ± 0,28, *n*=5). Endothelium removal (Emax = 1,27 ± 0,78%, *n*=5), Pz (Emax = 1,71 ± 1,05%, *n*=5) and BMY (Emax = 1,64 ± 0,82%, *n*=5) abolished this response. Yohimbine (Emax = 41,73 ± 1,02%, *n*=5), propranolol (Emax = 39,49 ± 0,89%, *n*=5) and β-GA (Emax = 42,69 ± 1,66%, *n*=5) did not altere adrenergic relaxation. Pz and BMY produced a non-competitive antagonism on Phe-induced relaxation, but a competitive one on adrenergic contraction. In Schild's analysis, pA_2 values for Pz were 9,8 ± 0,67 (*n*=5) and 10,3 ± 0,72 (*n*=5) for adrenergic contraction and relaxation, respectively. pA_2 values for BMY were 9,4 ± 0,69 (*n*=5) and 10,3 ± 0,51 (*n*=5) for adrenergic contraction and relaxation, respectively. **Discussion:** Phe-induced relaxation in rat carotid is endothelium-dependent and mediated by α_{1D}-adrenoceptors that seems to be endothelial. pA_2 values for Pz and BMY showed a minor potency for these antagonists on α_{1D}-adrenoceptors that mediates Phe-induced relaxation than the mediators of Phe-induced contraction, suggesting greater affinity of these antagonists for the endothelial α_{1D}-adrenoceptors. Differences of antagonist's affinity for its receptor is related to structural differences on receptor, suggesting endothelial α_{1D}-adrenoceptors are structurally different from muscular ones. **Reference:** Filippi S, *et al*. Alpha (1D)-adrenoceptors cause endothelium-dependent vasodilatation in the rat mesenteric vascular bed. J Pharmacol Exp Ther, 296, 869-875, 2001. **Supported by:** CAPES

06.051

Synaptic transmission of chemoreflex afferents in second order NTS neurons. Accorsi-Mendonça D, Bonagamba LGH, Castania JA, Machado BH, Leão RX FMRP-USP - Fisiologia

Introduction: NTS receives glutamatergic inputs from afferents of aortic depressor nerve (ADN) with high temporal fidelity. However, this synaptic property of ADN may not be representative of overall second-order neurons in the NTS. Our aim was to compare the TS evoked EPSCs on ADN-NTS neurons with EPSCs on neurons that receive afferents from carotid body (CB), which are involved in the peripheral chemoreflex. **Methods:** We labeled the ADN and CB synapses on NTS by applying DiI in the ADN or CB of adult Wistar rats (Protocol 02-12-2003). In some rats NTS neurons were also labeled with green retrobeads microinjected into RVLM or CVLM. Tractus solitarius (TS) evoked EPSCs were recorded using patch clamp in single- or double labeled NTS neurons. **Results:** The latencies of TS-eEPSCs were similar, but the mean jitter (standard deviation of latency) was higher in the CB-NTS (0.5 ± 0.04ms) and CB-NTS-RVLM neurons (0.6 ± 0.1ms) than in ADN-NTS (0.2 ± 0.05ms) or ADN-NTS-CVLM neurons (0.3 ± 0.05ms). All groups presented similar frequency-dependent depression of the TS-eEPSCs but the failure percentage was higher in CB-NTS (25 ± 5%) and CB-NTS-RVLM neurons (19 ± 6%) than in ADN-NTS (3 ± 2%) and ADN-NTS-CVLM (5 ± 2%) neurons. **Conclusion:** These data show that the second-order synapses from the CB are not as precise and efficient as second order ADN neurons in the NTS. **Financial Support:** FAPESP and CNPQ.

06.052

Hemodynamic effects of inducible nitric oxide synthase (iNOS) inhibitor combined with sildenafil during acute pulmonary embolism. Neto-Neves EM, Dias-Junior CAC, Montenegro MF, Tanus-Santos JE FMRP-USP - Farmacologia

Introduction: Activating the nitric oxide (NO)-cGMP pathway improves hemodynamics during acute pulmonary thromboembolism (APE). However, NO generated by iNOS may have a role in APE-induced oxidative stress, so that iNOS inhibition with S-methylisothiourea may attenuate APE-induced oxidative stress and pulmonary hypertension. No previous study has examined whether infusion of an iNOS inhibitor enhances the beneficial hemodynamic or antioxidant effects of sildenafil during APE. **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: 020/2007). Hemodynamic evaluations were performed in non-embolized dogs treated with saline (Sham group; n=4), or S-methylisothiourea (SMT group; n=4), or sildenafil (Sild group, n=4), or S-methylisothiourea followed by sildenafil (SMT+Sild group, n=4), and in dogs that received the same drugs and were embolized with silicon microspheres (Emb group, n=8; Emb+SMT group, n=8; Emb+Sild group, n=8 and Emb+SMT+Sild group, n=8). S-methylisothiourea (2 mg/kg) was infused over 5 min followed by a maintenance infusion of 1 mg/kg/h for the duration of the study. Sildenafil (0.3 mg/kg over 30 min) was infused intravenously. The plasma concentrations of nitrite/nitrate (NOx) and thiobarbituric acid reactive substances (TBARS) were determined by Griess reaction and by a fluorimetric assay, respectively. **Results**: APE increased mean pulmonary arterial pressure (MPAP) from 10±1 to 32±4 mmHg and pulmonary vascular resistance index (PVRI) from 137±26 to 853±65 mmHg respectively, thirty minutes after APT in the Emb group. S-methylisothiourea neither attenuated APE-induced pulmonary hypertension, nor enhanced the beneficial hemodynamic effects of sildenafil during APE (32% and 48% reduction in MPAP and in PVRI, respectively; both $P<0.05$). S-methylisothiourea alone blunted APE-induced increases NOx (change from 43 to -2 µmol/L, $P<0.05$). However, the combined infusion of S-methylisothiourea and sildenafil showed significant increases in NOx (change from 43 to 14, $P<0.05$). The increased of oxidative stress caused by APE was attenuated by S-methylisothiourea (change from 19.8 to 1.3), sildenafil (change from 19.8 to -2.9) and by the association of the drugs (19.8 to -5.3), both $P< 0.05$. **Discussion**: The iNOS inhibitor neither attenuated APE-induced pulmonary hypertension, nor improved the beneficial hemodynamic effects of sildenafil. These findings are consistent with idea that vasodilator drugs with antioxidant properties such as sildenafil produce beneficial effects during APE and that are not further improved by iNOS inhibitors. **Supported by:** CNPq, CAPES and FAPESP.

06.053

Temporal study of angiotensin II infusion on kinin B1-receptor expression in blood vessels. Giaquinto LR, Ceravolo G, Tostes RCA, Fortes ZB, Carvalho MHC USP - Farmacologia

Introduction The inducible kinin B1-receptor (B1R) is involved in chronic inflammatory process (McLean, et al, Cardiovasc.Res. n.48, p.194-210, 2000), but the role of this receptor in hypertension, wich is consider an inflammatory process, is still inconclusive. We had previously demonstrated that Ang II infusion in Wistar rats, during 14 days, can induce B1R expression in conductance vessel but it was not observed in mesenteric microvessels (Ceravolo et al, Hypertension 50; 756-761; 2007).The aim of this study was to evaluate the effect of prolonged Ang II infusion, during 28 days, upon B1R gene expression in rats isolated mesenteric arterioles as well as in aorta. All the procedures used in this study were approved and performed in accordance with the guidelines of the ethics committee of the Institute of Biomedical Sciences of the University of São Paulo (protocol number 98/04). **Methods** Male Wistar rats received Ang II (400ng/kg/min- Ang II rats, n=12) or saline (S rats, n=12) infusion during 28 days, via subcutaneously implanted mini-osmotic pump. The blood pressure (BP) was measured in conscious rats by indirect tail-cuff method at days 7, 14, 21 and 27 after mini-osmotic pump implants. At 28th day aorta and mesenteric arterioles were excised for determination of B1R expression by RT-PCR. It was also studied vascular reactivity to des-arg9-bradykinin (DABK), a B1R agonist, in isolated aortic rings with and without endothelium. For that cumulative concentration-curve to DABK (0.1nM a 1uM) was performed in aortic rings pre-contracted with phenylephrine (0.1uM; 10 min) a concentration which elicits an 80% of the maximum response (MR).The CCE was analyzed at the maximum response. **Results:** The Ang II rats blood pressure levels were higher than in S rats at days 7 (S: 123.1 ± 1.623 vs Ang II: 161.1 ± 8.439 mmHg), 14 (S: 128.5 ± 3.465 vs Ang II: 170.1 ± 9.379 mmHg), 21 (S: 122.4 ± 2.294 vs Ang II: 172.9 ± 8.904 mmHg), and 27 (S: 129.4 ± 2.564 vs Ang II: 199.9 ± 6.237 mmHg). The B1R gene expression in mesenteric arterioles was detected only after 28 but not 14 days of Ang II infusion, when compared with S rats. However in isolated aorta the B1R expression was early detected after 14 days and also 28 days of Ang II infusion. Either after 14 and 28 days of Ang II infusion, DABK a B1R agonist, elicited a concentration-dependent relaxation in Ang II aortic rings with endothelium only (MR 19.39 ± 5.091) and not in aortic rings from S rats. **Discussion** These results provide evidences that Ang II may have a temporal effect modulating B1R gene expression in macro and microvessels. These data confirm the existence of a new important site of interaction between the kinin and angiotensin systems in blood vessels of hypertensive rats. Supported by: FAPESP and CNPq

06.054

Participação do endotélio na manutenção da resposta vasoconstritora à noradrenalina em aortas de ratas diabéticas: papel do NO e da endotelina. Sartoretto SM[1], Akamine EH[2], Tostes RCA[1], Carvalho MHC[1], Fortes ZB[1] [1]USP - Farmacologia, [2]USP - Fisiologia e Biofísica

Introdução: A hiperglicemia crônica afeta a composição e estrutura de tecidos vasculares. Essas modificações levam à depressão cardiovascular, caracterizada pela diminuição da pressão arterial, da frequência cardíaca e da resposta pressórica a agentes vasoativos. As alterações vasculares, como a resposta vasoconstritora e os mecanismos envolvidos nesta alteração estão bem caracterizados em machos diabéticos, mas poucos estudos têm sido realizados em fêmeas. Nosso objetivo foi avaliar a mobilização de cálcio (Ca^{2+}) induzida pela noradrenalina (NA) em aortas de ratas diabéticas (D) e a capacidade contrátil do músculo liso, assim como a participação do óxido nítrico (NO), da endotelina (ET), da angiotensina II (Ang) e dos produtos da ciclo-oxigenase (COX) na resposta de contração à NA. **Métodos**: O diabetes foi induzido em ratas Wistar por injeção intravenosa de aloxana (40mg/kg). Após 30 dias da indução e caracterizado o diabetes, foram avaliadas a mobilização de Ca^{2+}, analisando a contração induzida pela NA (0,1µM) após a retirada e subseqüente reposição do Ca^{2+} extracelular (2,5mM), e a resposta contrátil, realizando curva concentração efeito (CCE) ao cloreto de potássio (KCl) (5mM-108mM) e à NA (0,1nM-30µM), em anéis de aorta com (E+) e sem (E-) endotélio de ratas controles (C) e D. Para avaliar a participação do NO, da ET, da Ang e dos produtos da COX, na resposta à NA, os anéis de aorta E+ foram incubados com L-NAME(100µM), inibidor da síntese de NO, tezosentan(0,01µM), bloqueador não seletivo dos receptores de ET, losartan (10µM), bloqueador dos receptores AT1 de Ang e indometacina(10µM), inibidor inespecífico da COX respectivamente, 30 minutos antes do início da CCE à NA. **Resultados:** Na ausência de Ca^{2+} extracelular, a contração à NA (0,1µM) em aortas E+ e E- de ratas D foi reduzida em 50% e a adição do Ca^{2+} ao meio manteve a resposta reduzida quando comparada às respectivas aortas de ratas C. A resposta contrátil ao KCl foi reduzida em aortas E+ (29%) e E- (43%) de ratas D. Em aortas E+, a resposta máxima (Rmáx) à NA de ratas D foi semelhante à de C. A retirada do endotélio promoveu aumento da resposta contrátil à NA em aortas de C (56%), porém esse aumento foi de menor magnitude em D (29 %). A inibição da síntese de NO com L-NAME aumentou a Rmáx à NA apenas em aortas de ratas C (45%). O bloqueio dos receptores de ET com tezosentan reduziu a Rmáx à NA somente em aortas de ratas D (35%). Tanto o losartan quanto a indometacina reduziram a Rmáx à NA em aortas de ratas C (24% e 48%, respectivamente) e D (30% e 45%, respectivamente). **Conclusões:** Alterações no aparato contrátil, como por exemplo, redução da liberação dos estoques intracelulares e do influxo de Ca^{2+} podem ser os responsáveis pela redução da resposta contrátil do músculo liso de aorta de ratas D. O endotélio de ratas D é capaz de manter a resposta vasoconstritora à NA semelhante à de C. A redução da modulação do NO sobre a resposta à NA ou o aumento da liberação de endotelina pelo endotélio podem ser os responsáveis pela manutenção da resposta contrátil à NA em aortas E+ de ratas D. A Ang e os produtos da COX participam de forma semelhante na resposta à NA em aortas de C e D, não podendo ser responsabilizados pela diferença observada. **Apoio Financeiro:** FAPESP/CNPq. 007/04/CEEA

06.055

Hemodynamic effects of acute infusion of recombinant matrix metalloproteinase-2 in anesthetized sheep. Santos Sousa O[1], Neto-Neves EM[1], Dias-Junior CAC[1], Gerlach RF[2], Tanus-Santos JE[1] [1]FMRP-USP - Farmacologia, [2]FORP-USP - Morfologia

Introduction: Mouting evidence indicates that upregulation of matrix metalloproteinases (MMPs) play a role in cardiovascular diseases. Indeed, increased MMP-2 levels have been reported in hypertension, preeclampsia, heart failure and other cardiovascular diseases. While experimental studies have shown that MMP-2 may affect the vascular tonus by modulating the concentrations of vasoactive peptides including endothelin-1, calcitonin gene related peptide, and adrenomedullin, no previous study has examine the hemodynamic effects of increased circulating MMP-2 concentrations. Here, we examined these effects in anesthetized sheep. **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 020/2007). Anesthetized Santa Ines sheep (N=5/group) received intravenous infusions of human recombinant MMP-2 (diluted in saline) at doses of 0.15, 0.45, 1.5, and 4.5 micrograms/kg every 15 min or vehicle (saline; Control group). Hemodynamic evaluations were carried every 15 min for two hours. The mean pulmonary arterial pressure (MPAP), mean arterial pressure (MAP), heart rate, and cardiac output (measured by thermodilution) 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:** Baseline hemodynamic variables were similar in both groups. We found no significant hemodynamic changes in the Control group of sheep throughout the study period. However, significant reductions in MAP (from 94±4 mmHg to 73±15 mmHg;$P<0.05$) and in SVRI (from 3099±328 $dyn.s.cm^{-5}.m^{-2}$ to 2228 ± 422 $dyn.s.cm^{-5}.m^{-2}$ $P<0.05$) were found in sheep receiving MMP-2 infusions by the end of the highest dose. HR and CI showed no significant changes throughout study period. **Discussion:** These findings suggest that MMP-2 may produce acute systemic vasodilation. While we have not examined the mechanisms that may explain these effects, it is possible that MMPs may modify the vascular concentrations of vasoactive peptides and thus regulate vascular function. **Acknowledgments**: FAPESP, CAPES and CNPq.

06.056

Effects of different diuretics in contractile mechanisms in rat cavernosal smooth muscle. Claudino MA[1], Takeshi FI[1], Antunes E[1], Lopes AG[2], De Nucci G[1] [1]UNICAMP - Farmacologia, [2]IBCCF-UFRJ - Fisiologia Renal

Introduction: Erectile dysfunction is a highly prevalent health problem that impacts on the quality of life of the patients. Several diuretics have been associated with sexual dysfunction in men, including decreased libido, difficult ejaculation and impotence, but these adverse mechanisms remain poorly understood. The aim of this study was determine the involvement of different diuretics in the contractile mechanisms of rat corpus cavernosum smooth muscle. **Methods:** Male Wistar rats (300 g) were anaesthetized and corpus cavernosum removed. Concentration-responses curves to phenylephrine (PE; 10 nM - 100 μM) and potassium chloride (KCl; 1 mM - 100 mM) in corpus cavernosum were obtained in the absence (control) or in presence of furosemide (loop diuretic), hydrochlorothiazide (thiazide diuretic), amiloride (potassium-sparing diuretic) and methazolamide (carbonic anhydrase inhibitor; 10 μM each). The values of potency (pEC_{50}) and maximal responses (E_{max}) were calculated. Neurogenic contractile responses induced by electrical-field stimulation (EFS; 1-32 Hz) were also studied in absence or in presence of all diuretics. **Results:** Cumulative addition of the PE produced concentration-dependent contractile responses in the rat cavernosal tissues. Furosemide and hydrochlorothiazide caused no significant change in the pEC_{50} for PE (5.54±0.06 and 5.58±0.07, respectively) compared with control groups (5.51±0.02), but significantly increased the E_{max} (2.97±0.22 mN and 3.65±0.39 mN, respectively) when compared to control groups (2.41 ± 0.10 mN). Amiloride induced a significantly reduced in the pEC_{50} for PE (5.37±0.08); however, it did not change the E_{max} values (2.48±0.29 mN) compared to control group. In contrast, neither the pEC_{50} nor the E_{max} for PE were affected by methazolamide. Cumulative addition of the KCl produced concentration-dependent contractile responses in the rat cavernosal tissues. Furosemide and hydrochlorothiazide did not altered the pEC_{50} for KCL (1.37±0.03 and 1.31±0.08, respectively) compared with control groups (1.35 ± 0.10); however, they significantly increased the E_{max} values (1.24±0.16 mN and 1.41±0.06 mN, respectively) when compared to control groups (0.65±0.05 mN). Amiloride caused a greater reduction in the pEC_{50} for KCl (1.06±0.04) however, not induced changes in the E_{max} from KCl (0.84±0.16 mN). Methazolamide caused no significant alterations in the pEC_{50} and E_{max} for KCl. EFS induced frequency-dependent contractile response in the rat cavernosal tissue. Furosemide promoted a significant increase in the contraction magnitude in all frequencies. Hydrochlorothiazide significantly increased the EFS-induced contractions only at the highest frequencies (8, 16 and 32 Hz). Amiloride significantly reduced the EFS-mediated contractions at the highest frequencies (16 and 32 Hz). Methazolamide did not change the EFS-induced contractile responses. **Conclusion:** The mechanisms by which different diuretics distinctively alter the *in vitro* contractile mechanisms of rat corpus cavernosum possibly involve changes in the $[Ca^{2+}]_i$ and $[Cl^-]_i$ content. **Financial support:** FAPESP and CNPQ.

