Session 01 − **Cellular and Molecular Pharmacology**

01.001

Pharmacologic evaluation of new alpha-1 adrenoceptor antagonists: structural characteristics of the derivatives N-phenylpiperazinics that affect the affinity for alpha-1 adrenoceptors. Nascimento JB[1], Romeiro LAS[2], Nascente LC[3], Lemes LFN[3], Noel F[1], Silva CLM[1] [1]UFRJ − Farmacologia Básica e Clínica, [2]FCS-UnB − Desenvolvimento de Estratégias Terapêuticas, [3]UCB-LADETER

Introduction: Alpha1-adrenoceptor (AR) antagonists are currently used in the treatment of hypertension and benign prostate hyperplasia. Previously, a new N-phenylpiperazine was synthesized and pharmacological evaluation unveiled a potent alpha1-adrenoceptor (AR) blockage, being selective for alpha-1A and alpha-1D AR (Romeiro et al., submitted). However, it also has a high affinity (nM) for 5-HT receptors. The objective of this study was to determine the affinity of new analogues (LTD series) for alpha1 AR subtypes and 5-HT receptors, and to determine some structure-activity relationships. **Methods**: All protocols are approved by the Ethics Committee of UFRJ (CAUAP; DFBC – ICB011). Animals were anesthetized and killed by decapitation. Binding assays: 150 ug of membrane proteins enriched in alpha-1B AR (rat liver), alpha-1A AR (rabbit liver), 5-HT2A receptors (rat cortex) and 5-HT1A receptors (rat hippocampus) were incubated with 0.1 nM [3H]-prazosin, 1 nM [3H]-ketanserin or 1 nM [3H] 8-OH-DPAT for 15 to 40 min at 37oC in the absence and presence of LTD 2-8 (1 – 30,000 nM). The reaction was stopped with the addition of cold Tris-HCl buffer (pH 7.4), followed by filtration under vacuum. The radioactivity was quantified in a liquid scintillation counter. Functional studies: isometric contraction experiments were performed as previously described using rat aorta (alpha-1D AR) (*Silva et al. 2002. J. Pharmacol. 135:293*). The rings (3 mm wide) with functional endothelium were contracted with phenylephrine (PE) (1 to 10000 nM) before and after incubation with LTDs (10 uM, 1h) to assess the vasodilator effect. The results were analyzed by non-linear regression to calculate the parameters EC50, IC50 and Emax. The KB value was calculated using Schild equation. **Results and discussion**: The analysis of the competition curves showed a concentration-dependent inhibition. Compared to the prototype the affinity of LDTs for 5-HT2A and 5-HT1A receptors was reduced except for LDT 8. The IC50 values of LTD2-6 for alpha-1B AR were respectively: 16.5, 10.9, 6.5, 5.3, 4.7 uM. LDT8 showed a mean IC50 value of 5.3 nM, indicating that substitutions at the aryl group reduce the affinity for alpha-1B AR. In rat aorta (alpha-1D AR), the contraction induced by PE was competitively inhibited by LTDs with KB values in the nM range. Particularly, LTD5 was the most potent (KB = 0.62 nM) whereas LDT6 was the least potent (KB >50 nM). In conclusion these new N-phenylpiperazine derivatives have a high affinity for alpha-1D AR and a reduced affinity for 5-HT receptors. **Acknowledgement**: CNPq and CAPES (fellowship), FAPERJ (financial support) and Orlando da Rocha Moreira (technical assistance).

01.002

Decreased bone resorption by low β2-adrenergic antagonist. Rodrigues WF[1], da-Silva-Filho VJ[1], Campos-Júnior JC[1], Dias da Silva VJ[2], Barbosa Neto O[2], Lopes AHP[1], Napimoga MH[3] [1]UNIUBE − Biopatologia e Biologia Molecular, [2]UMTM− Fisiologia, [3]UNIUBE − Biologia Celular e Molecular

Introduction: The propranolol is a non-selective β-adrenergic blocking receptor agent, used mainly as antihypertensive drug. The interaction between the activating factor NF-kB (RANK) and its ligand RANKL was shown to be critical for the occurrence of the formation of osteoclasts, thus, an unbalance of RANK/RANKL induce the formation of osteoclasts and bone resorption. The use of β2-blockers has been studied due to the association with decreasing bone fragility. Also, some studies have suggested that bone remodeling is under β-adrenergic control. Although, pharmacological studies on adrenergic receptors have provided controversial results. Thus, we hypothesized that the effect of the propranolol may modulate the bone resorption in a periodontal disease experimental model. **Methods**: All experimental procedures were approved by the Ethical Committee for Animal Research of the University of Uberaba (#048/2009). The experimental periodontitis was induced by a ligature placement around mandible first molars of each animal (ligated-animals). Rats were assigned to one of the following groups: 1) animals without ligature receiving vehicle; 2) ligated-animals receiving vehicle; 3) ligated-animals receiving 0.1 mg of propanolol; 4) ligated-animals receiving 5mg of propanolol and 6) ligated-animals receiving 20 mg of propanolol. Thirty days after induction of periodontal disease, heart rate, left ventricular systolic (LVSP) and end-diastolic pressures (LVEDP), and positive and negative LV *dP/dt* were measured by cardiac catheterization in anesthetized rats. Gingival tissues were removed and assessed for IL-1b and TNF-a quantification by ELISA and ICAM-1 and RANKL expression by western blot. The mandibles were fixed and decalcifed in EDTA and sliced into 6mm sections mounted on glass slides and stained with hematoxylin and eosin for the evaluation of bone resorption RESULTS: The results demonstrated that only propanolol at 0.1 and 5 mg/kg was able to reduce statistically the bone resorption (48% and 43% respectively) as well as ICAM-1 (50% and 28%, respectively) and RANKL (59% and 33%, respectively) expression. However, only 0.1 mg/kg was able to reduce significantly the IL-1b (51%) and TNF-a (63%) levels in comparison to ligated-animals receiving vehicle. Important, only 0.1 mg/kg of propanolol did not alter all the hemodynamics parameters. **Discussion/Conclusion**: Taken together we demonstrated that the use of a b receptor antagonist at a low dose (0.1 mg/kg) inhibits the bone resorption by decreasing inflammatory cytokines and bone-related activator in the periodontal tissue without interfering the heart functions. **Financial Support**: CNPq 471305/2009-0, PAPE-UNIUBE 2009/001 and FAPEMIG 097/09

01.003

The lidocaine analogue JMF2-1 prevents allergen-induced lung inflammation without causing immunosupression. Olsen PC[1], Ferreira TPT[1], Serra MF[1], Costa JCS[2], Cordeiro RSB[1], Silva PMR[1], Martins MA[1] [1]IOC-FIOCRUZ − Inflammation, [2]FIOCRUZ − Farmanguinhos

Introduction: We have previously shown that inhalation of JMF2-1, an analogue of lidocaine with reduced anesthetic activity, prevents cardinal features of asthma. Reduction of airway hyperresponsiveness, T_H2 cytokine generation and lung eosinophilic inflammatory infiltrate were likely due to an inhibition of T cell function and survival. In the current study, we tested the hypothesis that JMF2-1 is able to reduce lung inflammation in antigenic challenged mice without inducing systemic immunosupression. **Methods**: Sensitized BALB/c mice were exposed to aerosolized ovalbumin (OVA) from day 19 to 21 post-sensitization. JMF2-1 (1-2%) or lidocaine (2%) was aerosolized for 30 min twice a day, being administered concomitantly and 8 h post-challenge. Dexamethasone (1 mg/kg, intraperitoneal) was administered 1 h before challenge. Analyzes of JMF2-1, lidocaine or dexamethasone effects on inflammation were performed 24 h post-challenge. Phenotype and survival of T cells obtained from the bronchoalveolar lavage fluid (BALF) were analyzed by flow cytometry, by staining cells with anti-CD4 (PE) and annexin V (FITC). Lymph node T cells from treated and challenged mice were re-stimulated with OVA *in vitro*. After 72 h, proliferation was confirmed by staining DNA with propidium iodide and by analyzing $S+G_2$ population by flow cytometry. We also investigated size, weight and T cell population of lymph nodes and thyme from non-challenged mice treated as described previously. Protocol number of Animal Ethics Committee approval is 00085-02. **Results**: We found that JMF2-1 inhalation, as well as dexamethasone and lidocaine treatment, inhibited T cell influx into the BALF. Accordingly, JMF2-1 nebulization increased the percentage of apoptotic T CD4 cells in the BALF (mean= 5.69%, SEM= 0.68), when compared to the OVA-challenged group (mean= 2.63%, SEM=0,47). After re-stimulation *in vitro*, lymph node T cells from OVA-challenged mice re-called antigen showing increased proliferation. Lymph node T cells obtained from mice treated with lidocaine or JMF2-1 that were re-stimulated *in vitro* showed similar proliferation rates than cells obtained from non-treated mice. In contrast to dexamethasone (mean= 0.66%, SEM= 0.22), lidocaine (mean= 2.96%, SEM=0.20) and JMF2-1 (mean= 2.42%, SEM= 0.20) nebulization in non-challenged mice did not reduce lymph node and thymus sizes and weights, when compared to saline treated mice (mean= 2.2%, SEM= 0.39); also, they did not alter T cell subpopulation distribution on those organs. **Discussion**: These results show that JMF2-1, as well as lidocaine, inhibits T cell influx into the airways of OVA-challenged mice without interfering in the activation and survival of lymphocytes from primary and secondary lymphoid organs. Therefore, JMF2-1 should be considered as a new prototype in drug discovery for asthma with advantages over immunosuppressive corticoids. **Financial Support**: CAPES, CNPq.