06.057

Long-term treatment with BAY 41-2272 ameliorates the erectile responses in nitric oxide-deficient rats. Silva FH, Claudino MA, Monica FZT, Rojas-Moscoso JA, De Nucci G, Antunes E UNICAMP - Farmacologia

Introduction: Stimulation of nitrergic neurons and endothelial cells in the erectile tissue result in release of nitric oxide (NO) that activates the soluble guanylate cyclase (sGC), facilitating the conversion of GTP to cGMP. This second messenger diminishes the intracellular Ca^{2+} levels causing corpus cavernosum (CC) relaxation and penile erection. The deficiency of the NO-cGMP pathway is the primary cause of male impotence. The compound BAY 41-2272 is a potent NO-independent sGC stimulator (Stasch et al., 2001). BAY 41-2272 causes CC relaxations *in vitro* and *in vivo*, but no studies attempted to evaluate the potential beneficial effects of BAY 41-2272 in ameliorating the erectile dysfunction in conditions of chronic NO blockade. Thus, the aim of this work was evaluated the effect of long-term oral treatment with BAY 41-2272 in CC of NO-deficient rats. **Methods:** The experimental protocols were approved the Animal Ethical Committee of UNICAMP. Male Wistar rats were divided into four groups: (1) Control, (2) L-NAME (20 mg/rat/day), (3) BAY 41-2272 (20 mg/kg; given by gavage) and (4) L-NAME+BAY 41-2272 (same doses). Rats were treated with L-NAME concomitantly with BAY 41-2272 for 4 weeks. After the end of experimental program, concentration-response curves to acethylcholine (ACh), nitroprusside sodium (SNP) and phenylephrine (PE) were obtained in rat CC. The contractile and relaxant responses induced by electrical-field stimulator (EFS) were also obtained in all groups. **Results:** Chronic L-NAME administration promoted a significant increase in arterial pressure (146± 6mmHg) that was nearly normalized by concomitant treatment with BAY 41-2272 (93 ± 9 mmHg), demonstrating the efficacy of BAY 41-2272 treatment (Zanfolin et al., 2006). Relaxing responses induced by EFS (nitrergic relaxation) were significantly reduced by L-NAME treatment (32Hz: 16±3%; $P<0.05$) compared with control group (25±2%). The reduction of EFS-induced CC relaxations by L-NAME was significantly reversed concomitant treatment with BAY 41-2272 group (29±1%). On the other hand, BAY 41-2272 treatment (associated with L-NAME) did not significantly affect the ACh-induced relaxations (33±4%) compared with L-NAME group (27± 2%) . The relaxing responses to SNP remained unaltered in all investigated groups. The contractile responses to either phenylephrine or EFS also remained unaltered in all investigated groups. **Conclusion:** Our findings show that the impaired nitrergic relaxations in rats are ameliorated by long-term oral BAY 41-2272 treatment suggesting a potential value for direct activators of sGC in treating erectile dysfunction. **Financial support:** FAPESP and CAPES.

06.058

Testosterone-induced ROS mediates vascular smooth muscle cells migration and apoptosis: unique features from SHR-VSMC. Chignalia AZ[1], Camargo LL[1], Lopes LR[1], Curi R[2], Carvalho MHC[1], Fortes ZB[1], Touyz RM[3], Tostes RCA[1] [1]USP - Farmacologia, [2]USP - Fisiologia, [3]University of Ottawa - Kidney Research

Introduction: Testosterone (Test) has been associated with augmented blood pressure. Mechanisms whereby Test induces vascular effects remain unclear, but reactive oxygen species (ROS) may be important. In the present study we investigated whether Test stimulates ROS production in vascular smooth muscle cells (VSMC), the mechanisms involved and the cellular responses to this effect, exploring the differences in cells isolated from normotensive and hypertensive animals. **Methods:** VSMC from Wistar (W), Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats were stimulated with Test 10^{-7}M from 5 to 120 minutes (m) and up to 24 hours (h). ROS generation was assessed by dihydroethidium (DHE) fluorescence, measurement of DHE-derived oxidation products by HPLC and lucigenin enhanced chemiluminescence. Expression of p47phox (NADPH oxidase subunit), translocation of p47phox from the cytosol to the cellular membrane, translocation of p65 (nuclear factor kappa B, NFkB, subunit) from the cytosolic to the nuclear fraction and activation of c-Src were assessed by immunoblotting. p65 gene expression was investigated by real-time PCR. Cell migration was evaluated by the wound healing assay. DNA fragmentation was evaluated by flow cytometry analysis. **Results:** Test increased superoxide anion ($O_2^{\cdot-}$) generation at 30m in SHR, at 1h in WKY and at 2h in W-VSMC (0.5 fold, at least 4 experiments). This production was augmented 2 fold in SHR VSMC until 2h. Flutamide (Flu, androgen receptor antagonist) and actinomycin D (ActD, gene transcription inhibitor) were unable to block rapid $O_2^{\cdot-}$ formation, but blocked long-term ROS generation (n=6, $p<0.05$).Activation of c-Src was observed at 30m (SHR) and 2h (WKY and SHR). Test-induced ROS production was remarkably inhibited by PP2 (c-Src inhibitor) only in SHR (0.5 fold, n=6) and it was significantly reduced by apocynin (Apo) (0.5 fold-WKY/ 1.0 fold-SHR, n=5). Test increased p47phox translocation to membrane fraction (1.5 fold, n=5) and its expression after 2h of stimulation (1 fold, n=5), which was inhibited by ActD (n=5). Test decreased p65 gene expression and its translocation only in SHR. In WKY, Test increased p65 translocation, having no effect on its expression. Test induced VSMC migration after 4h of stimulation ($p<0.05$, n=3). This effect was blocked by Flu and Apo ($p<0.05$, n=4). PP2 inhibited SHR-VSMC migration ($p<0.05$, n=3). Test induced DNA fragmentation only in WKY ($p<0.05$, n=4), which was inhibited by Flu ($p<0.05$, n=4) and Apo ($p<0.01$, n=4). **Discussion:** Our findings demonstrate that Test induces ROS formation in VSMC via NADPH oxidase. This mechanism is augmented in SHR cells with the unique characteristic of c-Src mediating fast ROS generation via membrane AR. Long-term ROS generation in WKY and SHR cells is a genomic effect, mediated by classical AR. ROS production leads to VSMC migration via c-Src exclusively in SHR. In addition, Test-induced ROS mediates apoptosis only in WKY-VSMC by unknown mechanisms. We hypothesize that NFkB signaling might play a key role in this phenomena. In conclusion, Test exerts some vascular effects via ROS production. These effects, may contribute to vascular oxidative stress-related pathologies. This may be particularly important in sex-associated hypertension. **Funding**: FAPESP/ License from Ethics Committee on Animal Experimentation: 036.

06.059

Vasodilation by no donor is mainly due to inhibition of Ca^{+2} influx. Pereira AC, Biazzotto JC, Da Silva RS, Bendhack LM FCFRP-USP

Introduction: NO produced in the endothelial cells or released by NO donor migrates to vascular smooth muscle cells (SMC) where it causes vascular relaxation due to decrease cytosolic Ca^{+2} concentration ($[Ca^{+2}]_c$). Several mechanisms has been proposed for $[Ca^{+2}]_c$ decrease such as inhibition Ca^{2+} influx due to membrane hyperpolarization via activation of K^+ channels and Ca^{2+} channels blockade, stimulation of Ca^{+2} uptake into the store by sarcoplasmic reticulum Ca^{+2}-ATPase (SERCA), increase in Ca^{+2} extrusion from cells through the sarcolemmal Ca^{+2} pump and Ca^{2+}efflux through the forward mode of Na^+-Ca^{+2}. This study aimed to investigate how the NO donor, *cis*-$[Ru(bpy)_2(py)(NO_2)](PF_6)$ (PY), induces vasodilation in the rat aorta. **Methods:** Vascular reactivity to PY was studied in endothelial cells denuded rat aortic rings. **1)** Functional studies were made in aortic rings pre-contracted with phenylephrine (Phe 100nM) or 60mM KCl. Cumulative concentration-effect curves for this NO donor were made in the presence or absence of a selective soluble guanylyl cyclase (sGC) inhibitor (ODQ 1μM) or SERCA inhibitor (Thapsigargin (TG) 1μM). **2)** Cumulative concentration-response curves for $CaCl_2$ stimulated with 100nM Phe or 60mM KCl were made in the presence or absence of NO donor and with or without TG 1μM. **3)** Effect of caffeine (20mM) in aortic rings pre-incubated or not with PY (3μM). **4) C**apacitative Ca^{+2} influx was studied in aortas treated with TG (1μM for 30 min) in the presence or absence of NO donor (3μM). We have analyzed the maximum effect (ME) and the potency (pD_2) of PY. All these procedures were approved by the Ethics Committee of the University of Sao Paulo (CEUA 07.1.608.53.8). **Results:** PY induced concentration-dependent relaxation in rat aorta pre-contracted with Phe (ME: 105±1.08%; pD_2: 6.54±0.1; n=5) or KCl (ME: 67.5±4.6%; pD_2: 5.79±0.11; n=5). However, ME and pD_2 values were lower KCl-contracted aorta. ODQ reduced the ME (75±4.5%) and pD_2 (5.2±0.09) in aortas pre-contracted with Phe. The effect of ODQ plus KCl reduced ME (60.3±5.7%), but did not change pD_2. Tapsigargin reduced ME (82.1±4.6; n=5) and the potency of PY (5.82±0.21; n=5). The cumulative concentration-effect curves for $CaCl_2$ stimulated with 100nM Phe were similar to those stimulated with 60mM KCl (Phe ME: 1.4±0.16g; pD_2: 0.47±0.19, n=9 and KCl ME: 1.6±0.21g; pD_2:0.51±0.12, n=7). Incubation with the NO donor reduced ME of $CaCl_2$ stimulated with 100nM Phe or 60mM KCl (Phe: 0.1±0.06g and KCl: 0.7±0.11g). pD_2 values of $CaCl_2$ curves with TG was lower in the presence of PY than in absence (TG+PY: 0.09±0.07; TG: -0.07±0.01, n=7). Contraction induced by caffeine in preparation incubated with PY (0.24±0.01g, n=7) was lower than in absence of NO donor (0.66±0.05g, n=6). The capacitative Ca^{+2} influx was inhibited (from 2.1±0.24g to 1.1±0.07g) in presence of the NO donor studied. **Conclusion:** The relaxation induced by *cis*-$[Ru(bpy)_2(py)(NO_2)](PF_6)$ involves the activation of sGC and K^+ channels, where these two pathways seems to be independent. Ca^{2+} storage, via SERCA in a pool sensitive to TG, contributes with the relaxation induced by PY. This NO donor inhibits Ca^{2+} influx and consequent contractile response. Interestingly, contraction induced by caffeine and by capacitative Ca^{+2} influx are inhibited by PY. **Supported by:** FAPESP and CNPq.

06.060

Vasorelaxant action of the total alkaloids fraction from root bark of *Solanum paludosum* Moric. on rat aorta involves the nitric oxide pathway. Monteiro FS[1], Santos RF[1], Correia ACC[1], Cavalcante FA[2], Basílio IJLD[1], Agra MF[1], Bhattacharyya J[1], Silva BA[1] [1]DCF-LTF-UFPB, [2]UFAL-ICBS

Introduction: *Solanum paludosum* Moric. (Solanaceae) is herbaceous species, known popularly as "jurubeba-roxa" in the Northeast of Brazil. (AGRA, M.F., Royal Botanic Gardens, p. 341, 1999). Chemical studies of the root bark of this species showed the presence of steroid alkaloids and its glycosides (BASÍLIO, Dissertação (mestrado), 2008), and pharmacological studies of the ethanolic extract and its aqueous phase obtained from root bark of this species showed spasmolytic activity on rat jejunum and hypotension on rats (ATAÍDE, J.R. Dissertação (mestrado), 1982). Recently, total alkaloids fraction from root bark *of S. paludosum* Moric. (FAT-SP) showed spasmolytic effect on rat uterus, guinea-pig trachea and ileum, and rat aorta (MONTEIRO, F. S. Dissertação (mestrado), 2009). Therefore, we decided to investigate the vasorelaxant action of FAT-SP on rat aorta. **Methods:** Rings of rat aorta (n = 5) were suspended in organ bath containing Krebs solution (pH = 7.4) at 37 °C, bubbled with 95% O_2 and 5% CO_2 mixture and resting tension of 1 g. Isometric contractions were registered through force transducer coupled to amplifier, which was connected to a microcomputer. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0204/08). **Results**: FAT-SP relaxed the rat aorta pre-contracted with 3 x 10^{-7} M phenylephrine both in the presence (EC_{50} = 75.4 ± 6.2 µg/mL) and absence (EC_{50} = 242.8 ± 11.7 µg/mL) of functional endothelium, being most potent in the presence of endothelium. In search of a possible vasorelaxant action mechanism of the FAT-SP on rat aorta with endothelium was observed that in the presence of atropine (EC_{50} = 93.3 ± 10.0 µg/mL), non-selective muscarinic receptor antagonist, or indomethacin (EC_{50} = 90.7 ± 13.2 µg/mL), non-selective cyclooxygenase inhibitor, the relaxant effect of the FAT-SP (EC_{50} = 75.4 ± 6.2 µg/mL) not was modified. However in the presence of 10^{-5} M L-NAME (EC_{50} = 147.0 ± 22.1 µg/mL), nitric oxide (NO) synthase inhibitor, 3 x 10^{-5} M hydroxocobalamin (EC_{50} = 94.9 ± 20.4 µg/mL), scavenger of NO, or 10^{-5} M ODQ (EC_{50} = 228.2 ± 30.9 µg/mL), selective blocker of soluble guanylyl cyclase (GC_S) the relaxant effect of the FAT-SP was attenuated. **Discussion**: As the vasorelaxant effect of FAT-SP was most potent in the presence of functional endothelium, it's suggestive that the endothelium derivative relaxant factors are involved on this effect. The fact of the relaxation curves of the FAT-SP to have shifted to the right in presence of L-NAME is suggestive of involvement of the endothelial NO synthase. In addition, the attenuation of the relaxant effect of the FAT-SP in the presence of hydroxocobalamin and ODQ is suggestive of involvement of NO/CG pathway, respectively, in the vasorelaxant effect of the FAT-SP. **Financial support**: CNPq, CAPES, LTF/UFPB.

06.061

Neonatal hyperleptinaemia upregultes renal Na^+/K^+ATPase. Marques EB[1], Luzardo, R[2], Silva PA[2], Lara Morcillo LS[3], Costa ACR[2], Vieyra A[2], Scaramello C[1] [1]UFF - Fisiologia e Farmacologia, [2]UFRJ - Biofísica, [3]UFRJ - Farmacologia Básica e Clínica

Introduction: Altered plasma leptin levels are related to renal and cardiovascular diseases like chronic kidney disease and congestive heart failure - CHF (Kastarinenn et al. Scand. J. Clin. Lab. Invest., v. 69, p.401, 2009; Fernandes et al. Braz. J. Med. Biol. Res., v.40, p.1632, 2007). Previous work showed that neonatal leptin treatment programmes leptin hypothalamic resistance and intermediary metabolic parameters in adult rats, such serum insulin, glucose and triacylglycerol levels (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 programmes renal function and may be involved in development of chronic diseases. **Methods:** When the offspring were born,pups were divided into two groups. The Lep group, injected daily with leptin (8 µg/100g body weight subcutaneously) for the first 10 days of lactation and the Control group, injected daily with saline. After weaning (day 21), body weight and food intake were monitored until the rat was sacrified and the kidney removed for further analysis. The use of animals took place according to the guidelines of the Animal Care and Use Committee of Federal University of Rio de Janeiro (DFBCICB007). Kidneys of young rats (1 moth-old) were homogenized, submitted to several centrifugations and sodium and Na^+/K^+ATPase activity assays were performed (Caruso-Neves et al. Biochim. Biophys. Acta., v.1468, p.107, 2000;Taussky & Shorr, J. Biol. Chem., v.202, p.675, 1953, respectively). Na^+/K^+ATPase expression was also investigated by SDS-PAGE followed by immunodetection (Laemmli. Nature, v.227, p.680, 1970). **Results:** Until 30 days-old, no difference was observed in body weight neither food intake between groups. Although no statistic difference was observed in Na^+ATPase activity between Lep and Control group (57,4±6,8 and 64,3±9,0 nmoPi/mg/min, respectively, n=3), Na^+/K^+ATPase activity was significant higher in Lep group when compared to Control (471,0±17,2 and 399,2±10,5 nmoPi/mg/min, respectively, n=4). Western blot of a1 subunit isoform of Na^+/K^+ATPase was higher in leptin group (172,7±24,2% of Control). **Discussion:** In this early stage of development (30 day-old) just Na^+/K^+ATPase activity was raised. The overactivity/overexpression of this mass sodium transporter may be, in part, related to an elevation of extracellular volume that impairs cardiovascular function such as observed in cases of CHF and some cases of hypertension. Further biochemical and functional studies are being made to understand this profile and also the disproportional rise in a1 Na^+/K^+ATPase expression, the housekeeping renal isoform, compared to the minor rise of its activity in Lep group. The study will be also performed with 5 moth-old rats to analyze the effect of neonatal leptin treatment in older rats and if the alteration could be different that observed in younger animals. **Financial Support:** FAPERJ, CNPq, CAPES, PROPPI/UFF.

06.062

Neonatal hyperleptinaemia upregultes renal na$^+$/K$^+$ATPase. Marques EB[1], Luzardo, R[2], Silva PA[2], Lara Morcillo LS[3], Costa ACR[2], Vieyra A[2], Scaramello C[1] [1]UFF - Fisiologia e Farmacologia, [2]UFRJ - Biofísica, [3]UFRJ - Farmacologia Básica e Clínica

Introduction: Ingestion of a salt meal induces secretion of guanylin (GN) and uroguanylin (UGN) into the intestinal lumen, where they inhibit Na^+ absorption and induces Cl^-, HCO_3^-, and water secretion (Kita, Am. J. Physiol. 277:960, 1999). Simultaneously, these hormones stimulate renal electrolyte excretion by inducing natriuresis, kaliuresis, and diuresis (Fonteles, Braz. J. Med. Biol. Res. 29:267,1998). The highly integrated mechanism allows the organism to maintain sodium balance by eliminating the excess of NaCl in the urine. However, their physiological regulation within the kidney has not been studied (Sindice, J. Am. Soc. Nephrology 17:607,2006). The aim of this study was to investigate rat renal function under high sodium chloride ingestion. **Methods:** Renal effects were examined using wistar rats maintained for ten days in metabolic cages. Control group (C) received only water, while the treated group received 2% of sodium chloride solution (S). The renal function was evaluated using the isolated perfused rat kidney method. The kidneys were perfused using Krebs-Henseleit solution containing 6g% of a previously dialyzed bovine serum albumin (Fonteles, Am. J. Physiol. 244:235,1983). The perfusion pressure (PP) was kept fixed during the entire period of renal perfusion. All data were analyzed by ANOVA and Student *t*-test with level of significance set at *p<0,05. The experimental protocols were approved by the Ethics Committee on Animal Research of the Department of Physiology and Pharmacology, Federal University of Ceará with the number 68/08. **Results:** The treated kidneys showed a reduction compared with control group in renal vascular resistance (RVR) - (30min - RVR_C=4.8±0.2; RVR_S=2.7±0.1*; 60min - RVR_C=4.9±0.2; RVR_S=2.9±0.1*; 90min - RVR_C=4.5±0.2; RVR_S=2.9±0.1*; 120min - RVR_C=4.7±0.2; RVR_S=2.9±0.1* mmHg/ mL/g/min); in urinary flow (UF) - (30min - FU_C=0.16±0.02; FU_S=0.11±0.01*; mL/g/min); and in the glomerular filtration rate (GFR) - (30min - RFG_C=0.70±0.07; RFG_S=0.35±0.03*; 60min - RFG_C=0.71 ±0.05; RFG_S=0.47±0.05*; 90min - RFG_C=0.63±0.05; RFG_S=0.38±0.06*; 120min - RFG_C=0.69±0.08; RFG_S=0.39±0.09* mL/g/min). For the electrolytes transport, we observed that the salt-treated group showed a reduction in the percentage of sodium tubular transport (%TNa^+) - (30min - %TNa^+_C=81.9±1.2; %TNa^+_S= 71.8±3.4*; 120min - %TNa^+_C=79.8±0.6; %TNa^+_S=69.4±3.1*); potassium (%TK^+) - (30min - %TK^+_C=69.1±4.1; %TK^+_S=45.1±5.4*; 60min - %TK^+_C=69.1±5.7; %TK^+_S=45.36±5.9*; 90min - %TK^+_C=71.8±4.2; %TK^+_S=43.3±5.6*; 120min - %TK^+_C=69.9±6.9; %TK^+_S=40.9±5.5*) and chloride (%TCl^-) - (30min - %TCl^-_C= 79.9±0.9; %TCl^-_S=67.3±3.7*; 120min - %TCl^-_C=78.5±0.9; %TCl_S=64.2±3.2*). **Discussion:** These results suggest that a high salt ingestion on diet promote significant changes on rat renal function, possibly related to injury promoted by dehydration. It did also increase the excretion of sodium, potassium and chloride, indicating the probable systemic involvement of GN peptides in rat kidneys after its release from intestine. Financial Support: CNPq

06.063

Efeito vasorelaxante de *Sida santaremnensis* H. Monteiro em artéria mesentérica isolada de rato. Arcanjo DDR[1], Oliveira NNPM[1], Ferreira Filho ES[1], Alves AAR[1], Costa DA[2], Chaves MH[2], Borges ACR[3], Oliveira AP[1], Oliveira RCM[1] [1]UFPI - Plantas Medicinais, [2]UFPI - Química, [3]UFMA - Ciências Fisiológicas

Introdução: *Sida santaremnensis* H. Monteiro (Malvaceae) é conhecida popularmente por "guanxuma" no nordeste do Brasil. Estudos anteriores mostram atividades anti-inflamatória e gastroprotetora em ratos. O objetivo deste estudo foi investigar o efeito vasorelaxante do extrato etanólico de *S. santaremnensis* (SSan-EtOH) em artéria mesentérica isolada de ratos. **Métodos:** Os protocolos experimentais realizados foram aprovados pelo Comitê de Ética em Experiência Animal da UFPI, certidão 04/2008. Anéis de artéria mesentérica superior isolada de ratos Wistar machos (250 - 300g, n = 5-8), foram mantidos em cubas para órgãos isolados contendo solução de Tyrode a 37º C aerados com carbogênio, pH 7,4, sob tensão de 0,75 g/1h. Os anéis foram suspensos por linhas de algodão e fixados a um transdutor de força acoplado a um sistema de aquisição (AECAD 1604, AQCAD 1.4.6., AVS Projetos-SP) para registro de tensões isométricas. Após esse período, induziram-se contrações com fenilefrina (10 µM). A presença ou ausência do endotélio vascular foi verificada pela adição de ACh (10 µM) na fase tônica das contrações. Após um período de 30 min e durante o componente tônico de uma terceira contração ao agente contrátil, SSan-EtOH (0,1 µg/ml - 500 µg/ml) foi adicionado, cumulativamente, à cuba, em preparações diferentes. O relaxamento foi expresso como a percentagem reversa da contração induzida por fenilefrina ou KCl 80 mM. Os valores de CE_{50} e $E_{máx}$ foram obtidos por regressão não-linear a partir das curvas concentrações-resposta individuais, foram considerados significantes valores *p<0,05; **p<0,01, ***p<0,001. **Resultados e Discussão:** SSan-EtOH promoveu efeito vasorelaxante de maneira concentração-dependente em preparações com endotélio vascular (CE_{50} =18,84 ± 4,11 µg/ml), este efeito foi atenuado após a remoção do endotélio (CE_{50}= 92,50 ± 21,69 µg/ml**), sem alteração no efeito máximo ($E_{máx}$= 100,0 ± 0,0 %). Resposta semelhante foi observada em preparações com endotélio vascular na presença do inibidor da NO-sintase L-NAME 100 µM (CE_{50} = 124,98 ± 15,33 µg/ml***), ou do inibidor da ciclooxigenase indometacina 10 µM (CE_{50} = 247,02 ± 35,50 µg/ml) ou do inibidor não seletivo dos receptores muscarínicos atropina 1 nM (CE_{50}= 97,29 ± 17,84 µg/ml**). Em anéis pré-contraídos com KCl 80 mM, a adição cumulativa de SSan-EtOH promoveu efeito vasorelaxante concentração-dependente (CE_{50}= 44,46 ± 7,21 µg/ml). Em meio despolarizante nominalmente sem cálcio, SSan-EtOH (27, 81, 243 e 500 µg/ml) inibiu de maneira concentração-dependente as contrações cumulativas induzidas pela adição de $CaCl_2$ (10^{-6} - $3x10^{-2}$ M); ($E_{máx}$= 95,59 ± 5,84; 38,37 ± 2,81**; 40,03 ± 1,37** e 22,88 ± 9,55 %***, respectivamente). O efeito vasorelaxante promovido por SSan-EtOH em preparações com endotélio vascular envolve provavelmente a participação da enzima NO-sintase e dos metabólitos da ciclooxigenase bem como a participação dos receptores muscarínicos. O efeito independente do endotélio ocorre provavelmente pela inibição do influxo de cálcio através dos canais para cálcio sensíveis a voltagem. **Apoio:** UFPI/CAPES/FAPEPI/CNPq.

06.064

Perfil radiográfico do reparo alveolar de incisivos superiores de ratos wistar submetidos ao tratamento com antagonista B-adrenérgico. Cursino NM[1], Pereira CCS[2], Garcia LMG[3], Micaroni S[3], Okamoto R[2], Carvalho AAF[4], Antoniali C[3] [1]FOA-UNESP - Odontologia Infantil e Social, [2]FOA-UNESP - Cirurgia e Clinica Integrada, [3]FOA-UNESP - Ciência Básicas, [4]FOA-UNESP - Patologia e Propedêutica Clinica,

Introdução: O processo de reparo do alvéolo dental após exodontia pode ser definido em três principais fases: fase inicial (1 a 5 dias) na qual a organização do coágulo seria completada e o alvéolo seria parcialmente coberto por epitélio diferenciado; fase de formação óssea (5 a 20 dias) e fase de remodelação óssea (20 a 60 dias). Diferentes estudos demonstraram que a cronologia do reparo alveolar pode ser alterada por diferentes medicamentos, entre eles alguns utilizados para tratamento da hipertensão. Estudos recentes tem sugerido que o tratamento de pacientes e animais experimentais com antagonistas b-adrenérgicos inespecíficos estaria diretamente envolvido com ganho de massa óssea, aumento de densidade óssea e formação óssea. Neste estudo, avaliamos o efeito do atenolol sobre o reparo alveolar. **Materiais e métodos**: Ratos Wistar tratados, ou não, com Atenolol (100 mg/kg/dia, via oral) foram submetidos à cirurgia de exodontia do incisivo superior direito. O tratamento foi iniciado uma semana antes da cirurgia e mantido até o dia de sacrifício feito ao 7°, 14°, 21°, 28° e 42° dia após a exodontia. As maxilas direitas foram removidas e radiografadas com o aparelho de raios-X GE-100, com 50 kvp, 10 mA, 10 impulsos, distância foco-filme de 40 cm. A imagem digital foi obtida com placa óptica do sistema Digora e analisada no software Digora for Windows 1.51. Os experimentos foram conduzidos com a aprovação do Comitê de Ética local (CEEA-FOA no. 2008-001397). O terço médio (TM) e apical (TA) do alvéolo foram analisados. Os resultados foram expressos em Densidade Mineral Óssea (DMO) média, mínima e máxima e foram comparados pelo teste ANOVA. **Resultados**: No TM e TA do grupo não tratado foram observadas diferenças entre a DMO média do reparo alveolar. No TA, a DMO mínima diferiu entre o 28° (166,8±1,8) e 42° dia (149,8±2,6). Estes resultados demonstraram que um aumento significativo de formação óssea pode ser detectado no TA no final do reparo alveolar, e que aos 42 dias a quantidade menor de tecido mineralizado observada poderia estar associada à remodelação óssea. No grupo tratado com atenolol foi observada diferença entre a DMO média do TM ao 14° (155,1 ± 3,2) e 28° dia (167,3 ± 4,2), DMO máxima ao 14° (170,4 ± 2,8) e 28° dia (186,2 ± 3,7) e 14° e 42° dia (184,4±1,6). No TA do mesmo grupo houve diferença entre DMO média ao 14° (164,2±3,0) e 28° (176,2±3,6). **Discussão**: Os dados obtidos sugerem que a metodologia para a análise radiográfica não foi a mais adequada, uma vez que não conseguiu detectar uma correlação direta entre a cronologia do processo de reparo alveolar e a densidade mineral óssea no grupo controle. Diferentes fatores poderiam estar influenciando estes resultados como a quantidade de tecido ósseo maxilar, que por se sobrepor ao alvéolo poderia ter interferido na imagem analisada. No entanto, sob as mesmas condições, no grupo tratado com atenolol, valores DMO mais expressivos foram observados, sugerindo um possível aumento na quantidade de tecido ósseo formado. Nossos dados sugerem que o atenolol favoreceria uma melhor a formação óssea no processo de reparo alveolar. **Apoio**: FAPESP. Comitê de Ética: 2008-001397

06.065

Involvement of potassium channels and protein kinase a on the vasorelaxant effect of a standardized fraction from leaves of *Hancornia speciosa* in mice small mesenteric arteries. Silva GC[1], Rezende BA[2], Braga FC[3], Lemos VS[2], Côrtes SF[1] [1]UFMG - Farmacologia, [2]UFMG - Fisiologia e Biofísica, [3]FaFar-UFMG

Introduction: Vasorelaxant activities of *Hancornia speciosa* leaves have been previously demonstrated by our group [1]. We aimed to investigate the mechanism involved in the vasorelaxant effect of a standardized fraction (SFH) derived from the EtOH extract of *H. speciosa* leaves. **Methods:** Male normotensive Swiss mice were used. First branch of small mesenteric arteries mounted in myograph were used for vasorelaxation procedures. All experimental procedures were performed in at least five animals and approved by ethics committee of with protocol 227/08. All results are expressed as mean ± standard error of the mean. Two-way ANOVA for concentration-response curves and Student's *t*-test were used for statistical analysis. **Results and Discussion:** In endothelium-intact mesenteric arteries, pre-contracted with phenylephrine (3 μM), SFH produced a concentration-dependent vasorelaxation (IC_{50} = 32.0 ± 0.24 μg/mL). Removal of the endothelium and the pre-treatment with L-NAME (300 μM) significantly shifted the curve of SFH to the right (IC_{50} = 54.4 ± 5.06 and 50.1 ± 0.5 μg/mL, respectively; P< 0.001). In arteries with endothelium and pre-contracted with KCl (50 mM), SFH vasorelaxation curve was also shifted to the right (IC_{50} = 54.0 ± 1.7 μg/mL; P< 0.001) compared to vessels contracted with phenylephrine. However, in endothelium denuded vessels, the SFH vasorelaxant effect was abolished in arteries pre-contracted with 50 mM KCl. Tetraethylammonium (10 mM), a non-selective inhibitor for K channels, and 4-aminopyridine (1 mM), an inhibitor of Kv channels, shifted the concentration-response curve to the right (IC_{50} > 300 μg/mL, for both inhibitors) and reduced the maximal response (E_{max}) of SFH-induced vasorelaxation. Glibenclamide (1 μM), an inhibitor of K_{ATP} channels, shifted the curve to the right (IC_{50} = 61.2 ± 3.2 μg/mL; P< 0.001) without affecting the E_{max}. $BaCl_2$ (100 μM), at this concentration acting as an inhibitor of Kir channels, did not change the effect of SFH. H89 (0.1 mM), an inhibitor of protein kinase A (PKA), shifted the concentration-response curve of SFH to the right (IC_{50} > 300 μg/mL) and reduced the E_{max}, suggesting that PKA is involvement in the vasorelaxation response of SFH. In conclusion,SFH induced a vasorelaxant effect in mouse small mesenteric artery by a mechanism partially dependent on the presence of a functional endothelium and on production of nitric oxide. In addition, SFH also activated endothelium-independent mechanisms passing through activation of PKA and opening of K_{ATP} and K_V channels. **References:** Ferreira et al., *J Ethnopharmacol,* 109, 161. 2007. **Financial support:** CNPq and FAPEMIG

06.066

Vasorelaxant effect of tiliroside in rat small mesenteric arteries. Amaral AP[1], Rezende BA[1], Souza MFV[2], Côrtes FS[3], Lemos VS[1] [1]UFMG - Fisiologia e Biofísica, [2]UFPB - Tecnologia Farmacêutica, [3]UFMG - Farmacologia

Introduction: The consumption of polyphenolic compounds is associated to reduction of morbidity and mortality from cardiovascular diseases. Tiliroside (kaempferol 3-*O*-b-D-(6"-E-P-coumaroyl) glucoside) is a glycosyl flavonoid isolated from *Herissantia tiubae*, a plant found in the Northeast of Brazil. This flavonoid is described as having anti-obese and antioxidant effects. In the present work, we aimed to investigate the vascular effects of tiliroside in resistance vessels. **Methods:** Second branch of small mesenteric arteries were isolated from male Wistar rats, mounted in myograph and used for vasorelaxation procedures. All experimental procedures were performed in at least five animals. All results are expressed as mean ± standard error of the mean. Two-way ANOVA was used for statistical analysis. **Result and Discussion:** In the presence of an intact endothelium, tiliroside induced a concentration-dependent vasorelaxation in mesenteric arteries pre-contracted with phenylephrine (3 μM), with pIC_{50} =5.24 ± 0.09. Removal of the endothelium (pIC_{50} = 5.14 ± 0.09), the pre-treatment with L-NAME (300 μM; pIC_{50} = 5.59 ± 0.03) or indometacin (10 μM; pIC_{50} = 5.37 ± 0.13) did not change the response to tiliroside. In arteries pre-contracted with KCl (50 mM; pIC_{50} = 4,99 ± 0,07), tiliroside induced a vasorelaxant effect similar to that observed in arteries pre-contracted with phenylephrine. The present results allow us to conclude that tiliroside induces a concentration-dependent vasorelaxant effect in mesenteric arteries, through a mechanism independent on the presence of a functional endothelium, on production of nitric oxide or by activation of potassium channels. These results suggest the inhibition of calcium influx through voltage-dependent calcium channels as the mechanism involved on the vasorelaxant effect of tiliroside. **Financial support:** CNPq and FAPEMIG

06.067

Cardiovascular effects induced by alpha-terpineol in normotensive rats. Siqueira RJB[1], Silva MTB[1], Marques RB[2], Oliveira FA[2], Almeida FRC[3], Lahlou S[4], Santos AA[1], Magalhães PJC[1] [1]UFC - Fisiologia e Farmacologia, [2]NPPM-CCS-UFPI, [3]UFPI - Bioquímica e Farmacologia, [4]ISCB-UECE

Introduction: Alpha-terpineol (TERP) is a terpenoid constituent of essential oils found in a widespread variety of plants species, such as *Croton nepetaefolius*, which exhibit both myorelaxant and hypotensive effects on rats (Fundam Clin Pharmacol, 2008, 22, p.446). However, little is known about the pharmacological actions of TERP on the cardiovascular system. Thus, the present study investigated the cardiovascular effects elicited by the intravenous (i.v.) treatment of either anaesthetized or awake normotensive rats, as well as the mechanisms underlying TERP-induced effects on cardiovascular parameters. **Methods**: All experiments were performed according to ethical concerns (CEPA-UFC, 047/09). Male Wistar rats (280-320g) were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.), and vascular catheters (PE-10 fused to PE-50) were implanted in the abdominal aorta and in the inferior vena cava through the left femoral artery and vein, respectively. The heparinized (125 IU/mL) catheters were exteriorized at the dorsal neck level. Rats were housed individually in plastic cages and allowed to recover for 24h before experiments. They were divided in two groups to evaluate the effects of TERP on either pentobarbital-anaesthetized or conscious rats. At the time of experiment the catheters were connected to a data acquisition system (Power Lab®, ADInstruments, Australia). The following parameters were recorded: mean aortic pressure (MAP) and heart rate (HR). The effects induced by TERP were also evaluated in conscious animals treated with indomethacin (INDO), methylatropine (MA), hexamethonium (HEX). **Results and Discussion**: In conscious rats, control values for MAP and HR were 119±2 mmHg and 364±5 b.p.m., respectively (n=5). Under anesthesia, MAP and HR were 114±2 mmHg and 367±4 b.p.m., respectively. In general, TERP (1, 5 and 10 mg/k) induced hypotension and bradicardia, in a dose-dependent manner ($p < 0.001$, ANOVA), For instance, in either pentobarbital-anaesthetized or conscious rats, i.v. bolus injection of TERP (10 mg/kg) reduced significantly ($p < 0.05$, Bonferroni's_test) the MAP to values of -46.8±5.3 mmHg and -49.8±4.1 mmHg, respectively. It also showed bradicardic effects reducing the HR to -36.3±11.9 b.p.m. and -70.5±10.2 b.p.m., respectively. In conscious rats, TERP (10 mg/kg)-induced hypotension was significantly ($p<0.05$, Bonferroni's test) reduced by the pretreatment with INDO (5 mg/kg), but it was unchanged by MA (1 mg/kg) or HEX (30 mg/kg). On the other hand, bradicardic response was almost abolished by MA and reduced significantly by either HEX or INDO. In conclusion, these data suggest that TERP induces dose-dependent hypotension and bradicardia, which occurred independently. The hypotension appears occur due to a vascular relaxation related to prostaglandin release. The bradicardic response appears mainly dependent upon the presence of an operational and functional parasympathetic drive to the heart and probably is mediated by the release of a prostanoid. Supported by: FUNCAP, CAPES, CNPq.