01.004

Pharmacologic evaluation of new alpha adrenoceptor antagonists. Chagas-Silva F[1], Nascimento JB[1], Vieira RO[2], Romeiro LAS[3], Barberato LC[4], Noel F[5], Silva CLM[5] [1]ICB-UFRJ, [2]UFRJ − Farmacologia Celular e Molecular, [3]UCB − Química Bioorgânica e Medicinal, [4]UCB − Desenvolvimento de Estratégias Terapêuticas, [5]UFRJ − Farmacologia Básica e Clínica

Introduction: Previously, it was synthesized an alpha1-adrenoceptor (AR) antagonist, an *N*-phenylpiperazine and aryl sulfonamide hybrid, selective for alpha-1A and alpha-1D AR subtypes (Romeiro et al, submitted). However, it also has affinity for 5-HT receptors. The objective of this study was to investigate the affinity of new analogues, with substitutions at the nitrogen 4 of phenylpiperazine and position 2 of the aromatic ring (named LTD series), for alpha1-AR and 5-HT receptors, and to determine some of the structure-activity relationship. **Methodology**: All protocols are approved by the Ethics Committee of UFRJ (CAUAP; DFBC – ICB011). Animals were anesthetized and killed by decapitation. Binding assays: 150 ug of membrane proteins enriched in alpha-1B AR (rat liver), alpha-1A AR (rabbit liver), 5-HT2A receptors (rat cortex) and 5-HT1A receptors (rat hippocampus) were incubated with 0.1 nM [3H]-prazosin, 1 nM [3H]-ketanserin or 1 nM [3H] 8-OH-DPAT for 15 to 40 min at 37oC in the absence and presence of LTD39, LDT62 -70 or 2-OH-FPZ (1 − 50 uM). The reaction was stopped with the addition of cold Tris-HCl buffer (pH 7.4), followed by filtration under vacuum. The radioactivity was quantified in a liquid scintillation counter. Functional studies: Isometric contraction experiments were performed as previously described (*Silva et al. Br.J.Pharmacol. 135:293.2002*), where rat aortic rings (alpha1D-AR) were contracted with phenylephrine (PE) (1 to 10000 nM) before and after incubation with LTDs (50 nM) to assess the vasodilator effect. The results were analyzed by non-linear regression to calculate the parameters IC50, CE50 and Emax. The KB value was calculated using Schild equation. **Results and discussion**: The analysis of inhibition curves showed a concentration-dependent inhibition. Compared with the prototype (product of prototype simplification) the affinity of LDT62 (Romeiro et al., submitted) was reduced 110 fold for 5-HT1A, 23 fold for 5-HT-2A receptors and 1188 fold for alpha-1A AR. However, comparing to LDT62, the affinity of the new analogues for alpha1B-AR was maintained (uM). The mean IC50 values for 5-HT-1A, 5-HT-2A receptors, alpha1A-AR and alpha1B-AR for LTD62 were: 0.33, 10, 0.31 and 0.54 uM, respectively and for LDT69: 0.14, 9.55, 0.04, 0.22 uM, indicating that the replacement of aromatic ring in the position 2 of phenylpiperazine did not alter the affinity for 5-HT receptors and alpha1B-AR, but it increased the affinity for alpha1A-AR. In rat aorta (alpha-1D AR), the contraction induced by PE was competitively inhibited by all LTDs, with KB values in the nM range. Particularly, LDT69 has the same KB value of LDT62 (KB 8 nM approximately). Indeed LDT66 and LDT67 were the most potent (KB=1.66 and 1.88 nM, respectively). In conclusion, the modifications in the new analogues increased more effectively the affinity for alfa1A-AR and/or alfa1D-AR rather than 5-HT2A and 5-HT1A receptors, without affecting the affinity for alpha1B-AR. **Financial support**: CNPq, FAPERJ. **Acknowledgements**: Orlando da Rocha Moreira (technical assistance), CNPq (fellowship).

01.005

Morphological study of protective effect of glutamine and alanil-glutamine injury induced by TxA from *Clostridium difficile* in rat intestinal epithelial cells. Santos AAQA[1], Leite LL[2], Brito GAC[3], Oliveira MR[4], Ribeiro RA[4], Braga Neto MB[4], Barreto LRF[4] [1]UFC − Ciências Médicas, [2]UFC − Medicina, [3]UFC − Morfologia, [4]UFC − Fisiologia e Farmacologia

Introduction: *Clostridium difficille* is considered the most frequent cause of diarrhea associated to the use of antibiotics in industrialized countries. The Toxin A (TxA) causes the monoglicolisation of proteins from Rho family. This reaction inactivates Rho, Rac and Cds42 proteins, resulting in a disorganization of actin cytoskeleton, consequently it also results into a cellular restrain, loss of adhesion and the round shape of the cells in culture. Our research analyses how glutamine or alanil-glutamine influences the morphological characteristics of the intestinal epithelium cells damaged by TxA of *Clostridium difficille*. **Methods**: Rat intestinal epithelial cells (IEC-6) were grown for 24 h then were incubated with medium only (control) or for 1 hour with TxA (10 ng/mL),TxA + GLN (10 mM) and TxA + AGL (10 mM). After this period, the test and control group were fixed in 4% formaldehyde for 14 h and images were made on the Atomic Force Microscopy. The cell area and height and the cell volume and surface roughness were calculated using Nanoscope 5.30 R3.SR3. IEC-6 were cultured at the concentration of 2 x 10^4 cells/ml per 24h and then incubated in medium for 1 hour with TxA (10 ng / mL),TxA + GLN (10 mM) and TxA + AGL (10 mM). After this period, the cells were fixed in 4% formaldehyde for 10 minutes, permeabilized with 0, 1% triton for 20 minutes and were stained with phalloidin conjugated to rhodamine to analyze the f-actin cytoskeleton fibers distribution´s. The slides were analyzed using high-resolution microscope with rhodamine filter. **Results and Discussion**: The treatment of IEC-6 cells with TxA led to severe retraction of the cytoplasm, which aggregated around the nucleus. The control group showed morphological pattern to the cytoplasm and nucleus. Samples with GLN and AGL showed partial preservation of the cell morphology. TxA significantly ($P < 0.05$) altered all measured parameters, resulting in a reduction of 66.2% and 58.9% in volume and area of cells, respectively, and an increase of 79.7% and 73,5% at the height and roughness cell . AGL induced an 92.9%, 65.4% and 77.0% increase of in volume, area and roughness, respectively, and a reduction of 16.9% at height when compared to group treated with TxA. GLN promoted an increase of 46.3%, 67.6% and 73.1% in volume, area and roughness, respectively, and a reduction of 38.6% at height when compared to group treated with TxA. Immunofluorescence showed that Tx A caused cell shrinkage and concentration of F-actin around the nucleus. These alterations were prevented by GLN and AGL. Our results therefore, suggest a protective effect of GLN and AGL in epithelial injury induced by TxA. **Support**: CNPQ/PIBIC

01.006

Effect of annexin-1 derived peptide AC 2-26 on mice pulmonary fibroblasts. Trentin PG[1], Ferreira TPT[1], Pires ALA[1], Ciambarella BT[1], Flower RJ[2], Perretti M[2], Martins MA[1], Silva PMR[1] [1]FIOCRUZ − Inflamação, [2]William Harvey Institute − Biochemical Pharmacology

Introduction: Silicosis is a chronic occupational disease caused by inhalation of free crystalline silica particles and is characterized by intense inflammation and fibrosis. Fibroblasts are considered crucial cells involved in fibrogenesis and granuloma formation. There are several endogenous mediators able to regulate negatively some inflammatory responses, in order to guarantee the control of such processes. Glucocorticoids are considered as important agents based on their anti-inflammatory activity, which has been shown to be, at least partially, dependent on the generation of an intermediate protein named annexin-1. In this study we investigated the effect of annexin 1 N-terminal derived peptide Ac2-26 on the reactivity of pulmonary fibroblasts. **Methods**: Fibroblasts were obtained from lungs of normal Swiss-Webster mice by means of enzymatic dissociation with collagenase type 1. Cells were cultivated in DEMEN medium supplemented by SBF 10% until the 3rd passage. The analyses included cellular proliferation, ii) chemokine (MCP-1) generation and extracellular matrix (collagen) secretion and were performed by means of ^{3}H-thymidine incorporation, ELISA and Sircol technique, respectively. The cells were treated with Ac2-26 peptide (50 − 400 µg/mL) 1 h before stimulation with the profibrotic cytokine IL-13 (40 ng/ml). All the analyses were performed 24 h post-provocation. All experimental procedures used were in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (license 0213-4). **Results**: We noted that pulmonary fibroblasts showed an increased proliferation rate, collagen secretion and MCP-1 generation, after stimulation with IL-13. Incubation of the cels with the Ac2-26 peptide did not interfere with IL-13 induced proliferation. In contrast, the peptide a concentration-dependent inhibited the production of collagen and the profibrotic chemokine MCP-1. **Conclusion**: Our findings show that annexin 1 derived peptide Ac2-26 directly inhibited pulmonary fibroblast activation by IL-13, though failing against proliferation. They also suggest that treatment with Ac2-26 peptide may possibly constitute a promising pharmacological tool chronic fibrotic lung diseases such as silicosis. **Financial support**: FIOCRUZ, CNPq, CAPES, FAPERJ (Brazil).