06.068

Granulocyte colony-stimulating factor (G-CSF) increases prostanoids release in isolated aortic rings from spontaneous hypertensive rats. Padilha AS, Siman FDM, Baldo MP, Vassallo DV, Mill JG UFES - Physiological Sciences

Introduction: Several nonhematopoietic effects of G-CSF have been described in the last years. Cardiovascular effects include angiogenesis, antiapoptotic pathway activation and enhancement in cardiac function. Even so, effects on vascular function were not demonstrated. Thus, our aim was to evaluate the effects of G-CSF in the vascular reactivity in aortic from female spontaneous hypertensive rats (SHR). **Methods:** Aortic rings were isolated from three months old female SRH to evaluate the vascular reactivity to phenilephrine (10^{-10}- 3-10^{-4}M) before (CT) and after incubation with G-CSF for 40 min, with or without endothelium. The contribution of prostanoids in the effects of G-CSF on phenilephrine reactivity was evaluated after indometacin (10μM) incubation for 40 min in the bath. Moreover, acetylcholine (ACh,10^{-11}– 3.10^{-4}M) and sodium nitroprusside (SNP,10^{-11}-10^{-4}M) were used to test the endothelium-dependent or independent relaxation, respectively. Data are show as mean ± SEM, and significance established in $P<0.05$. **Results:** G-CSF increased the sensitivity (pD2) to phenilephrine (CT: -6.23±0.09 vs G-CSF: -6.55±0.10; n=10/13). In the absence of endothelium, the increase found in phenilephrine reactivity was similar in CT (n=7) and G-CSF (n=10) aortic (dAUC%- CT: 127.7±33.2 vs G-CSF: 80.8±1816.9). Indometacin reduced the reactivity to phenilephrine in both groups (n=5/6), however, more pronounced in presence of G-CSF (dAUC%- CT: 72.65±6.175 vs G-CSF: 216.0±38.76). Only the pD2 to ACh was reduced in presence of G-CSF (CT: -7.17±0.128 vs G-CSF: -6.04±0.311, n=6/5) and the relaxation to SNP was not altered. **Discussion:** Our data demonstrate that in a hypertensive condition such in these female SHR, G-CSF increases the vascular responsiveness to phenilephrine and reduces the acetylcholine-mediated relaxation. This may be due to increased vasoconstrictors prostanoids release. **Financial Support:** CNPq and FAPES/FUNCITEC

06.069

Cardiovascular responses to *Bothrops Jararacussu* snake venom. Smaal A, Rodrigues MAP, Dias L, Rennó AL, Hyslop S UNICAMP - Farmacologia

Introdução: Envenoming by *Bothrops* species can result in systemic alterations such as spontaneous systemic bleeding, shock and renal failure that, combined, are the main causes of death in humans bitten by this genus. In this study, we investigated the effects of *B. jararacussu* venom on cardiovascular parameters (blood pressure, heart rate, electrocardiogram - ECG and respiratory frequency) and marker enzyme (creatine kinase - CK; lactate dehydrogenase - LDH) levels in rats. We also examined the histological alterations caused by the venom. **Methods:** 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 (Dixtal® recorder, São Paulo, SP, Brazil). Venom (0.25, 0.5 or 0.75 mg/kg; a single dose per rat; n=6 each) was administered via a femoral vein in a fixed volume of 100 ml that was washed in with a further 100 ml of 0.9% NaCl. Changes in cardiovascular parameters were monitored for 120 min. At 0, 15, 30, 60 and 120 min post-venom, blood samples (0.3 ml/sample) were obtained for the quantification of CK and LDH with commercial kits (LabLabor, São Paulo, SP, Brazil); blood samples were replaced with an equal volume of 0.9% NaCl. After 120 min, the rats were killed and samples of heart, lungs, liver and kidneys were processed for histological analysis. The results (mean±S.E.M.) were analyzed statistically by using ANOVA followed by the Tukey-Kramer test. A value of $p<0.05$ indicated significance. These experiments were approved by the institutional Committee for Ethics in Animal Research (CEEA/UNICAMP, protocol no. 1739/1) **Results:** Intravenous injection of venom produced significant ($p<0.05$) immediate hypotension that was maximal within 30 s (from 92±7 to 56±12 and 54±10 mmHg for 0.25 and 0.5 mg/kg, respectively; n= 6), followed by gradual incomplete recovery. There were no significant alterations in heart rate, ECG or respiratory rate with these two doses. At the highest dose (0.75 mg/kg), rats died of irreversible shock and respiratory failure within 15 min, and this was accompanied by increased QRS, QT and QTC intervals in the ECG. There was a progressive increase in CK and LDH levels throughout the experiment. Macroscopic examination at necropsy revealed extensive pulmonary hemorrhage that was confirmed by histological analysis. Cardiac myonecrosis, edema and varying degrees of inflammatory infiltrates were seen with the two lowest doses. The liver showed loss of the normal architecture and the presence of an inflammatory infiltrate, but only with the dose of 0.5 mg/kg. In the kidneys, this venom dose (0.5 mg/kg) produced inflammation in the medullar cortex and cylinders in the cortical tubules; the lowest venom dose produced no marked renal alterations. No histological analysis done in rats injected with the highest venom dose. **Discussion:** These results show that *B. jararacussu* venom causes cardiovascular alterations that vary with the venom dose. These responses probably involve a mixture of vascular and direct cardiac effects, particularly at the highest venom dose. The increase in marker enzyme levels and the histological analysis confirmed that the venom also causes important tissue damage. **Financial support:** CNPq, CAPES, FAPESP

06.070

Liposomes prolong the cardioprotective activity of pyridostigmine. Vidal AT[1], Souza ACM[1], De Paula DCC[1], Silva-Barcellos NM[1], Leite R[2], Guimarães HN[3], Cardoso ASV[3], Frezard F[4], Grabe-Guimarães A[1] [1]UFOP - Farmácia, [2]MCG - Physiology, [3]UFMG - Engenharia Elétrica, [4]UFMG - Fisiologia e Biofísica

Introduction: The cardioprotective action of orally administered Pyridostigmine (Pyr), a reversible anticholinesterase agent, was demonstrated by its capacity in reducing some excitatory cardiovascular responses in rats, such as cardiac contractility and myocardium oxygen consumption (Grabe-Guimarães, 1999). Furthermore, Pyr reduces the QT dispersion during ECG rest (Castro, 2000) and enhances the left ventricular diastolic function during mental stress (Sant'anna, 2003). Long-circulating liposome is a powerful formulation to prolong the release of drugs to the tissues (Woofle, 1995) and was found to accumulate into the ischemic myocardium (Torchilin, 1996). In this context, the encapsulation of Pyr in liposomes has the potential to prevent or treat ischemic heart disease. **Objective:** To investigate the cardioprotective action of the Pyr encapsulated in long-circulating liposomes utilizing a sympathetic peripherial stimulation model in rats. **Methods:** The encapsulation of Pyr was carried out by freeze-thawed and extrusion. The formulation obtained was characterized and administrated intravenous (IV) to male Wistar rats (0.1, 0.3 and 1.0 mg/kg). The sympathetic stimulation was conducted by IV administration of 1 or 3 mg of noradrenaline (NA) after 1, 2, 4 or 6 hours. Blood pressure and electrocardiogram (ECG - limb lead II) were monitored, and the cardiovascular parameters were compared to animals that received intravenous injection of Pyr in free form or saline. All procedures related to the use of animals in these studies were approved by the local ethics committee under number 2009/11. **Results:** The efficiency of Pyr encapsulation into liposomes was 15.5%, with a medium size of 174 nm and zeta potential -50 mV. In those animals receiving saline, NA (3mg) induced a significant increase in systolic and diastolic pressure (76% and 70%, respectively). PR and QRS intervals of ECG were not significantly affected, while the QT interval increased 22%. Pyr in free and liposomal forms did not inhibit the increase in blood pressure. However, treatment with Pyr prevented significantly the increase of QT interval after sympathetic stimulation. Maximum effect of free Pyr preventing QT interval increase was observed after 1 hour (6.6% to the dose of 0 3 mg/kg) but was no longer observed after 2 hours of the treatment. On the other hand, maximum effect of liposomal formulation preventing QT interval increase was observed 2 hours after treatment (8.5% to the dose of 1.0 mg/kg) and was still present after 6 hours. **Conclusion:** The intravenous administration of free Pyr had a cardioprotective activity, by inhibiting the QT interval increase. Pyr administered as long-circulating liposomes was able to prolong its cardioprotective effect and may be a potential therapeutic alternative to prevent cardiovascular complications resulting from sympathetic hyperactivity in patients with ischemic heat disease. Acknoledgements: FAPEMIG, CNPq, UFMG, CIPHARMA-UFOP

06.071

Effects of GAP junctions modulators on oscillatory contractions and vascular reactivity in aortas from sinoaortic denervated rats. Rocha ML[1], Araújo AV[2], Bendhack LM[3] [1]UFG - Ciências Farmacêuticas, [2]USP - Farmacologia, [3]FCFRP-USP

Introduction: Following sinoaortic denervation (SAD) isolated rat aortas present oscillatory contractions and an augmented expression of gap junction communications. Therefore, this study aimed to verify the effects of heptanol (a gap-junction blocker) and tetraethylammonium (TEA, a gap-junction activator) on oscillatory contractions and vascular reactivity in the aortas isolated from SAD and Sham-operated (SO) rats. **Methods:** All the procedures were carried out in accordance with the Ethical Animal committee, of the University of São Paulo, Brazil (Process: 05.1.259.53.1 CEUA). Oscillatory contractions were induced by phenylephrine in denuded-aortic rings from SAD rats. We verified the effects of the gap-junction modulators heptanol (0.3 mM) or TEA (5 mM) on the frequency and amplitude of these oscillations. In another series of experiments, concentration-effect curves were constructed in denuded arteries from SAD and SO rats, pre-contracted with the EC_{50} of phenylephrine to TEA (10 µM to 100 mM) and heptanol (5 to 300 mM) in order to verify the effects of those gap-junction modulators. **Results:** Oscillatory contractions were observed in 10/10 SAD rat aortas vs. 2/10 controls. The treatment with heptanol completely abolished the emergency of oscillations in a concentration-dependent manner. The addition of TEA increased the amplitude (from 0.42±0.05 to 0.74±0.11grams) and it intensified the frequency of oscillations (from 4.5±0.5 to 11.5±1.5 cycles/min). In the experiments of concentration-effect curves to TEA, the maximal contractile effect was similar in both groups, although the potency was lower in SAD (pD_2: 2.64 ± 0.04) than in SO rat aortas (pD_2: 3.19 ± 0.11). The relaxation to heptanol in pre-contracted preparations was different between the groups. Heptanol presented higher potency (pD_2) in SAD as (3.61±0.05) than in SO rat pre-contracted aorta (2.88±0.04). **Conclusion:** Arterial pressure lability occurs only in SAD rats, and their isolated aortas exhibits intense oscillatory contractions. The oscillatory contractions seem to be dependent of the gap-junction communication, since these oscillations are intensified by TEA and completely inhibited by heptanol. The SAD rat aortas are more sensitive to heptanol and less sensitive to TEA than SO rat aortas. **Financial Support:** FAPESP and CNPq.

06.072

Does neonatal hyperleptinaemia modulate cardiovascular function? Marques EB[1], Pereira Toste F[2], Toste FP[2], Raimundo JM[3], Sudo RT[3], Zapata-Sudo G[3], Marques SA[4], Brito FCF[1], Vieyra A[5], Scaramello C[1] [1]UFF - Fisiologia e Farmacologia, [2]UFF - Ciências do Exercício, [3]UFRJ - Farmacologia Básica e Clínica, [4]UFF - Neurobiologia, [5]IBCCF-UFRJ

Introduction: Altered leptin activities are related to cardiovascular diseases like congestive heart failure - CHF (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). It was also shown that neonatal leptin treatment is related to leptin resistance and metabolic changes in adult rats (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 programmes cardiovascular function and could be involved in the development of chronic diseases. **Methods:** Pups were divided into two groups. Lep group, injected daily with leptin (8µg/100g sc) for the first 10 days of lactation and Control group, injected saline at the same period. After weaning, body weight and food intake were monitored. One month-old rat heart and aorta were removed for morphological and functional analysis. Histological assays were performed using Hematoxilineosine (HE) and Gomori methods. Heart tension was recorded before and after 3µM isoproterenol(Langendorff method).Vascular smooth muscle activity was measured using phenylephrine (10^{-9}-10^{-4}M) and acetylcholine (10^{-9}-10^{-4}M). Maximal effort ergometer test was also performed and both group were submitted to the same pace (initial rate 1Km/h raising 0.5 km/h each 1 min and 0.1% inclination each 2min) to monitor distance and time. The use of animals took place according to the guidelines of the Animal Care and Use Committee of Federal University of Rio de Janeiro (DFBCICB007). **Results:** Until 30 days-old, no difference was observed in body weight, food intake neither in histological profiles. Striated cardiac tissue and its central nucleus, volume, colagen and cardiomyocytes were preserved. Although no statistic difference were observed in heart isometric tension between Lep and Control group in the absence (3.86±0.46 and 3.26±0.36g, respectively, n=4) and in the presence of 3µM isoproterenol (7.25±1.08 and 7.00±0.14g, respectively, n=4), it seems to be a minor increment of isometric tension by isoproterenol in Lep group (86.38±7.26% versus 120.1±21.54% of Control group). In maximal effort ergometer test, Lep group performance appears to be worse compared to Control (running time: 4.15±0.76min, n=4, versus 5.03±0.53 min, n=5; distance: 0.14±0.04Km, n=4, versus 0.19±0.03min, n=5, respectively). No difference was observed in phenylephrine dependent aorta contraction. However, acetylcholine induced relaxation EC_{50} was statistically lower for Lep ($2{,}28x10^{-7}$M) compared to Control group ($2{,}4x10^{-6}$M). **Discussion:** Preliminary data suggest that cardiac function is decreased in Lep group. These results are in agreement with the literature that leptin has cardioprotective effects (Dixon et al. J.Cardiovasc.Pharmacol.,v.53,p.311,2009), being an interesting diagnostic tool in CHF (Schulze & Kratzsch, Clin.Chim.Acta,v.362,p.1,2005). The higher potency of acetylcholine in Lep group also agrees with previous work that shows leptin decreasing blood pressure via NO production. Further assays will be performed also to investigate the molecular mechanism involved in cardiac and vascular changed activity in this model. Additionally leptin influence will be investigated in 5 moth-old rats to analyze the effect of neonatal leptin treatment in older rats. **FINANCIAL SUPPORT:** FAPERJ, CNPq, CAPES, PROPPI/UFF.

06.073

Cardioprotection of a self emulsifying drug delivery system of ipriflavone. Albuquerque K[1], Sales GS[2], Almeida TM[2], Leite R[3], Mosqueira VCF[3], Guimarães HN[4], Grabe-Guimarães A[3] [1]UFOP - Cipharma, [2]EFAR-UFOP, [3]UFOP - Farmácia, [4]UFMG - Engenharia Elétrica

Introduction: Ipriflavone, an isoflavone synthesized from daidzein (soy derived isoflavone) holds great promise to prevent and treat osteoporosis and other metabolic bone diseases. Its cardioprotection activity in rats and rabbits (250 mg/kg, P.O.) was reported in 1981 with no follow up studies. The results involving the use of ipriflavone for other purposes present high variability, possible due to its low oral bioavailability. This low bioavailability could be attributed to its hydrophobicity, which compromises its solubilization in the intestinal fluids, and consequently, its absorption. In order to enhance ipriflavone bioavailability, a self emulsifying drug delivery system (SEDDS) is proposed in this study. This system is an isotropic mixture of oil, surfactant, co-surfactant, sometimes including co-solvent that emulsify under conditions of gentle agitation, similar to those encountered in the gastrointestinal tract. **Objectives:** Evaluate the cardioprotection, in rats, of oral administered ipriflavone in a SEDDS using the model of heart ischemia induced by coronary artery occlusion technique. **Methods:** Female *Wistar* rats (220 - 250 g), provided by the animal facilities of the Federal University of Ouro Preto, received either vehicle or ipriflavone in a SEDDS (240 mg/kg) by gavage one hour before the beginning of experiments. Under pentobarbital anesthesia (60 mg/kg, I.P.), ECG lead II and blood pressure were digitally recorded (1200 Hz with a resolution of 16 bits) for 5 minutes (control period). At the end of the 5 minutes, the animals were submitted to a left anterior descending coronary occlusion with both parameters being recorded continuously for 30 min. Systolic and diastolic blood pressure; heart rate and ECG lead II were evaluated. **Results:** Blood pressure was dramatically reduced after coronary occlusion. Systolic and diastolic blood pressure in non-treated animals were reduced about 57% and 68%, while in ipriflavone-treated animals the reduction was 57% and 77%, respectively. The drop in systolic and diastolic blood pressure remained constant throughout the next 30 min of experiment. There was no significant difference regarding the changes in blood pressure between groups. At 5 minutes of occlusion, it was observed an elevation of the ST segment of 312% and 130%, in non-treated and ipriflavone-treated rats, respectively. At 30 minutes of occlusion, the elevation of the ST segment was 337% and 56%, for the non-treated and ipriflavone-treated rats, respectively. **Conclusion:** Ipriflavone orally administered through a SEDDS was able to reduce the changes in the ST segment induced by coronary occlusion-induced ischemia in female rats, suggesting a potential cardioprotection activity. *Financial support:* UFOP, FAPEMIG, CAPES. Protocolo de aprovação no comitê de ética da UFOP: 2009/11

06.074

Cardiac dysfunctions associated with compensatory remodeling are ameliorated by metalloproteinase inhibition in 2K-1C hypertension. Rizzi E[1], Castro MM[2], Prado CM[2], Aguiar Silva CA[3], Fazan Jr R[3], Rossi MA[3], Tanus-Santos JE[1], Gerlach RF[4] [1]FMRP-USP - Farmacologia, [2]FMRP-USP - Patologia, [3]FMRP-USP - Fisiologia, [4]FORP-USP - Morfologia

Introduction: Enhanced cardiac matrix metalloproteinase MMPs has been associated with ventricular remodeling and cardiac dysfunction. It is unknown whether MMPs contribute to systolic/diastolic dysfunction and compensatory remodeling in 2K1C hypertensive rats. **Methods:** To test this hypothesis we used 2K1C rats after 2 weeks of surgery treated or not with a non-specific inhibitor of MMPs (doxycycline) during 8 weeks**.** Maximum and minimum values of first derivative of left ventricular pressure (±dp/dt) and histological analyses were used to determination of cardiac and morphological alterations. To evaluate MMP-2 in ventricle of animals we used zymography, *in situ* zymography and imunofluorescence. Experimental protocols followed standards and policies of the University of Sao Paulo's Animal Care and Use Committee (196/2008). **Results:** The normotensive Wistar rats were used as control. We found blood pressure and ±dp/dt increased in 2K1C rats compared with sham groups that were attenuated by doxycycline treatment ($P<0.05$). Doxycycline treatment reversed cardiac hypertrophy observed in 2K1C rats ($P<0.05$). Hypertensive rats showed increased MMP-2 levels in zymograms and in the tissue by immunofluorescence ($P<0.05$) compared with sham groups. Increased gelatinolytic activity was observed in untreated 2K1C rats by situ zymography when compared with sham groups ($P<0.05$). Doxycycline decreased gelatinolytic activity in 2K1C to control levels ($P<0.05$), but did not affect the MMP-2 levels in 2K1C or sham group. **Conclusion:** In conclusion, an imbalance in gelatinolytic activity, with increased MMP-2 levels and activity underlies the development of morphological and functional alterations found in the compensatory hypertrophy observed in 2K1C hearts. Since function and structure were restored by doxycycline, the inhibition of MMPs or their modulation may provide beneficial effects for therapeutic intervention in cardiac hypertrophy. **Financial support:** CNPq, CAPES and FAPESP.

06.075

The nitric oxide (NO) donor Ru(terpy)(bdq)$NO^+]^{3+}$ (Terpy) induces relaxation of the rat mesenteric resistance arteries. Araújo AV[1], Biazzotto JC[2], Da Silva RS[2], Bendhack LM[2]
[1]FMRP-USP - Farmacologia, [2]FCFRP-USP - Física e Química

Introduction: There is a great interest in the development of new compounds that release NO in biological systems. Nitrosyl ruthenium compounds such as Terpy are very attractive as NO donors, and as we previously reported, it induces rat aorta relaxation. The aim of this study was to verify if Terpy relaxes the isolated third branch of rat mesenteric artery, a resistance vessel. **Methods**: Cumulative concentration-effect curves for the contractile agonists phenylephrine (Phe 0.1mmol/L-0.1mmol/L) and thromboxane A_2 analogue U46619 (0.01 mmol/L-0.01mmol/L) were constructed in endothelium-denuded third branch of the mesenteric artery. Curves for of relaxation for sodium nitroprusside (SNP, 1nmol/L-0.1mmol/L) and terpy (0.01 mmol/L - 0.01mmol/L) were constructed in vessels pre-contracted with Phe (0.01 mmol/L). We have analyzed the maximum effect (ME) and the potency (pD_2) of the drugs. **Results:** Phe and U46619 induced concentration-dependent contractions, and Phe-induced ME (16.98±0.83mN, n=4) was greater than that for U46619 (ME: 11.03±1.00mN, n=5, $p<0.001$). On the other hand, U46619 was more potent (pD_2: 6.63±0.11, n=5) than Phe (pD_2: 5.45±0.09, n=4, $p<0.001$). We have used U46619 to pre-contract the vessels, but it presented taquifilaxy. For this reason, we have changed to Phe. Both SNP and Terpy induced relaxation in a concentration-dependent way with similar ME in vessels pre-contracted with Phe. Terpy was less potent than SNP (pD_2: 4.77±0.19, n=4 for Terpy and 6.56±0.28, n=4 for SNP, $p<0.01$). **Discussion:** Our results show that the new NO donor terpy induces vascular relaxation in endothelium-denuded mesenteric resistance artery, that is comparable to the relaxation induced by SNP. The suitable contractile agonist is Phe. Taken together, the new NO donor relaxes the rat mesenteric resistant arteries. This study was approved by Ethical Committee of the University of São Paulo (CETEA-FMRP, protocol number: 044/2008). Supported by FAPESP and CNPq.

06.076

Inhibition of proximal tubular salt and water reabsorption by the gut incretin glucagon-like peptide 1 (GLP-1). Oricchio, FT[1], Lessa LMA[2], Malnic G[2], Girardi AC[1] - [1]InCor-HC-FMUSP, [2]ICB-USP - Fisiologia e Biofísica

Introduction: Glucagon-like peptide 1 (GLP-1) is a gut incretin hormone considered a potential therapeutic agent for type 2 diabetes because it stimulates beta cell proliferation and insulin secretion in a glucose-dependent manner. Apart from its actions on insulin-producing pancreatic islet cells, GLP-1 has additional physiologic effects as a result of expression of GLP-1 receptor (GLP-1R) in many other tissues. GLP-1R has recently been detected in renal proximal tubule cells, where GLP-1 reduces sodium reabsorption. The present study was undertaken to evaluate the role of GLP-1 in modulating the activity of the Na^+/H^+ exchanger NHE3 representing the major route for proximal tubular sodium and bicarbonate reabsorption. **Methods:** Male Wistar rats (230-300 g) were anesthetized and placed on a heated surgical table to maintain body temperature. After tracheostomy, polyethylene catheters were inserted into the jugular vein and the urinary bladder for infusion of solutions and urine collection, respectively. 0.1 mg/kg×min GLP-1 or vehicle (0.9% saline) was intravenously infused at a rate of 3 mL/h for the period of 1 hour. Arterial blood was collected at the time of death. Urine output was measured gravimetrically and creatinine clearance was used to estimate glomerular filtration rate (GFR). The kidneys were removed for cortical microsomal membrane preparation and immunoblotting analyses. The activity of Na^+/H^+ exchanger NHE3 in rat renal proximal tubule was measured by in vivo stationary microperfusion. **Results and Discussion:** Rats treated with GLP-1 had significantly increased urine output compared to controls (45.3 ± 5.5 vs. 22.4 ± 2.5 mL/min×kg; P=0.001). The increase in urine output was accompanied by a significant increase of fractional sodium excretion (0.41 ± 0.12 vs. 0.14 ± 0.03%; P = 0.02) and glomerular filtration rate (12.6 ± 1.5 vs. 7.9 ± 1.1 mL/min×kg; P =0.03). Urine analyses revealed that rats treated with GLP-1 acidified their urine to a lesser extent than controls (6.52 ± 0.10 vs. 6.09 ± 0.07; P=0.006). Accordingly, fractional bicarbonate excretion was higher in GLP-1-treated rats (0.053 ± 0.012 vs. 0.007 ± 0.005%; P=0.01). A trend toward decline of blood pH and bicarbonate concentration was observed in rats treated with GLP-1. However, differences in systemic acid-base status were not statistically significant. Experiments of stationary microperfusion performed in rat renal proximal tubule revealed that GLP-1 significantly reduced NHE3-mediated bicarbonate reabsorption (1.16 ± 0.13 vs. 2.11 ± 0.17 nmol/cm2´s; P=0.002). Inhibition of NHE3 activity was associated with a significant increase (64 ± 9%; P=0.02) in the phosphorylation of the transporter. The data presented demonstrate that GLP-1 is a potent diuretic and natriuretic agent that acts to inhibit sodium, bicarbonate and water reabsorption in the rat renal proximal tubule. It also raises the feasibility of using GLP-1 analogs for the treatment and/or prevention of salt-sensitive hypertension. **Supported by** FAPESP and CNPq. Projeto aprovado em 14/11/2007 pela Comissão de Ética para Análise de Projeto de Pesquisa (CAPPesq, HC-FMUSP) - Registro 1425.

06.077

Evaluation of cardiovascular changes induced by chronic treatment with arsenic and antimony trivalents in rats *in vivo.* Reis PG[1], Almeida, WM[1], Guimarães HN[2], Leite R[1], Teixeira MC[1], Grabe-Guimarães A[1], Silva-Barcellos NM[1] [1]UFOP - Farmácia, [2]UFMG - Engenharia Elétrica

Introduction: Previous studies have shown that the semi metals arsenic and antimony have large chemical and toxicological similarities. The trivalent antimony was used in the clinical treatment of schistosomiasis, cutaneous leishmaniasis and kalazar. It was demonstrated recently that the encapsulation of tartar emetic, an antimony compound, in PEGylated liposomes increases its therapeutic efficacy and reduces toxicity which could make this compost an option for the treatment of these diseases again. The use of trivalent arsenic to treat cancer and parasite infection has been recently investigated, including the approval of the FDA (USA). Promising results were obtained in the treatment with arsenic trioxide for patients presenting multiple myeloma and recurrent cases of acute promyelocytic leukemia resistant to conventional therapy, presenting in most cases the total remission of the disease. The purpose of this study was to evaluate and compare the cardiovascular changes *in vivo* induced by arsenic and antimony trivalents in rats for the future evaluation of pharmaceutical formulations that enable the reduction this toxicity. **Methods:** Three groups of male Wistar rats (200 to 250g) were treated for 30 days with: 1) solution of $C_8H_4K_2Sb_2$ (3.5 mg/ kg ip), 2) solution of AsNaO2 (3.8mg/kg ip) and 3) vehicle (control group). On the 31th day, the animals were anesthetized with sodium thiopental (60mg/kg) in order to obtain arterial pressure (AP) and the ECG (limb lead II). The cardiovascular parameters extracted were: systolic arterial pressure (SAP), diastolic arterial pressure (DAP), heart rate (HR) and PR, QRS, QT intervals of the ECG. This project was approved by the local ethics committee (number 2009/11). **Results:** It was observed significant changes of some parameters evaluated. The $C_8H_4K_2Sb_2$ induced increases of QT (34%), PR (26%) and QRS (24%) intervals, and reduction of 23% in heart rate. There was significant difference regarding all intervals and heart rate between this group and control group. The $AsNaO_2$ induced increases of QT (46%), reducing of PR (2,6%) and QRS (5,0%) intervals and 3% of reduction in heart rate. In this case, there was significant difference regarding QT interval and heart rate between this group and control group. SAP and PAD didn't show significant changes in both groups. **Discussion**: The arsenic and antimony are capable of inducing cardiovascular changes indicative of their toxicity. Both semi metals can induce changes of heart rate and QT interval. It is known that changes in this last parameter are identified as the main cause of arrhythmia and sudden death during treatment with these compounds. The rat model could be useful for the future evaluation of pharmaceutical formulations that enable the reduction of arsenic and antimony trivalent toxicity. **Acknowledgments**: FAPEMIG, CNPq, UFOP.

06.078

Furosemide effects on isolated perfused rat kidney: a preliminary study. Leite CAVG[1], Alves RS[2], Ximenes RM[1], Jorge ARC[1], Sousa DF[1], Marinho AD[3], Sousa PCP[1], Mendonça ATB[1], Daher EF[4], Monteiro HSA[1] [1]UFC - Fisiologia e Farmacologia, [2]UFC - Análises Clínicas e Toxicológicas, [3]UFC - Farmácia, [4]UFC - Medicina Clínica

Introduction: Diuretic drugs are widely used for the treatment of patients with edema. Among these drugs, loop diuretics such as furosemide are perhaps the most frequently prescribed, and their clinical pharmacology is better understood than other diuretics (Brater, DC. NEJM. 339: 387-395, 1998). Loop diuretics block the sodium-potassium-chloride transporter (Odlind B. Clin Pharmacol Ther. 27:784-790, 1980). The aim of this work was to study the effect of furosemide on perfused isolated rat kidney. **Methods:** Isolated kidneys from Wistar rats, weighing 240 to 280g (n=4), were perfused with Krebs-Henseleit solution containing 6 g% of previously dialyzed bovine serum albumin (Fonteles, Am. J. Physiol. 244:235, 1983). The effects of furosemide (8 µg/mL) 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 were studied. All data were represented as mean ± SEM and analyzed by ANOVA and Student *t*-test with level of significance set at $p<0.05$. The drug was added to the system 30 minutes after an internal control. The experimental protocols were approved by the Ethics Committee on Animal Research of the Department of Physiology and Pharmacology, Federal University of Ceara (number 68/08). **Results:** Furosemide increased PP (PP_{30}= 114.40 ± 1.44; PP_{60}= 148.30 ± 6.63 *(p<0.05)*, PP_{90} = 184.10 ± 7.75 *(p<0.05)*; PP_{120} = 189.60 ± 7.25 mmHg *(p<0.05)*, RVR (RVR_{30} = 5.16 ± 0.08; RVR_{60} = 6.74 ± 0.34 *(p<0.05)*; RVR_{90} = 8.32 ± 0.41 *(p<0.05)*; RVR_{120} = 8.56 ± 0.38 $mmHg.g^{-1}.mL^{-1}.min^{-1}$ *(p<0.05)*, UF (UF_{30} = 0.12 ± 0.02; UF_{60} = 0.27 ± 0.05 *(p<0.05)*; UF_{90} = 0.43 ± 0.06 *(p<0.05)*; UF_{120} = 0.43 ± 0.05 $mL.g^{-1}.\ min^{-1}$ *(p<0.05)*, and increased GFR (GFR_{30} = 0.78 ± 0.10; GFR_{90} = 1.39 ± 0.21 *(p<0.05)*; GFR_{120} = 1.34 ± 0.18 $mL.g^{-1}.min^{-1}$ *(p<0.05)*. For the transport of electrolytes, we observed a reduction in %TNa^+ (TNa_{30} = 84.91 ± 1.00; TNa_{60} = 69.00 ± 1.61 *(p<0.05)*; TNa_{90} = 69.17 ± 2.48 *(p<0.05)*; TNa_{120} = 66.53 ± 1.62% *(p<0.05)*), %TK^+ (TK_{30} = 84.98 ± 0.97; TK_{60} = 69.87 ± 1.61 *(p<0.05)*; TK_{90} = 69.35 ± 2.30 *(p<0.05)*; TK_{120} = 66.23 ± 1.67% *(p<0.05)*) and %TCl^- (TCl_{30} = 83.85 ± 1.05; TCl_{60} = 67.11 ± 1.71 *(p<0.05)*; TCl_{90} = 67.87 ± 2.55 *(p<0.05)*; TCl_{120} = 66.10 ± 1.38% *(p<0.05)*. **Conclusion:** The results indicate that furosemide promoted significant changes on renal function as well as vascular alterations, phenomena probably due to furosemide effect on the release of renin in the rat kidney. **Financial Support**: CNPq

06.079

Chronic treatment with spironolactone and/or hydrochlorothiazide attenuates the vascular changes in renovascular hypertension. Ceron CS[1], Castro MM[1], Rizzi E[1], Salgado MCO[1], Gerlach RF[2], Tanus-Santos JE[1] [1]FMRP-USP - Farmacologia, [2]FORP-USP - Morfologia

Introduction: The structural and functional vascular changes in renovascular hypertension have been associated with upregulation of metalloproteinases, which are enzymes involved in vascular remodeling. We examined whether the treatment with spironolactone, hydrochlorothiazide, or both drugs modified two kidney-one clip (2K1C) hypertension-induced changes in arterial blood pressure, aortic remodeling, vascular reactivity, and metalloproteinase levels/activity. **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. After two weeks of surgery, hypertensive and sham rats were treated for 8 weeks with water, spironolactone (25 mg/kg/day), hydrochlorothiazide (20 mg/kg/day), or both drugs. Systolic blood pressure (SBP) was monitored weekly by tail-cuff plethysmography. Aortic rings were isolated to assess endothelium dependent and independent relaxations. Morphometry of structural changes of the aortic wall were studied in hematoxylin/eosin sections. Aortic MMP-2 levels/activity were determined by zymography, fluorimetry, gelatin and in situ zymography. This study was approved by Animal Ethics Committee of FMRP/USP (n 196/2008). **Results:** Treatment with spironolactone, hydrochlorothiazide, and the combination of drugs presented a small but significant reduction on blood pressure (189 ± 3.9 mmHg, 178 ± 2.4 mmHg, and 175 ± 2.4 mmHg, respectively, versus 200 ± 1.4 mmHg in hypertensive controls; all $P<0.05$), and reversed the reduction in endothelium-dependent vasorelaxation in hypertensive rats to acetylcholine (94.7 ± 3.2%, 95.7 ± 2.44%, 94.7 ± 2.3%, respectively, versus 76.3±5.4% of relaxation in hypertensive controls; all $P<0.05$). No significant differences were observed in endothelium-independent vasorelaxation to nitroprusside among the experimental groups. Both drugs and the combination reversed the vascular remodeling and the increases in aortic MMP-2 total levels/activity found in hypertensive rats. Aortic MMP-2 levels increased by 42% in hypertensive rats, and treatment with spironolactone, hydrochlorothiazide, and the combination reduced MMP-2 levels by 35%, 22%, and 25%, respectively in hypertensive animals (all $P<0.05$). Aortic MMP-2 activity increased by 20% in hypertensive rats, and treatment with spironolactone, hydrochlorothiazide, and the combination reduced MMP-2 activity by 18%, 17%, and 17%, respectively in hypertensive animals (all $P<0.05$). No significant changes were seen in Sham and Sham+treatment groups during the experiments in all the parameters evaluated. **Discussion:** These findings suggest that spironolactone, or hydrochlorothiazide, either alone or combined, decrease vascular metalloproteinase-2 upregulation and attenuate the vascular dysfunction and remodeling associated with renovascular hypertension. **Supported by:** CAPES, FAPESP, CNPq.

06.080

Contraction induced by angiotensin II (AII) is impaired by the endothelium in aorta from epileptic rat. Restini CA[1], Reis RI[2], Costa-Neto CM[2], Garcia-Cairasco N[3], Bendhack LM[1] [1]FCFRP-USP - Física e Química, [2]FMRP-USP - Bioquímica e Imunologia, [3]FMRP-USP - Fisiologia

Introduction: AII has been assumed to be the main peptide acting in the periphery and growing evidences indicate that the Renin-Angiotensin System (RAS) acts in specific neuronal pathways in the central nervous system. Its involvement has been demonstrated in some neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's disease. Up-regulation of AT1 receptor for AII is related to increased tissue excitability in the hippocampus and cortical areas. Concerning to cardiovascular system, RAS is related to vascular remodeling and has pivotal role in the blood pressure control. In addition, RAS is an important modulator of endothelial NO release. Since there is a tight relation between neurological problems, as the seizures observed in epilepsy, and cardiovascular alterations, we have hypothesized that in epileptic Wistar audiogenic rat strain (WAR) the AII-induced contractile response could be altered and the differential response could be due to contractile endothelial factor (EDCF). This study aimed to study the contractile response induced by AII and angiotensin I (AI) and the expression of AII receptors and angiotensin converting-enzyme (ACE) in aortas from WAR. **Methods:** Concentration-effect curves for AII were constructed in intact endothelium (E^+) and denuded (E^-) aortas from control (Wistar) and WAR. Western Blotting was designed to quantify the protein expression of AT_1 and AT2 receptors and ACE in both rat aortic groups. **Results:** AII induced contractile response in control rat aortas in both E^+ (1.13±0.04g, n=6) and E^- (1.20±0.05g, n=6). The maximum contractile response induced by AI in control rat aortas (n=6) were 0.45 ± 0.03g (E^+) and 1.13 ± 0.08g (E^-). However, in WAR E^+ aortic rings AI and AII failed to induce contraction. AT1 receptor expression was not different in WAR E+ (1.21±0.09), in control Wistar E+ (1.16±0.05) in WAR E^- (1.60±0.14) and in control E^- (1.50±0.09). AT2 receptor expression was increased in control E^- (0.83±0.10) comparing to the other groups. There are no differences between the AT2 expression in control E+ (0.58±0.02), WAR E^- (0.51±0.06) and WAR E^+ (0.52±0.07). ACE receptor expression was higher in WAR E^+ (1.41±0.4) and in control E^+ (1.15±0.05) than in WAR E^- (0.28±0.06) and control E^- (0.78±0.035). **Discussion:** The presence of the endothelium impaired the contractile response induced AI and AII in WAR. These results suggest that endothelial factors play an important role to prevent the aorta vascular smooth muscle contraction in this epileptic rat strain. However, in denuded arteries it did not produce the same amplitude of contractile response as compared to the aortas from control rats, which suggests that other mechanisms could be involved in the impaired contraction to AI and AII in WAR aortas. This study was approved by the Ethical Committee of University of São Paulo (CEUA124/2005). Supported by FAPESP and CNPq.