01.007

Investigation of the binding of trypanosomal FKBP12 to the ryanodine receptor-3 of rat vas deferens: possible implications in heart failure due to Chagas disease. Perissé L[1], Muzi-Filho H[2], Aido-Machado R[1], Cunha VMN[2], Salmon DJJ[1] [1]UFRJ − Bioquímica Médica, [2]ICB-UFRJ − Farmacologia Celular e Molecular

Introduction: FKBPs (FK506 Binding Proteins) belong to the super family of peptidyl prolyl cis-trans isomerases (e.g. immunophilin). The information obtained over the last 15 years reveals that FKBP are involved in diverse biological processes affecting the function and structure of target proteins and several signal transduction pathways (TOR and Ca^{2+} signalling pathways). In *Trypanosoma brucei*, it was recently shown that one of them, TbFKBP12, is involved in flagellar motility suggesting that this immunophilin might be involved in the regulation of the Ca^{2+} release through the modulation of the Ca^{2+} release channel (1,4,5-triphosphate receptor/ryanodine receptor (RyR)) activity. This FKBP12 is highly conserved in *T. cruzi*, the causative agent of the Chagas disease, an epidemic cause of dilated cardiomyopathy in South America. The aims of the present work were: 1 − evaluate the "*in vitro"* re-association of the recombinant TbFKBP12 to RyR3-FKBP12 complexes of the sarcoplasmic reticulum from rat vas deferent (RVD); 2 − investigate the expression of FKBP12 during the *T. cruzi* cell cycle. **Methods**: Five 3 months-old male Wistar rats were sacrificed (CEUA DFBCICB 007) and the RVD were removed, homogenized and submitted to a first ultracentrifugation (108000 x g for 1 h). Part of the pellet was treated to dissociate the FKBP12-RyR3 complex (Scaramello et *al*., 2009). The FKBP12-dissociated pellet was re-incubated overnight at 15^{o}C with the supernatant containing free rat FKBP12 or with *T. brucei* recombinant protein (TbFKBP12-associated fraction). Western blotting assays were performed using specific antibodies raised against recombinant TbFKBP12 or mammalian FKBP12. **Results**: The expression of TbFKBP12 seems to be stage-specific and it is overexpressed in the mammalian stages of the cell cycle (207% in amastigote and 192% in trypomastigote forms). According to previous data, rat FKBP12 was mechanically dissociated from RyR3 in vas deferens ((57±5% of control, n=2; $p<0.05$). Preliminary results show that the dissociated rat FKBP12 as well as TbFKBP12 seem to be re-associated to this binding site (96 % of control and 150% of control, respectively). **Discussion**: Our data show that the TbFKBP12 re-associate to vas deferens RyR3 very efficiently, as is the case of the endogenous rat FKBP12. This data suggests that the binding of the heterologous FKBP12 to this target is very tight and stable. In addition, the observation that this protein is overexpressed in both intracellular and bloodstream stages of the parasite suggests that this immunophilin might interact with host ryanodine- sensitive calcium channel causing pathological disorders as is the case of the cardiomyopathology observed in Chagas disease. **Financial support**: FAPERJ; PROAP.

01.008

Maternal protein deprivation during lactation increases leptin secretion and inhibits apoptosis of thymic cells from young offspring. Salama Rodrigues C[1], Renovato-Martins M[2], Vargas da Silva S[1], Barja Fidalgo TC[1] [1]UERJ − Farmacologia, [2]UERJ − Farmacologia e Psicobiologia

Introduction and objectives: Several studies have demonstrated that maternal nutritional imbalance during critical time windows of development leads to persistent effects on the health of the offspring. Our group has been studying the effects of metabolic programming induced by maternal protein deprivation during lactation on immune function of progeny. In this model, it has already demonstrated that serum leptin levels were higher in pups (21 days old) whose dams were fed with a diet containing 8% of protein (PD group) during lactation, when compared to the control group (whose dams received a regular 22% protein diet). It is known that leptin regulates different facets of immune response such as the promotion of enhanced peripheral T cell activity and proliferation. Likewise, this hormone has antiapoptotic effects on T cells by up-regulating prosurvival signaling, through the activation of Ob receptor. Thus, the objective of the present study was to investigate and characterize the effect of maternal protein deprivation (8% protein) during lactation on the response of thymocytes isolated from young rat offspring (30 days old) (CEA/047/2009), identifying the role of leptin on these alterations. **Methods**: Serum leptin levels were detected by ELISA, apoptosis and profile of T cell subsets were accessed by FACS, mRNA for ObRb was analyzed by RT-PCR and signal transduction pathway was evaluated by immunoblotting. **Results and discussion**: We showed that at 30 day of age, PD rats present body and thymus weights lower than controls, and maintain an increased serum leptin levels, confirming previous observation at 21st day. Although the mRNA level of ObRb was similar in both groups, ObRb protein expression was enhanced in the thymus of animals from PD group. No significant differences were observed in the contents of pJAK2 nor pSTAT3 in thymocytes isolated from PD and C group. There are no differences in the profile of T cell subsets between PD and control group neither after 24 hours of incubation with leptin (10^{-8} M – *in vitro*). However, thymic cells isolated from PD rats present a decrease in the rate of spontaneous apoptosis when compared to controls. Leptin (10^{-8} M – *in vitro,* 24 h) inhibited the spontaneous apoptosis of thymocytes in both groups. Noteworthy, it was observed that the expression anti-apoptotic protein Bcl-2 was higher in thymic cells of PD than control group, but similar pro-apoptotic protein Bad expression was detect in both groups. **Conclusion**: Metabolic programming induced by maternal protein deprivation during lactation increases the basal secretion of leptin, which, in turn, down-modulates the thymic cells apoptotic process in the progeny, inducing, independently of JAK2-STAT activation, the synthesis of anti-apoptotic protein, Bcl-2. **Financial support**: CAPES; CNPQ; FAPERJ.

01.009

LASSBio-1135: a multi-target antinociceptive imidazopyridinic derivative that is a TRPV1 antagonist. Silva RM[1], Guimarães MZP[1], Lima CKF[2], Lacerda RB[2], Barreiro EJ[2], Fraga CAM[2], Miranda ALP[2] [1]UFRJ − Farmacologia Básica e Clínica, [2]FF-UFRJ − Fármacos − LASSBio

Introduction: TRPV1 is a ligand-gated nonselective cation channel present in nociceptors which can be activated by capsaicin, the pungent chemical in chili peppers, and noxious heat, among other painful stimuli. Recently TRPV1 became a novel target for developing analgesics and some antagonists are effective against acute, inflammatory and neuropathic pain. LASSBio-1135 is a 3-arylamine-imidazo[1,2-*a*]pyridine derivative developed by hybridization of celecoxibe and SB-203580, a p38 MAPK inhibitor, which was able to revert capsaicin-induced thermal hypernociception (Lacerda, Bioorg. Med. Chem., vol. 17, p. 74, 2009). Because of LASSBio-1135 inhibition of the capsaicin effect and since TRPV1 is known to signal through p38, we asked whether this compound had any direct action on the TRPV1 channel. **Methods**: *Xenopus laevis* oocytes were obtained from adult female frogs by performing a small abdominal incision through anesthesia. After collagenase treatment and sorting, oocytes were injected with cRNA encoding rat TRPV1. Following 5 to 9 days of expression, these cells were used in electrophysiological experiments. We tested the effects of various concentrations of LASSBio-1135 against activation of TRPV1 by 1 microM capsaicin. To validate our findings *in vivo*, LASSBio-1135 was injected in rats' paws (50 nmol/10 mL/paw), 20 minutes before capsaicin (50 nmol/10 mL/paw). Next animals were evaluated for thermal hypersensitivity at different time points and were represented by the delta of latency to withdraw the injected paw (time to withdraw before treatments – time to withdraw after capsaicin). Results were expressed as average ± SEM. Animal procedures were approved by CEUA/UFRJ (DFBCICB 009). **Results**: LASSBio-1135 at 50 microM was able to inhibit the capsaicin-induced current by 44.8 ± 13.5% ($P<0.001$ by RM ANOVA followed by Tukey's, n=6). We also observed that LASSBio-1135 effect *in vitro* is concentration dependent. *In vivo*, locally applied LASSBio-1135 reduced capsaicin-evoked thermal hypernociception. At 2 minutes following capsaicin, the delta in latency was reduced from 6.5 ± 0.36s to 3.9 ± 0.55s and at 5 minutes from 5 ± 0.41s to 2.5 ± 0.72s. This reduction was statistically significant by 2-way ANOVA followed by Bonferroni posttest ($P<0.05$, n=8 for each group). **Discussion**: LASSBio-1135 was able to directly inhibit capsaicin currents *in vitro* and quickly reduced hypernociceptive responses *in vivo*. These results are consistent with the idea that at least some of the antinociceptive effects of LASSBio-1135 could be attributed to TRPV1 antagonism. Since it was also shown to inhibit p38 phosphorylation, we now plan to investigate whether this effect is due to TRPV1 antagonism. **Financial Support**: FAPERJ, Pronex and CNPq.

01.010

Effect of a new compound, thiophenacetamide, against *Mycobacterium bovis* (BCG) infection. Vergara FMF[1], Candea ALP[1], Rosas EC[1], de Souza MVN[2], Henriques MGMO[1] [1]FarManguinhos-FIOCRUZ − Farmacologia Aplicada, [2]FarManguinhos-FIOCRUZ − Síntese Orgânica

Introduction: Nowadays approximately 1.7 billion people are infected by *Mycobacterium tuberculosis* and about 20 million of these have active tuberculosis. According to the latest estimates from the World Health Organization in 2008 occurred 9.4 million new cases of TB infection (OMS, 2009). Today´s TB drug regimen was developed in 1960, takes 6 months to administer and requires too many pills, besides several side effects. In this context, thiophene nucleus represents a very important field in drug discovery. Our group presented a new class of compounds and demonstrated its activity against *M. tuberculosis.* (Lourenço. *Bioorg. Med. Chem. Lett*. (17) 6895, 2007). Here we describe the effects of thiophenacetamide, *in vitro*, intracellular infection and *in vivo,* infection with *M. bovis*. **Methods**: The direct growth inhibition of *M. bovis* was evaluated by Alamar blue® method. The bacteria suspension was incubated with different concentrations (0,1 1,0; 10; 25; 100 µg/mL) of the compound. Cytotoxicity of the compound was evaluated by MTT method (Mosman, *J Immunol Methods* (65) 55, 1985) after 48 hours treatment of infected cells. The indirect growth inhibition was evaluated by infection of macrophages with *M. bovis* (1/1) and the treatment for 48 hours. The bacterial growth was counted 30 days after been cultivated in Lowenstein Jensen (LJ) medium. Cytokines were evaluated by ELISA assay and CBA, and nitric oxide (NO) by Greiss method, using supernatant collected from infected and treated cells. Translocation of NF-κB factor was analyzed by confocal microscope. The anti-inflammatory and the antimicobacterial effect *in vivo* were evaluated in BCG-induced pleurisy model (Henriques MG, *Br J Pharmacol* (1) 164, 1990) in C57BL/6 mouse (CEUA, Fiocruz; licence n. L-0004/08), the compound was administrated orally at (5,0; 25; 50 mg/kg) and the effects was evaluated at 6 hours, 24 hours and 15 days post infection. **Results and Discussion**: Thiopheneacetamide wasn't cytotoxicity in infected macrophages in all concentrations used. *In vitro*, this compound was able to inhibit directly and the intracellular growth of *M. bovis* (BCG). The pre treatment with the compound was able to increase IL-10 and reduce TNF-α, IL-6, IL-12 and nitric oxide in infected macrophages and decreased the translocation of NF-κB factor, the after treatment with the tiophenacetamide was able to increase nitric oxide. *In vivo* treatment modulates the mononuclear and neutrophils influx to the pleural cavity but not interfere in eosinophil influx. We also show that thiopheneacetamide was able to inhibit the growth of *M. bovis* (BCG) at 6 hours post infection which get higher at 24 hours. We also demonstrated that tiophenacetamide was able to reduce nitric oxide in the first moment but increased at 24 hours post infection. Thiophenacetamide could decrease IL-1β since 6 hours post infection, IFN-γ since 24 hours and IL-6 only at 6 hours and was able to increase IL-6 since 24 hours and IL-12 in all moments evaluated, these was observed in the concentration range examined. Our data indicate that this new compound, tiophenacetamide*,* presents *in vitro* and *in vivo,* an important antimicobacterial activity and immunomodulatory effect. **Financial Support**: CNPq

01.011

Anti-inflammatory and antimicrobial activity of pyrazinamide analogs. Mendonça MSA[1], Candea ALP[1], Lima CHS[2], de Souza MVN[2], Henriques MGMO[2] [1]FIOCRUZ − Farmacologia Aplicada, [2]FarManguinhos-FIOCRUZ − Síntese Orgânica

Currently, tuberculosis is the leading cause of mortality due to a single infectious agent worldwide. Treatment for this infection is based on drugs developed in the 60´s and the increase in cases of infection with MDR (multidrug resistant) strains, leads to the urgent need for the discovery and development of new drugs against tuberculosis. In this contest, the synthesis of pyrazinamide analogs has been an important tool for medicine chemistry studies. Our group has recently described a series of pyrazinamide analogs with mycobacterial activity against *M. tuberculosis.*

The aim of this study was to evaluate the anti-mycobacterial activity of pyrazinamide analogs in macrophages infected with BCG in vitro and the immunomodulator effects evaluating the nitric oxide, cytokines and chemokines production, as well as evaluating the bactericidal potential of these analogues.

Initially the cells were infected for a period of four hours and subsequently treated with different concentrations of analogs 2, 25, 31, 33, 55 and 56, until 24 hours. The cell supernatant was collected to analyze the presence of chemical mediators. The analysis of the percentage of infected cells and quantification of bacteria inside macrophages after treatment with the analogs was performed using the Ziehl Neelsen staining. All procedures were approved by the Committee for Animal Care and Use (CEUA-FIOCRUZ) under L0052-2008 license.

All analogs demonstrated efficacy in reducing the number of infected cells and the amount of bacteria within these cells. We observed that the samples are able to inhibit the release of IL-6 and MCP-1 and showed different effects on the release of KC and NO.

The results presented indicate that among all the analogs, the compound 25 present the best antimicrobial profile, increasing the production of nitric oxide and reducing significantly, the number of infected cells and the rate of infection.

Supported by: CNPq, Fiocruz

01.012

O-glcnacylation contributes to the vascular effects of ET-1 via activation of the RHOA/RHO-kinase pathway. Lima VV[1], Giachini FR[1], Carneiro FS[1], Webb RC[2], Tostes RCA[1] [1]USP − Farmacologia, [2]Medical College of Georgia − Physiology

O-Linked attachment of ß-N-acetyl-glucosamine (O-GlcNAc) on serine and threonine residues of nuclear and cytoplasmic proteins is a highly dynamic post-translational modification that plays a key role in signal transduction pathways. O-GlcNAcylation augments vascular contractile responses and O-GlcNAc-proteins are increased in the vasculature of DOCA-salt rats. Since Endothelin-1 (ET-1) plays a major role in vascular dysfunction associated with salt-sensitive forms of hypertension, we hypothesized that ET-1 augments O-GlcNAc levels and this modification contributes to increased vascular contractile responses via activation of RhoA/Rho-kinase pathway. Incubation of rat aortas (007/04/CEEA) or vascular smooth muscle cells (VSMCs) with ET-1 (0.1µM) produced a time-dependent increase in O-GlcNAc levels and decreased expression of *O*-GlcNAc transferase (OGT) and β-N-acetylglucosaminidase (OGA), key enzymes in the O-GlcNAcylation process. Overnight treatment of aortas with ET-1 increased phenylephrine (PE) vasoconstriction [Emax (mN)= 19±5 vs. 11±2 vehicle]. ET-1 effects were not observed when vessels were previously instilled with anti-OGT antibody or after incubation with an OGT inhibitor (ST045849, 100µM). Aortas from DOCA-salt rats, which exhibit increased prepro-ET-1, displayed increased contractions to PE and augmented levels of O-GlcNAc proteins. Treatment of DOCA-salt rats with an atrasentan (ET_A antagonist) abrogated augmented vascular levels of O-GlcNAc and prevented increased PE vasoconstriction. Aortas from rats chronically infused with low rate of ET-1 (2pmol/kg/min, 14days) exhibited increased O-GlcNAc-proteins and enhanced PE responses [Emax (mN) = 18±2 vs. 10±3 control]. These changes are similar to those induced by PugNAc (OGA inhibitor which increases O-GlcNAc levels). Systolic blood pressure (mmHg) was similar between control and ET-1-infused rats (117±3 vs. 123±4; respectively). ET-1 as well as PugNAc augmented contractions to PE in endothelium-denuded rat aortas, an effect that was abolished by the Rho kinase inhibitor Y-27632. Incubation of VSMCs with ET-1 did not change expression of ROCK-α, ROCK-β, CPI-17, MYPT-1 or MLC, but increased phosphorylation levels of MYPT-1 (Thr^{853}), CPI-17 (Thr^{38}) and MLC (Thr^{18}/Ser^{19}). The effects of ET-1 on MYPT-1, CPI-17 and MLC phosphorylation were prevented by the OGT inhibitor and OGT siRNA transfection, as well as by atrasentan. ET-1 increased RhoA expression and activity in VSMCs, and this effect was abolished by OGT siRNA transfection and OGT inhibition. ET-1 also augmented expression of PDZ-Rho GEF and p115-Rho GEF in VSMCs and this was prevented by OGT siRNA, OGT inhibition (ST045849) and ET_A receptor blockade (atrasentan). In conclusion, our data strongly suggest that ET-1 augments O-GlcNAc levels and this modification contributes to increase vascular contractile responses, via activation of the RhoA/Rho-kinase pathway. The modulatory effect of ET-1 on O-GlcNAcylation may represent a novel mechanism underlying the vascular effects of the peptide. **Financial Support**: FAPESP. **Protocol of the Animal Use Ethic**: (007/04/CEEA)

01.013

Intravascular danger signals guide neutrophils to sites of sterile inflammation. Menezes GB[1], Braedon B[2], Pittman K[2], Teixeira MM[3], Kubes P[2] [1]UFMG − Morfologia, [2]University of Calgary − Immunology, [3]UFMG − Bioquímica e Imunologia

Introduction: Neutrophils are recruited from the blood to sites of inflammation where they contribute to wound healing, but may also mediate tissue damage. As such, uncontrolled neutrophil recruitment in response to sterile inflammatory stimuli is a fundamental contributor to the immunopathology observed in many diseases including ischemic injuries/infarction, trauma, autoimmunity, drug-induced liver injury, and many others. Therefore, understanding the mechanisms that allow neutrophils to respond to sterile tissue injury and cell death is fundamental to our understanding of both homeostatic innate immune functions and pathogenic immune responses in disease. Using confocal intravital microscopy, we examined the kinetics and molecular mechanisms of neutrophil recruitment to sites of focal hepatic necrosis *in vivo*. **Methods**: To investigate mechanisms of hepatic neutrophil recruitment to sterile lesion, we developed a novel surgical approach generating a small thermal injury in the liver surface and imaging the injured site for 4 hours under intravital confocal microscopy. Genetically manipulated mice strains (and blocking antibodies) were used to study the role of adhesion molecules (ICAM1, Mac1, LFA1 and CD44), chemokines (MIP-2 and KC) and DAMPs (ATP and formylated peptides). Adhesion, crawling and morphology of eGFP-expressing neutrophils (Lysm-eGFP mice) within hepatic sinusoids were analyzed by Velocity and ImageJ softwares. In another series of experiments, human circulating PMN were harvested to *in vitro* agarosis chemotaxis assay, using necrotic HEK 293 cells as a chemotactic stimulus. This project was previously approved by CETEA/UFMG (113/09). **Results and discussion**: Neutrophils crawled precisely to the focus of lesion using the intravascular route, which started after 1 hour and was maintained at least until 4 hours after lesion. Sterile injury generated danger associated molecular patterns (DAMPs), such as ATP, which activated the NLRP3 inflammasome, via P2X7 receptor. Apyrase (ATPase) treatment, NLRP3 -/-, ASC -/- and P2X7 -/- mice confirmed these observations. Inflammasome activation generated an inflammatory microenvironment, via IL-1beta release, that alerted circulating neutrophils to adhere within liver sinusoids. Subsequently, *in vivo* immunostaining revealed that an intravascular chemokine gradient (MIP-2 and KC) was generated to directed neutrophil migration within the vasculature through healthy tissue en route to foci of damage. Finally, "necrotactic" formyl peptide (FP) signals released from necrotic cells superseded chemokine signaling to guide neutrophils through a barrier of non-perfused platelet-plugged sinusoids into areas of injury, as revealed by using FPR1 -/- mice and FPR1 antagonists (Boc1 and cysclosporin H). Leukotriene B4 and PAF were not chemoattractants in this model. Using an *in vitro* agarose chemotaxis assay, human necrotic cells potently attracted human neutrophils, and this attraction superseded CXCR2 signals, as incubation of neutrophils in IL-8 did not inhibit chemotaxis towards necrotic cells. Using concentrations of anti-FPR1 antibody or cyclosporine H that abrogated chemotaxis towards the bacterial formyl peptide f-Met-Leu-Phe, we observed significantly attenuated neutrophil chemotaxis towards human necrotic cells. Thus, dynamic *in vivo* imaging revealed a multi-step hierarchy of directional cues that guide rapid and precise neutrophil localization to sites of sterile inflammation, indicating a potential therapeutic role for FPR1 antagonists in the treatment of liver diseases. **Financial Support**: INCT-Dengue (CNPq), CAPES and FAPEMIG.