06.081

Vasodilatation induced by atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) in isolated aorta from rats. Andrade FA[1], Bendhack LM[2] [1]USP - Farmacologia, [2]FCFRP-USP

Introduction: ANP and CNP are peptides that play a key role in cardiovascular homeostasis. The vasodilatation induced by these peptides has been mainly attributed to the activation of particulate guanylate-cyclase (GCp) and production of cGMP. The cGMP produced via GCp activates protein kinase G (PKG) in order to induce vascular relaxation. This study aimed to investigate the vascular smooth muscle relaxation activated by the natriuretic peptides ANP and CNP and the effect of the selective PKG inhibitor KT5823 in this relaxation in rat aortic rings. **Methods:** Cumulative concentration-effect curves for ANP and CNP were constructed in endothelium-denuded rat aortic rings, pre-contracted with phenylephrine (0.01mmol/L). The potency (pD_2) and maximum effect (ME) were analyzed for ANP e CNP in inducing vascular relaxation. **Results:** ANP e CNP induced complete relaxation of aortas pre-contracted with phenylephrine. The potency in inducing relaxation was greater to ANP (pD_2: 8.62 ± 0.08, n=5) than that obtained with CNP (pD_2: 7.08 ± 0.24, n=5, $P< 0.001$). However, the maximum effect was similar for both ANP (102.9 ± 2.1%) and CNP (108.2 ± 2.8%). Incubation with 1µmol/L KT5823 did not alter the relaxation induced by ANP (pD_2: 8.70 ± 0.06 and ME: 106.7± 4.9%, n=4) and to CNP (pD_2: 7.19 ± 0.17 and ME: 103.7 ± 3.8%, n=4). **Discussion:** Our results demonstrate that ANP and CNP induce rat aorta relaxation in a concentration-dependent way either in the presence and in the absence of the inhibitor of PKG in the concentration used. However, in order to prove that PKG activation pathway is involved in the relaxation induced by ANP and CNP in rat aortic rings, we intend to use higher concentration of KT5823. In conclusion, the natriuretic peptides ANP and CNP induce vascular smooth muscle relaxation, which effect is not sensitive to PKG inhibition. This study was approved by the Ethical Committee of the University of São Paulo (CETEA 044/2008). Supported by CAPES, CNPq and FAPESP.

06.082

Tratamento antioxidante com tempol e apocinina previne disfunção endotelial e desenvolvimento de hipertensão renovascular. Costa CA[1], Amaral TAS[1], Carvalho LCRM de[1], Ognibene DT[1], Silva AFE[1], Oliveira PRB[1], Freitas de Bem F[1], Valença SS[2], Resende AC[2], Soares de Moura R[1] [1]UERJ - Farmacologia e Psicobiologia, [2]IBRAG-UERJ - Histologia e Embriologia

Introdução: O modelo de hipertensão renal dois rins, um clip (2R-1C) está associado a uma disfunção endotelial. Como o estresse oxidativo parece estar elevado neste modelo, nós avaliamos o efeito do tratamento crônico de animais hipertensos 2R,1C com os antioxidantes Tempol e Apocinina sobre o desenvolvimento da hipertensão e disfunção endotelial; a atividade das enzimas antioxidantes superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx); a expressão da enzima SOD-1 e a peroxidação lipídica (TBARS). **Métodos**: Os experimentos foram aprovados pelo Comitê de Ética em Pesquisa da UERJ (projeto 1730- CEP/HUPE - CAAE:0018.0.228.000-07). Ratos Wistar machos utilizados para obtenção da hipertensão renovascular 2R-1C de Goldblatt e ratos controles 2R (sham) receberam tratamento com veículo ou Tempol ou Apocinina durante 40 dias, e tiveram a pressão arterial sistólica (PAS) aferida por pletismografia de cauda. Os efeitos vasodilatadores da acetilcolina (ACh) e nitroglicerina (NG) foram estudados em leito arterial mesentérico (LAM) pré-contraído com norepinefrina (30mM). A atividade das enzimas SOD, CAT, GPx e os níveis de TBARS foram avaliados por espectrofotometria e a expressão da enzima SOD-1 foi avaliada por Western blot nos animais estudados. **Resultados**: A PAS (mm Hg) foi maior nos animais 2R-1C, e o tratamento com Tempol (251±22 vs 156±12) e Apocinina (213±13 vs 148±11) preveniram o desenvolvimento da hipertensão ($p \leq 0.05$). O efeito vasodilatador reduzido da ACh (100 pmol) em animais 2R-1C foi revertido pelo Tempol (47±4 vs 73±3) e pela Apocinina (46±3 vs 77±3). O efeito vasodilatador da NG (100 nmol) foi menor em animais 2R-1C e o tratamento com Tempol (42±3 vs 44±2) não modificou a resposta, ao passo que, o tratamento com Apocinina (35±3 vs 52±5) aumentou o efeito vasodilatador da NG nos animais 2R-1C. Os níveis de TBARS foram maiores em amostras de mesentério (M) e plasma (P) dos animais 2R-1C, e o tratamento com Tempol (M:10,4±1,2 vs 5,5±1,4; P:1±0,05 vs 0,7±0,07) e Apocinina (P:2,8±0,2 vs 1,8±0,3) diminuiu os níveis de TBARS nestes animais. As atividades da SOD, CAT e GPx foram menores em amostras de M e P nos animais 2R-1C. O tratamento com Tempol (SOD M:37,2±2,4 vs 154,5±18,3; P:125,2±8,1 vs 193,8±28,3; CAT M:1,5±0,2 vs 1,5±0,1; P:0,1±0,07 vs 0,1±0,04; GPx M:0,0005±0,1 vs 0,0005±0,1; P:0,00002±0,1 vs 0,00007±0,1) aumentou apenas a atividade da SOD em amostras de M nos animais 2R-1C, não modificando a atividade das demais enzimas, ao passo que, o tratamento com Apocinina (SOD P:62,4±4,4 vs 72,7±5,7; CAT:0,003±0,1 vs 0,006±0,1; GPx:0,0007±0,1vs 0,0003±0,1) não modificou a atividade destas enzimas. A expressão da SOD-1 foi menor nos animais 2R-1C, e o tratamento com Tempol (1,2±0,1 vs 1,4±0,1) não modificou a expressão desta enzima. **Conclusão**: O tratamento crônico com Tempol e Apocinina previne o desenvolvimento da hipertensão e melhora a disfunção endotelial em ratos 2R-1C. A redução da atividade antioxidante e o aumento na peroxidação lipídica sugerem o envolvimento de um mecanismo deficiente da defesa antioxidante e de um dano oxidativo aumentado, os quais foram revertidos pelo Tempol e Apocinina. **Apoio Financeiro:** CNPq e FAPERJ.

06.083

Papel das cavéolas sobre o relaxamento em artérias de animais obesos: diferenças entre artérias de condutância e de resistência e gêneros. Akamine EH[1], Wenceslau CF[2], Fortes ZB[1], Carvalho CRO[2], Rossoni LV[2] [1]ICB-USP- Farmacologia, [2]ICB-USP-Fisiologia e Biofísica

Introdução: Cavéolas são domínios da membrana plasmática ricos em colesterol. Tem sido proposto que redução do número de cavéolas possa promover disfunção vascular, prejudicando a sinalização do óxido nítrico e do cálcio. Acúmulo de gordura visceral está positivamente correlacionado com disfunção endotelial e com risco para doenças cardiovasculares. O objetivo do presente trabalho foi avaliar a participação das cavéolas no relaxamento dependente e independente do endotélio na obesidade. Uma vez que, a obesidade pode afetar diferentemente a função das artérias de condutância e de resistência assim como o gênero, o estudo foi conduzido em ambas as artérias em fêmeas e machos. **Métodos**: Ratas e ratos Wistar de 8 semanas de idade foram submetidos à dieta rica em gordura (60 %) durante 16 semanas. Após esse período, foram avaliados: o peso corpóreo e da gordura retroperitoneal e a reatividade vascular em anéis de aortas torácicas e de artérias mesentéricas de resistência (AMR) aos vasodilatadores acetilcolina (ACh, 10 pM-300 μM), dependente do endotélio, e nitroprussiato de sódio (NPS, 1 pM-3 μM), independente do endotélio. Para avaliar a participação das cavéolas na reatividade vascular, os anéis de aortas e AMR foram incubados com metil-beta-ciclodextrina (MCD, 10 mM), 1 h antes do início das curvas concentração-efeito. **Resultados**: Ratas (n=7) e ratos (n=7) que foram submetidos à dieta rica em gordura apresentaram aumento do peso (fêmeas: 19%, $p<0,001$; machos: 29%, $p<0,05$) e da gordura retroperitoneal (fêmeas: 309%, $p<0,01$; machos: 241%, $p<0,0001$) em comparação com os respectivos controles (CT). A ACh induziu relaxamento de igual magnitude em aortas e AMR de CT e de obesos (OB) de ambos os gêneros. A obesidade não modificou a reposta ao NPS em aortas de ambos os gêneros ou em AMR de fêmeas. Por outro lado, a sensibilidade (pD2) a esse vasodilatador foi reduzida em AMR de machos OB (CT, n=5: 7,6 ± 0,10 vs OB, n=5: 7,0 ± 0,17; $p<0,05$). Em aortas de animais CT, a MCD reduziu a pD2 à ACh em fêmeas (CT: 7,4 ± 0,09 vs CT/MCD: 7,1 ± 0,11; n=6; $p<0,05$) e a resposta máxima (Rmax) em machos (16%; n=6; $p<0,01$), não modificando esses parâmetros nas aortas provenientes dos animais OB de ambos os gêneros. Por sua vez, a MCD não modificou a resposta ao NPS em aortas de fêmeas CT e OB. Porém, verificamos aumento da pD2 ao NPS em aortas de machos CT (CT: 7,6 ± 0,02 vs CT/MCD: 7,9 ± 0,05; n=5; $p<0,001$) e OB (OB: 7,5 ± 0,11 vs OB/MCD: 8,2 ± 0,09; n=5; $p<0,001$) após incubação com MCD. Em AMR, a MCD reduziu a Rmax à ACh apenas em animais OB (fêmeas, n=5: 10%, $p<0,001$; machos, n=6: 12%, $p<0,001$). Em relação à vasodilatação induzida pelo NPS, em AMR, a MCD reduziu a Rmax somente em fêmeas OB (15%; n=5; $p<0,001$) e em machos CT (14%; n=5; $p<0,05$). **Conclusão**: Nossos resultados mostram que os efeitos deletérios da obesidade sobre o relaxamento aparecem primeiro em artérias de resistência dos machos. Além do mais, a participação das cavéolas no relaxamento induzido pela acetilcolina está na dependência da artéria e não do gênero avaliado. Por sua vez, no relaxamento induzido pelo NPS, a participação das cavéolas é dependente tanto da artéria como do gênero. Número CEEA: 005 nas fls. 28 do livro 2 Apoio Financeiro: FAPESP e CNPq

06.084

Níveis aumentados de GMPc mantêm agregação plaquetária normal em ratas grávidas espontaneamente hipertensas. Ognibene DT, Costa CA, Moss MB, Okinga A, Matsuura C, Soares de Moura R, Brunini T, Mendes Ribeiro AC, Resende AC UERJ - Farmacologia e Psicobiologia,

Introdução: A gravidez é caracterizada por importantes modificações hemodinâmicas, dentre as quais destacam-se a redução da resistência vascular periférica e a queda da pressão arterial. Alguns dados sugerem que o NO é o principal mediador na queda da resistência vascular, assim como, estudos com modelos animais e com humanos mostram que a síntese endotelial de NO torna-se elevada durante a gravidez. Entretanto, não há evidências a respeito do impacto da gravidez sobre a via L-arginina-NO em plaquetas ou sobre a própria função plaquetária em ratas espontaneamente hipertensas (SHR). **Métodos:** Os experimentos foram aprovados pelo Comitê de Ética em Pesquisa da UERJ (projeto 1730- CEP/HUPE - CAAE:0018.0.228.000-07). No presente estudo, foram utilizadas fêmeas Wistar normotensas em diestro (ND) ou grávidas (NG) e SHR em diestro (HD) ou grávidas (HG). Os experimentos foram realizados no plasma rico em plaquetas (PRP) dos diferentes grupos. Foi avaliado o influxo de L-[^{3}H]-arginina (100 µM), assim como a atividade basal da enzima óxido nítrico sintase (NOS) intraplaquetária, mensurada pela conversão de L-[^{3}H]-arginina (1 µM) a L-[^{3}H]-citrulina. A expressão da NOS endotelial e NOS induzível, assim como da fosfodiasterase 5 (PDE5) foram avaliadas por Western Blotting. A mensuração de GMPc intraplaquetário, em condição basal, foi realizada pelo método ELISA (Kit Kayman) e a agregação plaquetária foi induzida por ADP (12 µM). **Resultados:** Os dados obtidos demonstraram que o transporte de L-arginina em plaquetas foi mantido na gravidez em condições de normotensão (ND: 0.55±0.06/ NG: 0.60±0.01), enquanto a atividade da enzima NOS foi reduzida nessas condições (ND: 0.27±0.02/ NG: 0.15±0.01). As expressões das isoformas eNOS (ND: 2.39±0.3/ NG: 1.53±0.16) e iNOS (ND: 5.84±0.92/ NG: 2.58±0.12) foram reduzidas na gestação normal. Embora seja aparente uma menor atividade da via de produção de NO, a produção de GMPc foi mantida (ND: 0.16±0.02/ NG: 0.21±0.01), assim como a expressão de PDE5 (ND: 6.25±0.16/ NG: 4.35±0.59) e a agregação plaquetária (ND: 46.28±3.65/ NG: 43.66±4.67). Na gravidez acompanhada por hipertensão, o transporte de L-arginina foi reduzido em relação ao grupo de ratas SHR não grávidas (HD: 0.68±0.07/ HG: 0.43±0.05), da mesma forma que a atividade da NOS em plaquetas foi reduzida nessas condições (HD: 0.28±0.01/ HG: 0.18±0.03). Embora a expressão da eNOS tenha sido mantida na gravidez (HD: 1.28±0.13/ HG: 1.56±0.17), a atividade reduzida da enzima pode estar relacionada, em parte, à diminuição da expressão da NOS induzível (HD: 6.72±0.53/ HG: 3.26±0.16). Apesar disso, foi observada uma maior produção de GMPc (HD: 0.26±0.03/ HG: 0.35±0.05) e a manutenção da agregação plaquetária na gravidez em condições de hipertensão (HD: 42.12±4.19/ HG: 38.62±3.27). Este dado correlaciona com uma redução da expressão de PDE5, enzima responsável pela sua metabolização (HD: 10.17±0.97/ HG: 6.44±0.26). **Conclusão:** O presente estudo sugere que a manutenção da agregação plaquetária em ratas SHR grávidas, apesar da menor atividade da via L-arginina-NO intraplaquetária, pode estar relacionada à redução da expressão de PDE5 e ao consequente aumento da produção de GMPc. **Apoio Financeiro:** CNPq e FAPERJ.

06.085

Metabolic and vascular reactivity changes in SHR+MSG: is that an experimental model of metabolic syndrome? Dos Santos RA, Ceravolo GS, Oliveira MA, Tostes RCA, Fortes ZB, Carvalho MHC USP - Farmacologia

Introduction: Metabolic Syndrome consists of insulin resistance as a primary defect associated with obesity, hypertension, compensatory hyperinsulinemia and dyslipidemia. The several interventions during early postnatal period can modify endocrine functions or metabolic processes later in life. Objective: We studied the metabolic characteristics and the vascular reactivity to angiotensin II (Ang II) in Spontaneously Hypertensive Rat (SHR) treated with monosodium glutamate (MSG). **Methods and Results**: All protocols involving animals were done in accordance with the guidelines of the institutional (ICB-USP) Committee for Ethics in Animal Experimentation (registration # 98, page 5, book 2). Neonatal male SHR received subcutaneous injections of MSG (4 g/kg, MSG group) or saline solution (control group) from day 2 to 6 of life. After eighteen weeks, the arterial pressure (PA) was measured by tail plethysmography and both groups maintained elevated levels of PA (Saline: 175.7±6.88 vs. MSG: 170±6.4 mmHg, n=7). The MSG group presented obesity, characterized by Lee Index (Saline: 29.52±0.15 vs. MSG: 30.267±0.13, n=5, $p<0.05$) and visceral [retroperitoneal (Saline: 0.965±0.05 vs. 1.567±0.08 g/100g of weight, n=6, $p<0.05$) and periepididimal (Saline: 0.86±0.024 vs. 1.102±0.033 g/100g of weight, n=6, $p<0.05$)] fat pad. The glucose disappearance constant (*KITT*), characterized by insulin tolerance test (ITT), was decreased in MSG group when compared with control group (Saline: 3.519±0.31 vs. MSG: 2.157±0.25, n=6, $p<0.05$). These data were confirmed by increased levels of serum insulin, determined by enzyme immuno assay (Saline: 3.79±0.2 vs. MSG: 5.45±0.37 ng/mL, n=6, $p<0.05$). Serum levels of triglycerides were increased in MSG group (Saline: 23.6±1.69 vs. MSG: 38.5 vs. 4.63 mg/mL, n=5, $p<0.05$). In isolated arteriolar mesenteric bed of MSG group the dose-response curve to Ang II presented increased in maximal response (Rmax) in comparison with control group (Rmax, Saline: 82.93±6.71 (n=8) vs. MSG: 127.5±11.34 mmHg, n=5, $p<0.01$). In mesenteric arterioles of MSG group, Ang II stimulation promoted an increase in reactive oxygen species (ROS) generation in comparison with control group (Saline: 6.198±0.11 vs. MSG: 7.913±0.30 arbitrary units, n=4, $p<0.05$). **Conclusion:** SHR treated with MSG maintains the hypertension profile; it develops obesity plus increased levels of triglycerides and insulin resistance. Taken altogether, these characteristics allow us to classify SHR+MSG as an experimental model of metabolic syndrome. The increased vascular reactivity to Ang II in resistance vessels of MSG group might be associated to a ROS generation increase. **Financial support**: FAPESP and CNPq.