01.014

Modulation of VEGF effects by a synthetic analogue of 15-epi-lipoxins: involvement of the enzyme heme oxygenase-1. Vieira AM[1], Barja Fidalgo TC[2], Fierro IM[2] [1]DFP-UERJ, [2]UERJ − Farmacologia

Introduction: Lipoxins (LX) and 15-epi-LX (ATL) are eicosanoids generated via biosynthetic routes that have anti-inflammatory and pro-resolution bioactions. Heme oxygenase (HO)-1, the enzyme responsible for heme catabolism generates metabolites with anti-inflammatory and antiapoptotic actions. Our group previously demonstrated that a synthetic analog of ATL, 15-epi-16-(para-fluoro)-phenoxy-lipoxin A_4 (ATL-1), induces the expression of HO-1 in endothelial cells (EC). Besides, we also demonstrated that ATL-1 inhibits multiple steps of vascular endothelial growth factor (VEGF)-stimulated angiogenesis. However, the role of HO-1 in the angiogenic process is still controversial. In order to better understand the effects of ATL-1 and the angiogenic role of HO-1, the purpose of this study was to investigate the involvement of this enzyme in the effects of ATL-1 in EC, analyzing angiogenic factors such as VEGF, angiopoietin (Ang)-1 and chemokines as monocyte chemoattractant protein (MCP)-1. **Methods**: ECV 304 cells were pre-treated with ATL-1 (100nM) for 30 min and treated with VEGF (10ng/mL) for different periods of time. Protein levels of HO-1, Ang-1, Tie-2 were detected by western blot analysis and the mRNA levels were quantified by RT-PCR. To evaluate whether HO-1 can influence the expression of Ang-1 we performed western blot and immunocytochemistry analyses. The cells were pretreated with SnPPIX (50mM, an inhibitor of HO-1 activity) for 30 min and then incubated with ATL-1 before stimulation with VEGF. To investigate MCP-1 secretion, the levels of this chemokine in the supernatants were analyzed by ELISA. The mRNA levels for MCP-1 and HO-1 were also quantified by RT-PCR. In this work we used an immortalized cell line commercially available instead of primary cells. Research studies involving immortalized cells do not require ethical review by a Human Research Ethics Committee. **Results**: VEGF induced HO-1 mRNA expression in EC and this was inhibited by ATL-1. In addition, the induction of the growth factor Ang-1, or its receptor Tie-2, by VEGF was partially inhibited by the analog. HO-1 activity appears to be important for VEGF effect, since the treatment of the cells with SnPPIX abolished the expression of both Ang-1 and Tie-2. Furthermore, VEGF induced the transcription of mRNA and secretion of MCP-1, an effect decreased by ATL-1. The co-incubation of the cells with SnPPIX did not prevent the analog effect, however, inhibited VEGF-stimulated MCP-1 expression. **Discussion**: HO-1 activity is important for VEGF effects, since the inhibition of this enzyme decreased the expression of Ang-1 and Tie-2, as well as MCP-1. This study can contribute to a better understanding of diseases that involve the resolution of inflammation and the angiogenic process. **Supported by**: FAPERJ, CNPq and CAPES.

01.015

ATL-1, a synthetic analog of 15-epi-lipoxin A4, modulates foam cells activation: a novel potential tool for atherosclerosis treatment. Niconi-de-Almeida Y[1], Simões RL[1], Barja Fidalgo TC[2], Fierro IM[2] [1]UERJ − Farmacologia e Psicobiologia, [2]UERJ − Farmacologia

Introduction: Atherosclerosis is the major cause of death related to cardiovascular disease and now recognized as an inflammatory disease involving the vascular wall. Inside the blood vessel, atherosclerotic plaques are composed mainly by lipids, macrophage/foam cells and a necrotic core. The foam cell formation, occurring through oxidized LDL internalization by macrophages, is the origin of atherosclerosis. Foam cells are accumulated in the walls of blood vessels increasing inflammation response and atherosclerosis development. Monocyte chemotactic protein (MCP)-1 is a main chemokine responsible for monocyte recruitment and consequent foam cell formation, leading to the progression of atherotic plaque. Lipoxins (LX) and 15-epi-LX, a distinct group of arachidonic acid metabolites, are characterized as anti-inflammatory lipid mediators with potent effect in the resolution phase of inflammation. LX and their stable analogs act via interaction with a specific receptor, named ALX/FPR2. Recent evidences point to the involvement of these lipids in cardiovascular diseases. This work investigates the effect of ATL-1, a stable analog of 15-epi-lipoxin A4, in foam cells activation. **Methods**: Monocytes were isolated from healthy donors. A license request for Human Research Ethics Committee was submitted and it is on judgment. Cells were cultured for 7 days to maturate into macrophages, followed by incubation with oxidized LDL for 3 days inducing foam cells differentiation. To investigate which mechanisms are involved in foam cells activation, these cells were pretreated with specific inhibitor for NFkB (PDTC – 100nM), as well as ALX antagonist (Boc-2 – 100mM) before treatment with ATL-1 (10nM). Whole cell extracts were obtained and the samples submited to western blotting. MCP-1 levels were determined by ELISA. **Results and Discussion**: We demonstrated that foam cells express ALX, which is upregulated by ATL-1 stimulation. The analog, within 1 hour post-treatment, increased ERK-2 phosphorylation, an important protein involved on the atherosclerotic process. Furthermore, ATL-1 prevented IkBa degradation, withholding NFkB translocation, a transcriptional factor related to atherosclerosis development. Foam cells release higher levels of MCP-1 when compared to macrophages. The treatment of the cells with ATL-1 decreased chemokine secretion, the first evidence of an anti-atherosclerotic effect of lipoxins. **Conclusion**: These results show for the first time the effects of ATL-1 in foam cells, highlighting an important role for lipoxins in atherosclerosis and suggesting that these lipids may become a new tool in reducing the development and progression of this important disease. **Financial support**: FAPERJ, CNPq, SR-2/UERJ

01.016

Characterization of the antimuscarinic effect of LASSBio-767 in HT-29 cells. Gambôa NF[1], Pimentel LSB[1], Fraga CAM[2], Barreiro EJ[2], Bolzani V[3], Castro NG[1] [1]ICB-UFRJ Farmacologia Molecular, [2]FF-UFRJ − LASSBio, [3]UFRJ − LASSBio, UFRJ, [3]NuBBe-UNESP−Araraquara − Química Orgânica

Introduction: Central cholinergic hypofunction is a hallmark of the initial stages of Alzheimer's disease and is the target of current therapy with anticholinesterase drugs. LASSBio-767 was recently described as a semi-synthetic cholinesterase inhibitor orally effective and which presents a remarkable balance between central anticholinesterase activity and low peripheral toxicity in rodents (Castro et al. *Eur. J. Pharmacol.,* 580:339, 2008), which is not common among cholinesterase inhibitors available for treatment of AD. We have investigated the action of LASSBio-767 on the type 3 muscarinic receptor (M3), because these receptors are widely distributed in the peripheral nervous system and increase salivation, gastrointestinal motility and lacrimation (Abrams et al. *Br. J. Pharmacol*., 148:565-78, 2006). We performed Ca^{2+} assays by ratiometric microfluorimetry in continuously perfused cells and population fluorimetry in microplates. The latter has the advantage of allowing a quantitative analysis more efficiently, yielding a concentration-response curve and IC_{50} estimates of the substance. **Methods**: Human colonic epithelial adenocarcinoma HT-29 cells natively expressing the M3 were used in both experiments. Cells were plated onto glass coverslips or 96-well plates and were loaded with the calcium indicators fura-2 AM or fluo-4 AM, respectively. The coverslips were mounted in a small-volume perfusion chamber and the fluorescence intensity was measured by a photodiode-based dual-excitation optical system on an upright microscope with a 40x water immersion objective. Compounds were applied in 30-second pulses by switching between perfusing solutions. For inhibition tests, the antagonist was applied alone for 30 s before switching to the solution that also contained the agonist. The cells in the 96-well plates were evaluated in the presence of carbachol 10 microM and in the absence or in the presence of LASSBio-767. Maximum fluorescence was obtained by the addition of ionophore A23187-I 1 microM. All the experiments were performed in triplicate. **Results and Discussion**: The HT-29 cells showed a concentration-dependent increase in intracellular calcium concentration when stimulated by carbachol with EC_{50} of 5 microM. LASSBio-767 up to 100 microM did not induce calcium changes in either experiment. However, this compound inhibited the calcium responses induced by carbachol, with 50% inhibition between 1 and 10 microM in the microfluorimetry. The percent inhibition by LASSBio-767 at 10 microM was smaller with 500 microM than with 100 microM carbachol, suggesting a competitive antagonism. The substance also presented a concentration-dependent inhibition of the calcium responses induced by carbachol 10 microM in the microplate assay with IC_{50} of 0.20 microM. This additional M3 antagonism seems ideally suited to minimize gastrointestinal intolerance in the setting of Alzheimer's disease therapy. **Support**: Finep (Ações Transversais) and Apsen Farmacêutica

01.017

In vitro characterization of six new 1,4-benzodiazepines compounds. Thibaut JPB[1], Vieira RO[1], Menezes CMS[2], Barreiro EJ[2], Lima LM[2], Noel F[1] [1]UFRJ − Farmacologia Básica e Clínica, [2]FF-UFRJ − LASSBio

Introduction: Benzodiazepines (BZP) are central nervous system depressants, being one of the most prescribed drugs in the world. Although well tolerated in therapeutic doses, their chronic use presents clinical issues such as dependence, rebound anxiety, memory impairment and discontinuation syndrome. As the different behavioral responses to BZP are mediated by specific receptor subtypes, the search for new compounds with a more restricted activity profile (anxiolytic effect without sedation, for example) is currently a hot topic in medicinal chemistry. The objective of present work was to characterize the binding profile of new benzodiazepines. **Methods**: Six new N1-modified clonazepam analogues were synthesized using clonazepam as starting material and exploring a chemoselective N-alkylation. To estimate the affinities of our compounds, we performed competition assays using 0.2 nM [^{3}H]flunitrazepam or 0.5 nM [^{3}H]PK11195 as ligands for the central and peripheral benzodiazepine receptors (PBR), respectively. Rat brain (without cerebellum) or cerebellum synaptosomes were used as sources of receptors containing mainly the alpha 2 or alpha1 subtypes of the $GABA_A$ receptors, respectively. For the PBR assays, we used membrane preparations form rat kidneys. To investigate the intrinsic efficacy at the central BZP receptors, we performed competition curves for [^{3}H]-flunitrazepam binding in a washed preparation of brain synaptosomes, in the absence and presence of 200 µM GABA (GABA-shift assay) and observed if the curve were shifted to the left (agonists), to the right (inverse agonists) or superimposed (antagonists). **Results and Discussion**: Analysis of ^{1}H NMR spectra confirmed the substitution of the hydrogen present at the N_1 atom of clonazepam by ethanoic, methyl, allyl, benzyl and carboxymethylene groups. The affinity profile presented by each benzodiazepine showed that substitution at the N1 caused substantial variations in the affinity and selectivity towards the central and peripheral BZP receptors. The GABA-shift (GS) assay showed that BZP substituted with CH3 and CH2CH=CH were agonists (GS≈3,0); CH2CO2H, CH2Ph and CH2CO2Me were parcial agonists (GS≈1,4); CH2Co2Et was antagonist (GS=1). The BZP N1-substituted by CH2CO2H and CH2CO2Me had a 10-50 fold lower affinity for the central BZP receptors present in the cerebellum (mainly alpha1 subtype) than for the BZP receptors present in the brain synaptosomes, indicating a higher affinity for the subtypes alpha 2 or alpha 5. Our results indicate that some of our new BZP could have anxiolytic activity with less sedation. In vivo assays with mice are now being designed in order to test such hypothesis. **Supported by**: CAPES, FAPERJ, CNPq

01.018

Signaling function of Na/K-ATPase in ouabain-induced a decrease in LPS inflammation model *in vivo*. Kinoshita PF[1], Yshii LM[1], Sa Lima L[1], Davel APC[2], Rossoni LV[2], Kawamoto EM[1], Scavone C[1] [1]ICB-USP − Farmacologia, [2]ICB-USP − Fisiologia e Biofísica

Introduction: Ouabain, which is a compound extracted from *Strophantus gratus* has also been described as a new hormone synthesized in the adrenal cortex and hypothalamus. Ouabain inhibits Na,K-ATPase activity and in pathological conditions can cause enzyme dysfunction in Central Nervous System (CNS). Na,K-ATPase has an important role in the maintenance of the ionic homeostasis and also it has other cellular functions such as gene expression and cell growth. Objective: This study aims to evaluate the alterations induced by a low dose of ouabain in an inflammation model induced by LPS injection in rats. **Methods**: Adult male Wistar rats were separated in 4 groups which received acute administration of ouabain (1.8mg/kg, i.p.) or saline 20 minutes before the injection of LPS (200 mg/kg, i.p.) or saline. Some animals were submitted to the catheterization of carotid artery to assess the arterial blood pressure values before and after ouabain administration. Two hours after the LPS injection animals were killed by decapitation following procedure approved by the Ethical Committee for Animal Research (CEEA) of the Biomedical Sciences Institute of the University of São Paulo (registered under number 77 in the pages 72 of the book 2, 2009), and hippocampus was dissected. Na,K-ATPase activity was measured by colorimetric assay (Kawamoto et al., Neurobiology of Aging, 9:1149-1159, 2007). Semi-quantitative RT-PCR was done to measure mRNA expression of inducible NOS (iNOS), tumor necrosis factor (TNF) and B-cell leukemia/lymphoma 2 (BCL2) and BAX mRNA level (Munhoz et al., Journal of Neuroscience, 26(14):3813-20, 2006.). **Results**: Ouabain administration did not alter arterial blood pressure of rats when compare to control group. Data also showed that Ouabain when given before LPS reduced the mRNA expression of iNOS (OUA+LPS= 0.60±0.22 vs Control=0.44 vsLPS=1.59±0.23, $p<0.01$), IL-1b (OUA+LPS= 1.34± 0.37vs Control= 0.35vs LPS=2.41±0.18, $p<0.01$), and the BAX/BCL2 ratio (OUA+LPS=0.62 ± 0.08 vs Control=0.99vsLPS=1.813±0.14, $p<0.05$). At this concentration, ouabain did not alter Na,K-ATPase activity in hippocampus. Statistical comparisons for RT-PCR and Na,K-ATPase assays were performed by one-way ANOVA, followed by the Newman-Keuls test. Blood pressure was analysed by Student´s t-test. **Discussion**: Our results suggest that a low dose of ouabain which does not cause alteration on Na,K-ATPase activity seems to have an important anti-inflammatory effect in rat hippocampus. Therefore, the present findings suggest that Na,K-ATPase in addition to this established role as an ion pump, can also function as a signal transducer modulating genes that may play important roles in adaptative neuroplasticity and neuroprotection. **Financial support**: FAPESP, CNPq.

01.019

Protective effects of resveratrol on hepatotoxicity induced by antituberculosis drugs. Nicoletti NF[1], Santos Jr AA[2], Rodrigues-Junior VS[2], Campos MM[3], Leite CE[4], Basso LA[2], Santos DS[5], Souto AA[6] [1]INCTTB-PUCRS − Biologia Molecular e Funcional, [2]INCTB-PUCRS, [3]PUCRS, [4]PUCRS − Toxicologia, [5]PUCRS − Farmácia, [6]PUCRS − Química

Introduction: Tuberculosis (TB) is a major infectious disease that causes nearly two million deaths every year. Isoniazid (INH) and rifampin (RIF) are first line drugs used to prevent and treat TB. RIF has been extensively reported to exacerbate the hepatotoxicity caused by INH in patients with TB. Anti-TB drug-induced hepatotoxicity causes substantial morbidity and diminishes treatment effectiveness because they contribute to non-adherence, treatment failure and the emergence of drug-resistance (Tostmann *et al*., *Gastroenterol Hepatol*., 23, 192, 2008). Resveratrol (RSV) is a naturally occurring polyphenol with significant anti-inflammatory and antioxidant properties. Moreover, hepatoprotective effects for RSV have been previously demonstrated (Baur *et al*., Nat Rev Drug Discov., 5, 493 ,2006). In this work we have investigated the effects of RSV in the hepatotoxicity caused by INH and RIF in mice. **Methods**: All the experimental protocols were approved by the local Animal Ethics Committee (09/00107). Mice were distributed into four groups: (i) control, (ii) RSV-treated control, (iii) INH-RIF, and (iv) RSV-treated + INH-RIF. Liver damage was acutely induced by dosing INH (50 mg/kg) and RIF (100 mg/kg) in male BALB/c mice (25–30 g), both p.o., for three consecutive days. RSV (100 mg/kg) was administered orally 30 min prior to INH-RIF administration, and 2 times daily, each 6 h, until the third day after hepatotoxicity induction. Serum biochemical tests for liver function, histopathological examination, oxidative stress tests and myeloperoxidase (MPO) activity of liver tissues were determined. **Results**: Treatment of mice with INH+RIF induced hepatotoxicity as it was evidenced by elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Histopathological examination of liver tissue showed steatosis and increased apoptosis in the animals that received anti-TB drugs, which was accompanied by a marked increase of tissue MPO activity. As expected, catalase and glutathione activities were found to be reduced by INH-RIF treatment. The administration of RSV significantly decreased ALT and AST levels (58 ± 4 % and 36 ± 8 %, respectively), as well as the MPO activity (19 ± 3 %). Furthermore, the treatment with RSV completely reverted the decrease of both catalase and glutathione activities, and generally ameliorated the histopathological alterations associated to hepatotoxicity evoked by anti-TB drugs. **Discussion**: Recent data pointed out RSV as an antioxidant and anti-inflammatory agent able to prevent acetaminophen-induced hepatic failure in mice. Other studies show that RSV reduced mortality and liver damage induced by alcohol and partial hepatectomy in rats. In conclusion, the present study demonstrates, for the first time, that RSV protects against hepatotoxicity induced by INH-RIF. It is tempting to suggest that this natural compound might be useful for the treatment of liver injury due to anti-TB drugs. **Financial support**: CNPq-INCT, BNDES.

01.020

The time-points of *Bothrops lanceolatus* venom molecular effects on rat gastrocnemius.