06.086

Cardiotoxic effect of bothropstoxin I and II: antagonism by heparin. Ricardo HD[1], Machado MM[2], Martins V[1], Cons, BL[3], Fernandes FFA[1], Strauch MA[1], Cintra ACO[3], Melo PA[1] [1]UFRJ - Farmacologia Básica e Clínica, [2]FMC/UFRJ - Farmácia / Farmacologia Básica e Clínica, [3]FCFRP-USP - Departamento de Análises Clínicas Toxicológicas e Bromatológicas

Introduction: Investigated the cardiotoxic activity in vitro of Bothropstoxin toxins I and II (BthTX I and II) of the venom of Bothrops jararacussu and the effect of heparin to antagonize the cardiotoxic effects on isolated rat hearts. **Methods**: cardiotoxicity was evaluated in a Langendorff preparation with the heart of adult rats and bathed continuously perfused (2-5 mL / min) with Ringer´s solution at 37 ° C. The cardiac voltage was recorded continuously with a transducer, connected to a Grass 7D Polygraph and the electrocardiogram (ECG). Difference statistics with p <00.5. **Results:** In preparation of the isolated heart, the toxins in the concentration (10 mg / ml) induced a negative inotropic effect gradually over time. The BthTX I (10 mg / ml) decreased to 0% strain the heart after 60 min, a 20% increase in pressure of perfusãoe reaching 57.9% at 60 minutes, decrease of QRS amplitude to 83.6 % compared to the control, with changes in ECG waves. The BthTX II (10 mg / ml) decreased to 0% strain the heart after 1 min, the increase (600%) perfusion pressure, increase (130.5%) PR interval, QRS amplitude decreasing (7.6% of control), with changes in ECG waves. The addition of heparin 100 mg / ml decreased the cardiotoxic effects on cardiac voltage reaching 100% inhibition with 100 mg / ml (not BthTX II which was 48.3%), reversed the changes in perfusion pressure and the waves of the electrocardiogram. **Discussion**: The 10 mg / mL BthTX I induced the same effect observed with the crude venom on record since the BthTX II produced only depression of cardiac stress. Both effects were completely neutralized by the same concentrations of heparin. The heart was then removed from the Langendorff apparatus and the ventricles were cut and incubated in 1% triphenyl tetrazolium chloride (TTC) at 37 °C (pH 7.4) for 4 min. At the end of the incubation period, the heart was sliced and placed in formaldehyde solution with the objective of improving the contrast of color. **Conclusion**: Heparin was able to completely antagonize the cardiac arrest, changes in the electrocardiogram and the damage caused by toxins BthTX I and II in the preparation of isolated heart. **Financial Support by**: CAPES, CNPq, PRONEX e FAPERJ. Número da licença do Comitê de Ética: DFBCICB 026

06.087

Alterations in rat atrial rate and contractile force caused by *Bothrops jararacussu* snake venom. Rodrigues MAP, Dias L, Smaal A, Rennó AL, da Silva DA, Hyslop S UNICAMP - Farmacologia

Introduction: *Bothrops* venoms produce systemic effects such as coagulopathy, systemic hemorrhage, hypotension, cardiovascular shock and renal failure, although little is known about the direct cardiac actions of these venoms. In this work, we investigated the alterations caused by *Bothrops jararacussu* venom in rat isolated right atria. **Methods:** Male Wistar rats (300-400 g) were anesthetized with isoflurane, exsanguinated and the hearts quickly removed and placed in modified Krebs-Henseleit solution (KH; composition, in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 0.45; $NaHCO_3$, 25; KH_2PO_4, 1.03; D-glucose, 11.1; ascorbic acid, 0.14. The bath solution was maintained at 37°C and continuously gassed with 5% CO_2 and 95% O_2. The atria were dissected and mounted (resting tension: 1 g) in 10 ml organ baths containing KH. Changes in contractile force were measured isometrically and atrial rate was calculated from this record. Creatine kinase and creatine kinase MB (CK, CK-MB) levels were measured using commercial kits (LabLabor, São Paulo, SP, Brazil). At the end of the experiments, the atria were fixed and processed for histological analysis. The results (mean±SEM) were compared statistically by using Student´s *t*-test or ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. These experiments were approved by the institutional Committee for Ethics in Animal Research (CEEA/UNICAMP, protocol no.1433-1). **Results:** Venom (0.025, 0.05, 0.1 and 0.2 mg/ml; n=8) caused a progressive decrease in contractile force, with complete reduction (from 0.18±0.02 g to 0.00±0.00 g; $p<0.05$) after a 60 min incubation with 0.2 mg/ml; the responses to the other three venom concentrations were not significantly different from this. Atrial rate decreased significantly with 0.2 mg of venom/ml compared to control preparations incubated with KH alone. There were significant ($p<0.05$) increases in CK (from 6.7±0.8 to 24.1±1.2 U/ml for 0.025 mg/ml, 4.1±1.6 to 32.5±5.9 U/ml for 0.05 mg/ml, 17.7±2.2 to 135.8±10.1 U/ml for 0.1 mg/ml, and 24.5±5.9 to 251.2±44.5 U/ml for 0.2 mg/ml; n=5 each) and CK-MB (from 0.6±0.04 to 2.2±0.3 U/ml for 0.025 mg/ml, 1.4±0.5 to 2.6±1.0 U/ml for 0,5 mg/ml, 0.6±0.3 to 4.2±0.3 U/ml for 0.1 mg/ml, and 0.7±0.2 to 17.0±2.9 U/mL for 0.2 mg/ml; n=5 each) after 60 min. Histological analysis showed extensive myonecrosis with all venom concentrations. Dialysis of the venom (24 h, 4°C; membrane nominal cut-off ~2 kDa) did not affect the decrease in contractile force compared with non-dialyzed venom (0.2 mg/ml) whereas heating (100°C, 10 min) abolished this decrease. **Discussion:** These results indicate that *B. jararacussu* venom has direct negative chronotropic and inotropic effects in isolated atria. This decrease in contractile force is mediated by heat-labile components in the venom and may involve myonecrosis, as indicated by histological analysis and the release of marker enzymes. **Financial support:** CAPES, CNPq, FAPESP

06.088

On the mechanisms involved in the negative inotropism and chronotropism caused by *Bothrops jararacussu* snake venom in rat isolated right atria. Rodrigues MAP, Dias L, Smaal A, Rennó AL, da Silva DA, Hyslop S UNICAMP - Farmacologia

Introduction: Envenoming by *Bothrops jararacussu* leads to edema, hemorrhage and necrosis. Hypotension and cardiovascular shock are common complications after bites by this species. *Bothrops jararacussu* venom causes negative inotropism and chronotropism in rat isolated right atria. In this work, we examined the possible mechanisms involved in this response and the ability of bothropic antivenom to neutralize these effects. **Methods:** Male Wistar rats (300-400 g) were anesthetized with isoflurane, exsanguinated and the hearts quickly removed and placed in modified Krebs-Henseleit solution (KH; composition in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 0.45; $NaHCO_3$, 25; KH_2PO_4, 1.03; D-glucose, 11.1; ascorbic acid, 0.14. The bath solution was maintained at 37°C and continuously gassed with 5% CO_2 and 95% O_2. The atria were dissected free and mounted under a resting tension of 1 g in 20 ml organ baths containing KH. Changes in contractile force were measured isometrically (PowerLab recording system, ADInstruments) and atrial rate (beats/min) was calculated from this record. A single concentration of venom (0.2mg/ml) was tested and, when required, antagonists (cimetidine - 10μM, indomethacin - 5.6mM, L-NAME - 300mM, pyrilamine - 1μM, atropine - 10μM and propranolol - 5μM) were preincubated with the preparations for 10min before venom addition. In some experiments, neutralization by bothropic antivenom was tested by preincubating venom with antivenom (37°C, 60 min) at the ratio recommended for therapeutic use (1ml of antivenom neutralizes 5mg of venom; Instituto Butantan, São Paulo) prior to testing, while in other cases, antivenom (0.8ml) was added 1min after venom. The results (mean±SEM) were compared statistically by using ANOVA followed by the Tukey-Kramer test. A value of $p<0.05$ indicated significance. These experiments were approved by the institutional Committee for Ethics in Animal Research (CEEA/UNICAMP, protocol no.1433-1). **Results:** Venom (0.2mg/ml; n=6-8) caused a progressive decrease in contractile force (from 0.18±0.02 to 0.00±0.00 g after 60min; $p<0.05$) and atrial rate (from 298±4.1 to 119±31 bpm after 60min; $p<0.05$). None of these alterations were significantly affected by indomethacin (a non-selective cyclooxygenase inhibitor), L-NAME (a non-selective inhibitor of nitric oxide synthase) or pyrilamine (a histamine H_1 receptor antagonist)**.** In contrast, cimetidine (a histamine H_2 receptor antagonist) completely prevented the decrease in heart rate, without significantly affecting the decrease in contractile force. Addition of antivenom to the organ bath after venom attenuated the decrease in contractile force (before: 0.21±0.03 g; after: 0.16±0.01 g; $p<0.05$) and abolished the decrease in atrial rate (before: 339±4 bpm; after: 354±13 bpm; $p<0.05$); less effective neutralization was seen when venom and antivenom were pre-incubated before testing. **Discussion:** The negative inotropic and chronotropic effects of *B. jararacussu* venom are not mediated by arachidonic acid metabolites, nitric oxide or activation of H_1 histamine receptors. However, the negative inotropism may involve H_2 histamine receptors. The neutralization by antivenom indicates that the therapeutically recommended venom:antivenom ratio can protect against venom-induced atrial alterations. **Financial support:** CAPES, CNPq, FAPESP.

06.089

Atividade vasodilatadora de LASSBio-1020: participação da via óxido nítrico/GMP_c.

Louback LS[1], Lima LM[1], Monge A[2], Barreiro EJ[1], Miranda ALP[1] [1]LASSBio-FF-UFRJ - Fármacos, [2]CIFA-UNAV

Introdução: Doenças cardiovasculares são as principais causas de mortalidade e morbidade no mundo ocidental, diante deste quadro a busca por novos protótipos vasoativos se torna cada vez mais relevante. Derivados *N*-acilidrazônicos já foram descritos por sua atividade vasodilatadora, como LASSBio-294 e análogos (Silva et al., *Bioorganic & Medicinal Chemistry, 13, 3431,* 2005), objetivo deste trabalho foi investigar e contribuir com o estudo de possíveis mecanismos de ação de LASSBio-1020, um derivado *N*-acilidrazônico quinoxalínico. **Metodologia**: Foram utilizados aorta de ratos wistar (250-300g), com protocolo experimental submetido à comissão CEUA-UFRJ. O efeito vasodilatador foi avaliado sobre anéis com e sem endodélio funcional pré-contraídos com fenilefrina (10 µM) e KCl (80 mM). Inibidores de guanilato ciclase (ODQ), adenilato ciclase (DDA), óxido nítrico sintase (L-NAME), bem como antagonista muscarínico (atropina), foram empregados no estudo do mecanismo de ação de LASSBio-1020, foi observada a interferência deles na atividade de LASSBio-1020 sobre anéis com endotélio pré contraídos com fenilefrina. **Resultados**: LASSBio-1020 (100uM) foi capaz de relaxar anéis de aorta pré-contraídos com fenilefrina e KCl (65,30% e 70,77%, respectivamente). O efeito vasodilatador de LASSBio-1020 se mostrou parcialmente dependente do endotélio (efeito máximo de 71,88% com endotélio e 29,97% sem endotélio funcional). A pré-incubação dos anéis com endotélio com DDA (58,28%) não alterou o efeito de LASSBio-1020, enquanto que ODQ (23,34%) e L-NAME (34,95%) bloqueiam a atividade vasodilatadora endotélio depedendente de LASSBio-1020. Além disso, o antagonista muscarínico atropina não reverte a vasodilatação provocada por LASSBio-1020. Discussão: A perda parcial do efeito de LASSBio-1020, sobre a contração por fenilefrina, pela retirada do endotélio indica a participação de fatores relaxantes derivados do endotélio, este dado juntamente com a vasodilatação de anéis pré-contraídos com KCl exclui antagonismo adrenérgico. O óxido nítrico produzido pelo endotélio se difunde até a célula muscular lisa onde ativa a guanilato ciclase solúvel que produz GMPc, este ativa a proteína cinase dependente de GMPc (PKC) provocando relaxamento destas células. Esta via foi investigada como possível mecanismo de ação de LASSBio-1020. Os resultados mostraram o derivado estudado depende de NO e GMPc, já que L-NAME e ODQ bloqueiam seu efeito. A ativação de receptores muscarínicos estimula a produção de NO no endotélio, mas este não é o mecanismo de LASSBio-1020, já que a atropina não reverteu seu efeito. Estes resultados permitem concluir que LASSBio-1020 provoca vasodilatação em aorta de rato de forma dependente da via NO/GMPc, sem atuar nos receptores muscarínicos. Como prováveis mecanismos de LASSBio-1020 restam outra possibiliades como: estímulo da produção de NO, modulação dependente de NO da GCs, inibição de fosfodiesterases que degradem GMPc. Apoio: CNPq, FAPERJ, FUJB, IM-INOFAR, INCT.

06.090

Relationship between nitric oxide consumption and plasma hemoglobin in preeclampsia. Palei ACT[1], Sandrim VC[1], Metzger IF[1], Cavalli RC[2], Tanus-Santos JE[1]
[1]FMRP-USP - Farmacologia, [2]FMRP-USP - Ginecologia e Obstetrícia

Introduction: Preeclampsia pregnant (PE) present lower bioavailability of nitric oxide (NO) compared with healthy pregnant. Many factors may contribute to reduction observed, including ADMA levels, anti-angiogenic factors, genetic variation and oxidative stress. Another factor that impairs NO actions, and which evidences have been rising, it is NO consumption by cell-free hemoglobin. This mechanism prevents NO diffusion from plasma to smooth muscle and consequently decreases vasodilation. None study evaluated this parameter in PE. Therefore, in the present study we hypothesized that higher concentration of plasma hemoglobin and NO consumption would be found in PE compared with those found in HP. Moreover, we hypothesized that a direct relationship exists between cell-free hemoglobin levels (plasma) and NO consumption, which may interfere in NO bioavailability. **Methods:** A total of 82 pregnant (38 HP and 44 PE) were enrolled in the Department of Obstetrics and Gynecology, FMRP. Whole blood (WB) and plasma nitrite measurement were analyzed using an ozone-based chemiluminescence assay (NO analyzer). To measure NO consumption by plasma, we used a solution of DETA NONOate that produced a steady-state NO signal of 50-70 mV. Then we injected each plasma sample separately and observed a decay of baseline NO signal generating an AUC that was compared between groups. This study was approved by the local Ethics Review Board (CEP HCRP n° 4682/2006). **Results:** WB and plasma nitrite levels were significantly lower in PE (-48% and -39%, $P<0.05$) compared with HP. Conversely, plasma from PE consumes 63% more NO ($P=0.003$) and present hemoglobin level 53% higher than HP ($P=0.01$; 55.1±5.6 versus 36.2±3.7 µg/ml). Taking into consideration all pregnant women, we found positive correlations between plasma hemoglobin and NO consumption (Fig. 3; $P<0.0001$, Pearson $r=0.61$); and an inverse correlation between NO consumption and WB and plasma nitrite (respectively, $P=0.02$, $r=-0.32$; $P=0.01$, $r=-0.34$) and between plasma hemoglobin and WB and plasma nitrite (respectively, $P=0.03$, $r=-0.36$; $P=0.01$, $r=-0.38$). **Discussion:** We found that cell-free hemoglobin levels and NO consumption are higher in preeclampsia compared with HP. Moreover, a positive correlation found between these two parameters may suggest that cell-free hemoglobin is an important molecule that regulates NO consumption by the plasma, altering its bioavailability. Therefore, we suggest that a protocol based in our hypothesis be evaluated in other groups of PE and mainly in HELLP pregnant. The reproduction of our results may firstly suggest the plasmatic hemoglobin measurement as a biomarker and consequently direct novel therapeutic approaches in PE/HELLP pregnant, including inhaled nitric oxide, sodium nitrite, phosphodiesterase 5-inhibitors, among others.

06.091

The influence of 4-week physical training on platelet aggregation of rats. Monteiro PF[1], Prada Morganti R[1], Valgas da Silva CP[1], Gómez-Campos RA[2], Priviero FBM[1], Zanesco A[2], Antunes E[1] [1]FCM-UNICAMP - Farmacologia, [2]UNESP - Educação Física

Introduction: A healthy lifestyle has been strongly associated with the practice of regular physical activity. Evidence has shown that physically active subjects have more longevity with reduction of morbidity and mortality. Physical exercise prevents or reduces the deleterious effects of pathological conditions, such as arterial hypertension, coronary artery disease, atherosclerosis, diabetes mellitus, osteoporosis, Parkinson´s disease, and Alzheimer disease. (Zanesco and Antunes, 2007). Moreover, epidemiological and clinical findings clearly show that a physically active life style lead to lower risks of thrombotic events by decreasing the platelet sensitivity and aggregability (Lakka et al, 1994). However, the influence of regular physical exercise in the platelet functions has been poorly investigated. Therefore, the aim of this work was to evaluate the effects of 4-week regular physical exercise in the rat washed platelet aggregation *in vitro*. **Methods**: Male Wistar rats (250-280 g) were submitted to 4-week run training program, 5 days/week, 60 min/day, at a speed of 1.2 Km/h. Arterial blood was collected in the presence of the anticoagulant sodium citrate 3.8%, at 48 h after the last training session. The collected blood was centrifuged (300 g, 20ºC, 15 min) and the platelet-rich plasma (PRP) was added to washing buffer and centrifuged at 800 g, 20ºC, for 13 min. The pellet was resuspended in Krebs-Ringer, and platelet number was adjusted to $1.2x10^8$ plat/mL in presence of 1 mM $CaCl_2$. Aggregation assays were performed using an light transmission aggregometer and platelets were activated with either thrombin (100-300 mU/mL) or ADP (10-50 µM). **Results**: After 4-weeks physical training, the platelet aggregation induced by thrombin was significantly lower (36.5±6.8%, 63.8±5.3% and 68.4±3% for 100, 200 and 300 mU/mL thrombin, respectively) compared with the sedentary group (73.8±3.35%, 89.5±3.5% and 89.8±2.8% for 100, 200 and 300 mU/mL thrombin, respectively; n=4-5). ADP-induced platelet aggregation remained unchanged in all rats (n= 4-5). **Discussion**: Our preliminary data showed that 4-week physical exercise attenuates the *in vitro* thrombin-induced platelet aggregation suggesting that physical exercise might be beneficial for preventing atherosclerotic events. **References:** Zanesco A, Antunes E. Pharmacol. Ther., 2007, 114:307-317. Lakka TA, et al. N Engl J Med, 1994, 330:1549-1554

06.092

Cardiotoxic effects induced by crotalid snake venoms. Ricardo HD[1], Strauch MA[1], Machado MM[2], Martins VV[1], Cons, BL[1], Dip EC[4], Melo PA[1] [1]UFRJ - Farmacologia Básica e Clínica, [2]FMC / UFRJ - Farmácia / Farmacologia Básica e Clínica

Introdution: Snake venoms induce local damage such as hemorrhage, edema and myonecrosis as a consequence of the interaction of their components with tissues (Mebs and Ownby, Pharmacol. Ther. 48: 223, 1990). Some crotalid venoms induce myonecrosis by the direct effect of components named myotoxins, which are described to act specifically on skeletal muscle sarcolemma. We investigated the in vitro cardiotoxic activity of B. jararacussu, B. leucurus, B. asper and Agkistrodon contortrix laticinctus crude venom on isolated rat heart. **Methods**: The venoms were obtained from different sources: i) B. jararacussu, B.leucurus from CEPLAC-BA, Brazil, ii) B. asper from Clodomiro Picado Institute of the University of Costa Rica and iii) Agkistrodon contortrix laticinctus from Oklahoma State University, USA). Cardiotoxicity was evaluated on the Langendorff preparation by using adult Wistar rat heart, which was continuously perfused (2-5 mL/min) with Physiological Saline Solution at 370C. Heart tension was recorded continuously by a transducer coupled in a 7D Grass Polygraph, as well as by an electrocardiogram (EKG). **Results:** In the heart preparation, venoms at different concentrations (1 -10 5g/mL) induced progressive negative inotropic effect, that was time- and concentration-dependent. The crude venoms (10 5g/mL) induced a stoppage and decreased to 0% the heart tension, increased the perfusion pressure, PR interval and QRS amplitude, with changes of the EKG waves. The heart was removed from the Langendorff apparatus and the ventricles were sliced and incubated in 1% triphenyl tetrazolium chloride (TTC) at 370C (pH 7.4) for 4 min. At the end of the incubation period, the heart slice was placed in formaldehyde solution, which enhanced the color contrast. The normal myocardium was stained and the damaged area did not stain and showed a pale appearance. **Conclusions** : Our data showed that all the crude crotalid snake venoms that we tested presented cardiotoxic effects, by depressing the mechanical and electrical patterns of the rat isolated heart. These effects are corroborated by morphological changes observed with the TTC staining and the EKG waves patterns. **Financial Support**: CAPES; CNPq; PRONEX; FAPERJ.