Barbosa-Souza V[1], Contin DK[1], Bonventi W[2], Lôbo de Araújo A[1], Irazusta SP[1], Cruz-Höfling MA[3] [1]UNICAMP − Farmacologia, [2]CEETEPS, [3]IB-UNICAMP − Biologia Celular e Estrutural

Introduction*:* Poor muscle regeneration following intoxication by venomous snakebites results from fast and loss of sarcomeric proteins and deficit in protein re-synthesis and fiber regeneration. Cytokines and growth factors are key-mediators in the process. The knowledge of cellular signaling molecules has long been a matter of interest for understanding the severe local envenoming disturbances. *Methods*: The follow-up of CD68, osteopontin (OPN) and myogenin expression was studied by immunohistochemistry (IHC) at 1, 3, 6, 18 hours, 1, 2, 3, 7, 14 and 21 days after *B. lanceolatus* venom injection (100µg/100µl PBS) or PBS (100µl) in rat gastrocnemius. OPN immunolabeling was measured by GIMP 2.6.4 software (CNET Networks, Inc. Australia), whereas CD68 and myogenin positive fibers were counted in 10 tissue fields (one-way ANOVA plus Bonferroni, $p<0.05$). The research was approved by CEUA-IB-UNICAMP, protocol 945-1. **Results and Discussion**: CD68 protein, a marker of resident macrophages, achieved its apex at 24 h (461.7±144.7); OPN increased from 1 to 6 hours, stood still at 6-24 hours interval, and increased again up to 72 hours. The highest OPN expression in muscle fibers occurred at 3 (31±3.1), 7 (27±1.2) and 14 days (24.2±3.2%). Double CD68-OPN labeling in tissue showed that both protein were co-expressed by resident macrophages demonstrating by the first time the secretion of OPN by macrophages and muscle fibers in snake envenoming. Myogenin presented significant higher expression values at the 18 h-14 d interval, with peak attaining on day 7 (152.6±60.5). Our findings showed myogenin being expressed both in cytoplasm and nucleus of both myoblasts and myotubes. Higher figures of activated macrophages coincided with the onset and progress of muscle healing and gradual increase of pro-inflammatory cytokines such as OPN and high values of myogenin. OPN has been shown to be an adhesion glycoprotein molecule which expresses its adhesive domains through the presence of Arg-Gly-Asp (RGD) (Wang and Denhardt, Cytokine and Growth Factor Reviews 19:333, 2008) and is able to promote adhesion of myoblasts to other matrix proteins facilitating myogenesis (Uaesoontrachoon et al., *Int J Biochem Cell Biol* 40: 2303, 2008). Myogenin belongs to a family of transcriptional factors, collectively named as myogenic regulatory factors (MRF) that are key-signalings of activation, proliferation and differentiation of cells of the myogenic lineage. Myogenin is a marker of late stages of muscle differentiation. Concluding, the proteins studied were differentially expressed along the degenerative and regenerative time-points, permitting to infer about their role along the pathogenesis of changes caused by *B. lanceolatus* venom. The high expression of myogenin coincident with high levels of OPN and peak of resident macrophages gives strong evidence on their role in signaling pathways associated with activation, differentiation and remodeling processes underwent by skeletal muscle tissue after *B. lanceolatus* envenoming. **Support**: FAEPEX-UNICAMP/FAPESP/CNPq/CAPES

01.021

An autocrine/paracrine role for relaxin system in testis: Sertoli cell proliferation via activation of ERK1/2 pathway and a possible role in early steps of spermatogenesis. Nascimento AR, Pimenta MT, Royer C, Lucas TFG, Porto CS, Lazari MFM UNIFESP − Farmacologia

Introduction: Relaxin is an insulin-related peptide that activates the G-protein coupled receptor RXFP1. We previously reported higher levels of relaxin mRNA in testis of 15-day old animals than in adult rats and that Sertoli cells constitute an important source of relaxin in the testis (Lais CC. Reprod. 139; 185, 2010). In addition, expression of the protein relaxin has been detected in Sertoli cells of young and adult rats. Similarly, mRNA for RXFP1 has been localized to Sertoli cells and higher expression of the receptor has been immune-detected in late stage spermatids (Filonzi M. Reprod. Biol. Endocrinol. 5; 29, 2007). We hypothesized that relaxin could affect spermatogenesis through two main mechanisms: 1) a direct action of the hormone on Sertoli cell function; 2) a direct action of the hormone on germ cells in early and late stages of the spermatogenesis. **Methods**: All experimental procedures were approved by the Ethical Committee from UNIFESP (CEP1118). We first analyzed the effects of relaxin on the activation of the mitogenic pathways ERK1/2 and PI3K/AKT. Primary culture of Sertoli cells was obtained from 15-day old Wistar rats (Lucas TF. *Life Sci*. 75; 1761, 2004). Cells were incubated in the absence or presence of increasing concentrations of relaxin (25-200 ng/ml), for different periods (5, 10, and 30 min), at 35°C. To characterize upstream pathways to ERK1/2 phosphorylation, cells were previously treated with the inhibitor of the kinase activity of the EGF receptor (EGFR) AG1478 (50 μM for 15 minutes; N=6), the PI3K inhibitor wortmannin (1 μM for 30 minutes; N=2), or the general PKC inhibitor GF109203X (5 μM for 30 minutes; N= 4). ERK1/2 and AKT phosphorylation was determined by Western Blot analysis (Lucas TF. Biol. Reprod. 78; 101, 2008). To evaluate the possible action of relaxin on early steps of spermatogenesis we set up a co-culture of Sertoli and germ cells from 7-day old rats. Immunofluorescence with an anti-rat relaxin antibody (Abcam) and a secondary fluorescent antibody (Alexafluor) was conducted in 5-day old cultures. **Results**: Relaxin increased ERK1/2 phosphorylation in a time and concentration-dependent fashion. The peak of ERK1/2 phosphorylation occurred at 5 minutes, and with 50 ng/ml of relaxin (N=3). ERK1/2 phosphorylation was inhibited by pre-treatment with AG1478, wortmannin and GF 109203X. Incubation with relaxin (50 ng/ml) for 5, 10 or 30 min, failed to stimulate AKT phosphorylation. Analysis of the co-cultures by confocal microscopy revealed the expression of relaxin in cultured Sertoli cells and gonocytes from 7-day old rats. **Discussion**: Our results indicate that relaxin stimulates ERK1/2 but not AKT phosphorylation in rat Sertoli cells. Activation of ERK 1/2 phosphorylation seems to involve transactivation of EGFR, activation of PI3K and non atypical isoform(s) of PKC. The expression of relaxin in gonocytes suggests that the hormone may play a role in early steps of spermatogenesis, and the co-culture system will provide an important tool to investigate the role of relaxin in proliferation and differentiation of germ cells. **Financial support**: FAPESP, CAPES, CNPq.

01.022

β-adrenoceptor modulates skeletal muscle contraction by coupling to both Gs and Gi proteins: a new concept to cAMP signaling pathway. Rodrigues FSM, Bergantin LB, Pires-Oliveira M, Andrade-Lopes AL, Godinho RO UNIFESP − Farmacologia

Introduction: One of the most well known mechanisms to increase skeletal muscle contractile force relies on drugs that increase intracellular cAMP. Classically, intracellular cAMP accumulation is the result of activation of stimulatory G protein (Gs) coupled receptors, such as the □2 adrenoceptor, which in turn activates adenylyl cyclases (AC). Interestingly, phosphodiesterase inhibitors alone are also capable of enhancing contraction force without pharmacological activation of the Gas/AC/cAMP pathway, indicating that basal cAMP biosynthesis might be increased during contraction. In fact, β-adrenoceptors-mediated responses might be affected by complex pathway interactions. For example, in addition to coupling to Gs protein, human and murine heart β2-adrenoceptors can also couple to Gi protein (Heubach et al, *Mol Pharmacol* 65:1313, 2004). However, it is not known whether β-adrenoceptor agonists can activate alternative cell signaling pathways in skeletal muscle. In the present study we evaluated the effect of electromechanical activity on cAMP production in mice skeletal muscle. The effect of β-adrenoceptors activation on muscle contraction and its biased coupling to distinct G proteins was also evaluated. **Methods**: The effect of isoproterenol (ISO, β adrenoceptor agonist), IBMX (phosphodiesterase inhibitor) and SQ 22536 (AC inhibitor) on isometric contractility was studied in mouse diaphragm muscle under direct electrical stimulus (supramaximal voltage, 2 ms, 0.1 Hz; n = 3-6). To evaluate the effect of electromechanical activity on intracellular cAMP production, cAMP content was determined in diaphragm submitted or not to electrical stimulus, using a radiometric competitive binding assay to PKA regulatory subunits. **Results and Discussion**: Isoproterenol (3 nM-100 nM) increased by up to 51 % contraction force induced by electrical stimulation. However, this effect was attenuated when concentrations ≥ 300 nM were used, indicating a negative inotropic effect of Iso at high concentrations. Pre-incubation of diaphragm with the selective Gi protein inhibitor pertussis toxin (PTX, 1.0 μg/mL), prevented the negative inotropic effect of 1.0 μM isoproterenol, demonstrating that, at high concentration, isoproterenol decreases contractile force via activation of Gi protein. Addition of the non-selective phosphodiesterase inhibitor IBMX (50 and 100 μM) to the organ bath also increased by 10-20% the contractile force induced by electric stimulation, which was accompanied by parallel increase in cAMP production, an phenomenon inhibited by pre-incubation with SQ 22536. Conversely, IBMX failed to significantly increase intracellular cAMP content of untensioned diaphragm, indicating that skeletal muscle electromechanically stimulate AC and consequently increases muscle contractile force. In summary, our results show that isoproterenol has positive and negative inotropic effects on diaphragm muscle, respectively at low and high concentrations, which are consistent with activation of both Gs and Gi proteins. Besides, depolarization of muscle membrane is able to stimulate cAMP generation, which may affect the final contractile response. These new data redefine current concepts about cAMP signaling pathways in modulating muscle contraction. **Supported by**: FAPESP, CAPES and CNPq. **Ethical Committee**: 0011-08