06.093

Estudo da atividade antiagregante plaquetária de derivados *N*-metilacilidrazônicos análogos ao LASSBio-785. Fumian MM[1], Kummerle AE[1], Fraga CAM[1], Brito FCF[2], Barreiro EJ[1], Miranda ALP[1] [1]FF-LASSBio-UFRJ - Fármacos, [2]UFRJ - Farmacologia Básica e Clínica

Introdução: Inserido em uma linha de pesquisa em Química Medicinal, o Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio) possui um longo histórico de trabalhos científicos que descrevem o planejamento, a síntese e a avaliação farmacológica de derivados *N*-acilidrazônicos (NAH) (Barreiro et al, Quim. Nova 25, 129, 2002). Nesse sentido, o composto LASSBio-294 foi sintetizado, apresentando o grupamento farmacofórico *N*-tienilacilidrazona. Foram observadas propriedades farmacológicas anti-inflamatórias e cardiovasculares relevantes para o composto LASSBio-294 (Sudo *et al*, Br. J. Pharmacol 134, 603, 2001). Na busca pela otimização do composto LASSBio-294, foi desenvolvida uma nova série de derivados tienilacilidrazônicos dando origem a LASSBio-785 que apresentou potente atividade inibitória da agregação plaquetária. (Brito *et al*., SBFTE 2005). Tendo em vista estes resultados, foi proposta a síntese de uma nova série de derivados *N*-metilacilidrazona, análoga ao derivado LASSBio-785, visando a otimização de seu perfil farmacológico. Considerando o efeito antiplaquetário já observado para outros derivados NAH, este trabalho teve como objetivo a avaliação da atividade anti agregante plaquetária desta nova série, buscando elucidar sua atividade sobre a cascata do ácido araquidônico. **Métodos:** A atividade antiagregante plaquetária foi avaliada *in vitro*, pelo método turbidimétrico, em PRP citratado de coelho, na agregação induzida por AA (200 µM), colágeno (5µg/mL) e ADP (5 µM). Os compostos *N*-metilacilidrazônicos foram testados na concentração de triagem farmacológica de 100 µM (em DMSO). Para a realização da curva concentração resposta, foram utilizadas diferentes concentrações dos compostos em estudo. **Resultados:** Todos os dez derivados estudados apresentaram uma inibição de 100% da agregação plaquetária frente ao AA. Frente ao colágeno, os derivados LASSBio-1004, 1455, 1373, 1374, 1375, 1376, 1377, 1028 e 1289 apresentaram uma inibição significativa da agregação plaquetária. Na agregação plaquetária induzida por ADP nenhum dos derivados apresentou atividade inibitória significativa. A partir destes resultados, foram selecionados 5 derivados para o estudo da curva concentração-resposta frente ao AA e ao colágeno. Os derivados estudados apresentaram uma IC_{50} inferior a 10µM frente aos dois agonistas, o que ressalta o potente perfil antiplaquetário da série. **Conclusão:** Os novos derivados *N*-metilacilidrazônicos apresentaram importante atividade antiagregante plaquetária frente ao ácido araquidônico e ao colágeno, sem interferir na agregação plaquetária induzida por ADP, reproduzindo os resultados previamente observados para o composto protótipo, LASSBio 785. As modificações estruturais introduzidas na série não alteraram negativamente a atividade antiagregante do composto LASSBio-785, confirmando o grupamento *N*-metilacilidrazônico como um farmacóforo para a atividade antiagregante plaquetária. **Apoio Financeiro**: FAPERJ, FUJB, PRONEX, CNPq, IM-INOFAR, INCT.

06.094

Nitric oxide and potassium channels mediate GM1 ganglioside-induced vasorelaxation. Furian AF[1], Rattmann YD[1], Oliveira MS[1], Royes LF[3], Marques MCA[2], Santos ARS[3], Mello CF[1] [1]UFSM - Fisiologia e Farmacologia, [2]UFPR - Farmacologia, [3]UFSC - Ciências Fisiológicas

Introduction*:* Monosialoganglioside (GM1) is a glycosphingolipid present in most cell membranes that displays antioxidant and neuroprotective properties. It has been recently described that GM1 induces pial vessel vasodilation and increases NOx content in cerebral cortex, which are fully prevented by the nitric oxide synthase inhibitor L-NAME. However, it is not known whether GM1 relaxes larger vessels, as well as the mechanisms by which GM1 causes vasorelaxation. **Methods*:*** In this study we demonstrate that GM1 (10, 30, 100, 300 μM, 1 and 3 mM) induces vascular relaxation determined by isometric tension studies in rat mesenteric artery rings contracted with 1 μM phenylephrine. The procedures were approved by the Research Ethics Board of the UFPR (number 138). **Results:** The vasorelaxation induced by GM1 was abolished by endothelium removal, by incubation with L-NAME (1 μM) and partially inhibited by the blockade of potassium channels by 1 mM tetraethylammonium (TEA), 10 μM glibenclamide (GLB), by the soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-alpha]quinoxalin-1-one (10 μM ODQ), and by 50 nM charybdotoxin (ChTX), a blocker of large and intermediate conductance calcium-activated potassium channels. Moreover, GM1-induced relaxation was not affected by apamin (50 nM), a small conductance calcium-activated potassium channel blocker. **Discussion:** The results indicate that direct and indirect nitric oxide pathways play a pivotal role in vasorelaxation induced by GM1, which is mediated mainly by potassium channels activation. We suggest that vasodilation may underlie some of the biological effects of exogenous GM1 ganglioside. **Financial support**: CAPES, CNPq, TRB Pharma.

06.095

L-carnitine supplementation improves endothelium-dependent relaxation in mesenteric artery of exercised rats fed with hyperlipidic diet. Priviero FBM[1], Rojas-Moscoso JA[1], Gómez-Campos RA2[3], Valgas da Silva, CP[1], Antunes E[1], Zanesco A[2] [1]UNICAMP - Farmacologia, [2]UNESP - Educação Física

Background: Physical exercise is recommended for weight control but obese people who start an exercise program, usually quit it due to their reduced aerobic capacity. L-carnitine (L-Car) is a supplement that regulates body composition associated with lipid metabolism. Supplementation with L-Car improves tolerance to exercise in non-obese sedentary and trained rats. Furthermore, supplementations with fat burners are often taken to speed up the losing weight process. However, no studies exist investigating the effect of L-Car intake associated with dynamic exercise on the vascular responsiveness of obese rats. Thus, the aim of this work was to investigate the effect of L-Car supplementation on the contractile and relaxant responses of the superior mesenteric artery of sedentary and trained rats fed with hyperlipidic diet (HD). **Methods:** Male Wistar rats were fed with HD and divided into four groups: sedentary (SDHD), sedentary supplemented with L-Car (HDSD L-Car), exercised (HDEX) and exercised supplemented with L-Car (HDEX L-Car). Animals were trained in a treadmill with an intensity of 70- 80% of maximal oxygen consumption, in sessions of 60 minutes, 5 days a week. Run training was performed simultaneously to L-Car intake (0.2 g/kg daily, given in the drinking water) for 4 weeks. Concentration-response curves were obtained for acetylcholine (ACh), sodium nitroprusside (SNP) and phenylephrine (PE) in isolated superior mesenteric rings. **Results:** Experimental procedures were approved by the Animal Care and Use Co mmittee of the State University of Campinas (Protocol # 1307-1). Oral supplementation with L-Car provoked a significant reduction in body weight gain in sedentary rats (∆: 137 ± 4 g) compared with SDHD group (∆: 188 ± 10 g). However, L-Car supplementation did not affect the body weight gain in trained rats. In mesenteric rings, the potency (pEC_{50}) of ACh was reduced in HDEX rats (7.32 ± 0.05) compared with the HDSD group (7.65 ± 0.04). Nonetheless, L-Car supplementation increased the pEC_{50} of ACh in HDEX L-Car group (7.89 ± 0.03) but not in the HDSD L-Car (7.67 ± 0.06). Maximal responses (E_{MAX}) to ACh were not changed either by the physical exercise or L-Car supplementation. Neither the pEC_{50} nor the E_{MAX} of SNP and PE were changed in all studied groups. **Discussion:** L-Car supplementation reduced the body weight gain of sedentary rats fed with hyperlipidic diet with no impairment of the vascular reactivity. Additionally, in exercised rats fed with hyperlipidic diet, although L-Car supplementation did not reduce the body weight gain, L-Car improved the endothelium-dependent relaxation of the mesenteric artery. Financial Support: **FAPESP**

06.096

Angiotensin II induced reactive oxygen species generation in spontaneous hypertensive rats: role of protein disulfide isomerase. Dias AA[1], Ceravolo GS[1], Camargo LL[1], Androwiki ACD[1], Fernandes DC[2], Carvalho MHC[1], Laurindo FRM[2], Janiszewski M[1], Lopes LR[1] [1]ICB-USP Pharmacology, [2]InCor-HC-FMUSP - Vascular Biology

Introdution: NADPH oxidase is a major source of ROS in the cardiovascular system and Angiotensin II (AII)-induced ROS production contributes to the vascular alterations associated with hypertension. We recently demonstrated that PDI regulates ROS production in AII stimulated vascular smooth muscle cells (Janiszewski et al., 2005). Hypertension is associated to an increase in ROS generation which contributes to the vascular alterations observed in this pathology. The goal of the present study was to investigate the role of the PDI in ROS production and vascular reactivity in hypertensive rats. **Material and Methods:** ROS production in aortic rings from rats W and SHR was assessed by analysis of DHE-derived oxidation products by HPLC. PDI and $p47^{phox}$ expression in aortic segments was determied by immunohistochemstry and Western blot, respectively. The vascular reactivity to AII was evaluated in isolated aorta from Wistar (W) and SHR rats with (E+) or without (E-) endothelium. Superoxide dismutase (SOD; 150 U/ml) catalase (CAT; 300 U/ml) and PDI inhibitor, bacitracin (BAC; 1 mM) were used in some experiments. The protocol here applied has been approved by the Committee for Ethics and Animal Research /157. **Results:** PDI is expression was increased in SHR as compared to W. Increased ROS production (EOH:0,09±0,03 vs. 0,3±0,05; ET: 0,06±0,06 vs. 1,1±0,03) and p47phox expression (0,06±0,04 vs. 1,5±0,1) was observed in aorta from SHR rats as compared to W rats. SHR aortas showed a reduced response to AII in comparison to W, which was not affected by endothelium removal (0,4±0,04 vs 0,2±0,02; 1,5±0,1 vs. 0,6±0,01). In aortic E+ rings from W rats, the incubation with BAC, SOD, SOD+BAC, CAT, CAT+BAC and CAT+SOD decreased the contractile response to AII (0,4±0,04 vs. 0,2±0,02; 0,02±0,02; 0,3±0,05; 0,3±0,04; 0,2±0,02; 0,2±0,03). The same effect was observed after endothelium removal. On the other hand, in aortic E+ rings from SHR, the incubation with BAC, SOD, SOD+BAC and CAT+SOD had no effect on the contractile response to AII (0,2±0,02 vs. 0,2±0,02; 0,2±0,02; 0,18±0,02; 0,26±0,02). However, the incubation with CAT unexpectedly increased the contractile response to AII in SHR (0,2±0,02 vs. 0,7±0,04). This effect was reversed by the association of CAT+BAC and CAT+SOD (0,2±0,02 vs. 0,1±0,01; 0,02±0,02). Incubations with BAC, SOD in aortas E- rats had no effect on the contractile response to AII in SHR (0,6±0,01 vs. 0,5±0,01; 0,5±0,01). Vascular reactivity to AII in SHR E- was reduced only by association of SOD+BAC (0,6±0,01 vs. 0,2±0,01). **Discussion:** Altogether, our results show that PDI plays a role in ROS generation and vascular reactivity to AII in W but not in SHR animals. This effect is possibly related to the increased ROS generation, NADPH oxidase activity and PDI expression observed in these animals. Therefore, PDI could be contributing to the oxidative stress observed in SHR aortas. The fact that AII reactivity in SHR was decreased by the association of CAT+BAC indicates an important role of H_2O_2 in the maintenance of vascular tone in these animals. **Financial support:** CNPq, FAPESP

06.097

Vascular relaxation induced by 3-hydroxy-4-(hydroxyimino)-2-(3-methylbut-2-enyl)naphthalen-1(4h)-one in rat superior mesenteric arteries. Dantas BPV[1], Magalhães NM[1], Medeiros IA[1], Câmara CA[2], Alencar JL de[3] [1]UFPB - Tecnologia Farmacêutica, [2]UEPB, [3]UFPB - Medicina Interna

Introduction: The pharmacological effects of the oxime 3-hydroxy-4-(hydroxyimino)-2-(3-methylbut-2-enyl)naphthalen-1(4H)-one, a naftoquinona derivative of lapachol extracted from *Tabebuia avallanedae* Lor.Ex.Gris (Bignoniaceae), in the cardiovascular system, being the endothelium responsible from the release of a variety of relaxing and contracting factors. The most widely known endothelium-derived relaxing factor is nitric oxide (NO). Some compounds can act as donating agents, like oximes, organic nitrates, among others, whose biotransformation into NO or NO-related vasorelaxant species in blood vessels is independent from the activating of nitric oxide synthase (NOS). In the present work, the effect of a oxime synthesized in LTF was evaluate in isolated rat superior mesenteric arteries. **Methods:** All experiment were performed according to the pattern of the Ethics Committee in Animals Research (CEPA/LTF-UFPB, nº 0610/05). Isolated rat superior mesenteric artery rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in a Tyrode's solution at 37ºC and gassed with a 95% O_2 and 5% CO_2, under a resting tension of 0.75g. Concentration-responses curves to Oxime derivative of Lapachol (10^{-9} - 10^{-4}M) were obtained. **Results:** In isolated rat mesenteric rings with intact endothelium pre-contracted with phenylephrine (10 µM), Oxime derivative of Lapachol (10^{-9} - 10^{-4}M) induced concentration-dependent relaxations CE_{50}=6,37x10^{-7} (4,8x10^{-7} - 8,5x10^{-7} I.C.) and $E_{Máx}$= 85,81 ± 7,41% (n = 6) and after endothelium remove CE_{50}=2,4x10^{-6} (2,14x10^{-6} - 2,7x10^{-6} I.C.) and $E_{Máx}$= 99,05 ± 0,95% (n = 6). The vasorelaxant effect induced by oxime in isolated rat mesenteric rings without endothelium in presence of ODQ 10µM $E_{Máx}$= 77,08 ± 8,23%, PTIO 100µM $E_{Máx}$= 87,72 ± 5,88% and Proadifen 30µM $E_{Máx}$= 99,10 ± 0,90%, the $E_{Máx}$ was significantly attenuated in presence of ODQ with p=0,024. **Discussion:** These results suggest that the vasorelaxant effect induced by Oxime derivative of Lapachol appears to involve sGC pathway in vascular smooth muscle. **Supported by:** CNPq, CAPES, LTF, UFPB

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Protein dissulfide isomerase inhibition reduces microvascular reactivity to Angiotensin II: Role of NADPH oxidase. Camargo LL[1], Dias AA[1], Androwiki, ACD[1], Chinaglia, AZ[1], Ceravolo GS[1], Carvalho MHC[1], Fortes ZB[1], Janiszewski M[2], Lopes LR[1] [1]USP - Farmacologia, [2]IEP-Hospital Israelita Albert Einstein

Introduction: Angiotensin II (Ang II) signaling in vascular smooth muscle cells (VSMCs) is dependent on reactive oxygen species (ROS) through activation of the enzimatic complex NADPH oxidase. c-Src, a tyrosine kinase expressed in VSMCs, is rapidly activated by Ang II and plays an important role in the signaling events associated with contraction. Redox mechanisms have been shown to contribute to vascular remodeling and total peripheral resistance increase associated to hypertension (Paravincini & Touyz, 2006). We recently demonstrated that the redox chaperone protein disulfide isomerase (PDI) modulates Ang II dependent ROS generation in rabbit aortic VSMCs (Janiszewski et al, 2005). However, the role of PDI in the increased ROS generation and 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; protocol number 13 of CEEA-ICB-USP). To further investigate the mechanisms involved, VSMCs isolated from mesenteric resistance arteries from Wistar and SHR rats were cultured. Ang II-induced ROS production was assessed by dihydroethidium derived fluorescence (DHE, 5 μM) in the presence or absence of the PDI inhibitor bacitracin (0.5 10^{-3}M; 30 min). The expression of PDI in total cell homogenate was analysed by western blotting. In order to investigate the effect of PDI on Ang II-induced activation of c-Src, cells were stimulated with Ang II (10^{-7}M) and the activity of Src was determined by analysis of the phosphorylation of the kinase (P-Src) by western blot. Results: Vasoconstrictor responses to Ang II were similar in Wistar and SHR and inhibition of PDI with bacitracin substantially suppressed contraction in SHR ($P < 0.05$, n=3). ROS generation in response to Ang II and PDI expression were higher in VSMCs from SHR as compared to Wistar ($P<0.05$, n=3). The inhibition of PDI completely abolished Ang II stimulated ROS generation in both strains. Phosphorylation of c-Src (Tyr 416) after one minuite of Ang II stimulation was higher in SHR than Wistar VSMCs ($P < 0.01$, n=3). PDI inhibition significantly diminished P-Src in both Wistar and SHR cells ($P < 0.01$, n=3). **Discussion:** Altogether our results show that inhibition of PDI results in reduced mesenteric vasoconstriction which could be related to a decreased ROS production and c-Src phosphorylation in response to Ang II in mesenteric VSMC from Wistar and SHR animals. Recently, our group has shown that PDI overexpression is associated to an increase in oxidative stress and NADPH oxidase activity in rabbit aortic VSMCs (Fernandes et al., 2009). 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 contribute to the oxidative stress observed in hypertension. **Acknowledgements**: The authors thank Sidney Verissimo Filho e Ana Rita Araujo Gonçalves for technical support. **Financial Support:** FAPESP.