01.023

Role of $P2X_7$ receptor during *Mycobacterium tuberculosis*-infection in mice. Santos Jr AA[1], Rodrigues-Junior VS[2], Coutinho R[3], Santos DS[2], Campos MM[4], Morrone FB[1]
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Introduction: Tuberculosis (TB) continues to be one of the deadliest diseases in the world. The emergence of multi-drug-resistant strains of *M. tuberculosis*, the unbearable side effects of the available drugs and the frequent patient non-compliance in completing the therapy have increased the need for the identification of new molecular targets and other pharmacological strategies to treat this disease. It was previously demonstrated that treatment of *Mycobacterium bovis* (BCG)-infected human macrophages with ATP induced $P2X_7$ receptor-dependent killing of intracellular Mycobacteria (Biswas et al., *BMC Immunology*, 9, 35, 2008). The aim of this study was to investigate the role of the purinergic $P2X_7$ receptor during *Mycobacterium tuberculosis*-infection in mice. **Methods**: Firstly, we have evaluated the expression of $P2X_7$ in the lungs of infected and non-infected animals. Male Swiss mice (6 per group, 25-30 g) were used for these experiments. All the experimental protocols were approved by the Local Animal Ethics Committee (CEUA 09/00094-PUCRS). The infection model was accomplished according to the methodology described by Chambers et al. (*Antimicrobial Agents Chemotherapy*, 49, 2816, 2005). The animals were anesthetized and received an intravenous injection of 200 ml of a *M. tuberculosis* suspension (H37Rv strain; 5×10^8 CFU/ml). Control mice received the same volume of saline. The procedures were carried out in a level III security cabinet. The infection was confirmed in a lung homogenate, by using the specific Ziehl-Neelsen staining. Parafin-embedded lung sections obtained after 28 days of infection were used for both histological and immunohistochemical analysis. As a second approach, we evaluated the response to infection in $P2X_7$ knockout mice (C57BL/6 $P2X_7^{(-/-)}$) and C57BL/6 wild type (WT), and compared the profiles in infected and non-infected groups. After 28 days of infection, these animals were sacrificed and the spleens and lungs were weighed and submitted to clinical evaluation. **Results**: *M. tuberculosis* inoculation in Swiss mice resulted in a striking inflammatory response of both lungs, characterized by marked macrophage infiltration and typical TB-related granulomes, according to the Ziehl-Neelsen staining. Of high interest, immunohistochemical analysis revealed a significant increase of $P2X_7$ receptor detection, especially around the granuloma region. This augmentation corresponded to 180±3 %, in comparison to control non-infected animals. We have also found that infected WT mice showed a marked increase in the spleen weight, in comparison to non-infected animals. Of high interest, *M. tuberculosis*-infected $P2X_7^{(-/-)}$ mice showed an increase of 56 ± 3 % in the spleen weight when compared to infected WT mice. **Discussion**: The evidence presented herein points out, for the first time, the $P2X_7$ receptor as one target molecule for understanding the pathogenesis of TB. Whether selective agonists or antagonists of this receptor might be useful for improving TB complications remains a matter to be investigated. **Financial support**: CNPq-INCT-TB, CAPES, BNDES, PUCRS.

01.024

Creb response after caloric restriction in a LPS inflammation model in rat hippocampus.

Vasconcelos AR, Sá Lima L, Kawamoto EM, Scavone C ICB−USP − Farmacologia

Introduction: Studies have shown that caloric restriction without malnutrition can promote cellular growth and survival by hormetic mechanisms involving activation of adaptive cellular stress response pathways (ACSRPs)(1). Gene expression regulation is essential to hormetic adaptive response. Although different stressors elicit unique signature responses, comparison of prototypical hormetic inducers has highlighted the role played by a few transcription factor families. Periodic pulsatile activation of cyclic AMP-regulatory element binding protein (CREB) has been found to be essential for obtaining beneficial effects of various hormetic stimuli in different biological models. CREB is believed to play a key role in promoting neuronal survival, precursor proliferation, neurite outgrowth and neuronal differentiation in certain neuronal populations (2). Additionally, CREB signaling is involved in learning and memory processes in several organisms (3). The current work investigated adaptative effects of caloric restriction diet on CREB activation in rat hippocampus in the presence and absence of inflammatory stimulus (LPS). **Methods**: Adult male Wistar rats were placed on diet intermittently for 30 days (every other day with and without food) followed by injection of LPS (1mg/kg, i.v.) or saline after one day with food. The rats were weighted before and after diet. Two hours after LPS injection animals were killed by decapitation following procedure approved by the Ethical Committee for Animal Research of ICB-USP (registered under number 89, page 60, Book 2, 2008), and the hippocampus was dissected. Nuclear extracts were isolated and gel mobility shift assay was used to measure changes in CREB binding activity with a radiolabeled CRE consensus oligonucleotide probe. Western blot assay was used to evaluate constitutive levels total CREB and p-CREB in nuclear extract. The same blot was stripped and reprobed with an anti–β-actin antibody to show equal loading of samples. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Student Newmam-Keuls posthoc-test for CREB binding activity and CREB phosphorylation; and Student´s t test for the body mass difference (Δ). *Significance was set at $p<0.05$. **Results**: Rats gained body mass after one month of diet (Δ = 23.2 ± 3.0 g*), which shows that malnutrition has not occurred, but this gain was lower than the one of the control group (Δ = 60.1 ± 3.0 g). Caloric restriction reduces CREB binding activity (12.4 ± 1.2 % of control*) and increases CREB phosphorylation (867.6 ± 95,4 % of control*). LPS induced activation of CREB binding activity (17.2 ± 1.2 % of control*) without changing CREB phosphorylation when compared to control group, but not in animals under caloric restriction. No change was found in total CREB protein expression. **Discussion**: Our results suggest that intermittent diet during 30 days modulates CREB signaling pathways decreasing LPS induced activation of CREB binding activity in rat hippocampus. Strategies as caloric restriction that regulates target specific CREB-regulated genes could bring new contributions to the treatment of neuropsychiatry disorders. References: 1. Mattson M.P. (2008) *Ageing Res*. Rev, 7: 43-48; 2; Lonze, B.E. et al., (2002) Neuron 34, 371-85; 3. Dash, P.K. et al. (1990) *Nature* 345, 718-21. **Financial support**: FAPESP, CNPq.

01.025

Ovariectomy does not modulate chronic unpredictable stress (CUS) potentiation of lipopolysaccharide-induced NF-kB activity in striatum of female Wistar rats. Sá Lima L[1], Porto CS[2], Scavone C[1], Carolina DM[1] [1]ICB−USP − Farmacologia, [2]UNIFESP – Farmacologia

Introduction: *Stress* may be an underlying cause of *cardiovascular,* neuropsychiatric, neurological, and neurodegenerative diseases. We have shown that chronic unpredictable stress (CUS) enhances the lipopolysaccharide (LPS)-induced NFkB activation in the frontal cortex and hippocampus of male rats via activation of glucocorticoid receptors (Munhoz et al. *J Neurosci* . 2006). Experimental and clinical evidence suggest that estrogen, now widely used in hormone replacement therapy in women post-menopause, could reverse cognitive impairment caused by stress and prevent diseases. In addition, estrogen appears to have an anti-inflammatory action, since this hormone can inhibit the activation of microglia, increase the expression of antioxidant genes, and also modulate some parameters of inflammatory response, such as the activation of NFKB. The present study aimed to verify the effects of estrogen on the CUS potentiation of LPS-induced NFkB activation in the striatum of female rats. **Methods**: All experiments were conducted in accordance with the ethical principles in animal research adopted by the Biomedical College of Animal Experimentation (102/06/CEEA ICB-USP). Adult Female Wistar rats (200 – 250g) were either intact (INT), or ovariectomized (OVX). Seven days later, rats were randomly assigned to either control or CUS groups. CUS paradigm was applied as described in Munhoz et al. (*J. Neurosci.;* 26(14):3813, 2006). Twenty-four hours after the last stress section, rats were injected with LPS (1mg/kg, iv) or saline (SAL) and sacrificed 1h later. NFKB activation in the rat striatum was verified by the Electrophoretic Mobility Shift Assay (EMSA). **Results**: In the female rat striatum, in the INT group, LPS (1mg/kg iv) increased NFKB activation (58%, $p<0.05$) when compared to INT-SAL and CUS potentiated the LPS-induced NFkB activation (LPS = 58%, CUS + LPS = 99.5%, $p<0.05$). In the OVX groups, the absence of estrogen further increased the LPS-induced NFKB activation (LPS = 58%, OVX LPS = 118.2%; $p<0.05$) when compared with INT LPS but did not alter the CUS potentiation of LPS-induced NFkB when compared with CUS + LPS group (CUS + LPS = 99.5%, OVX+CUS+LPS = 126,9%; $p<0.05$). **Conclusion** : LPS increased NFkB activity in the striatum of female rats which was potentiated by CUS. Estrogen seemed to have an anti-inflammatory role in this LPS effect since OVX further potentiated the LPS-induced NFKB activation in that brain structure. On the other hand, OVX was not effective in modulate the CUS induced pontentiation of LPS effect on the striatum perhaps due to a ceiling effect of CUS in the brain. **Financial support**: FAPESP, CNPq and *Procontes-USP.

01.026

Effect of N^1-acetyl-N-formyl-5-methoxykynura-mine (AFMK) on the production nitric oxide by cultured endothelial cells. Freitas AH, Tamura EK, Markus RP IB-USP − Fisiologia

Introduction: Melatonin (MEL) blocks the expression of inducible nitric oxide synthase (iNOS) in endothelial cells stimulated by lipopolysaccharide (LPS) in the μM range (TAMURA, J Pineal Res, 46:268, 2009). Polymorphonuclear cells activated by LPS convert MEL to AFMK in concentrations compatible for blocking tumor necrosis factor (TNF) and interleukin-8 (IL-8) (Silva, *J Neuroimmunol*, 156:146, 2004). In macrophages, AFMK blocks LPS-induced activation of cyclooxigenase 2 (Mayo, *J Neuroimmuno*l, 165:139, 2005). As a matter of fact, AFMK has a higher efficiency than MEL in this process. Otherwise, MEL, but not AFMK, blocks the activity of the neuronal NOS obtained commercially or extracted from rat striatum (Léon*, J Neurochem*, 98:2023, 2006). Taking into account that LPS-induced expression of TNF, IL-8, COX-2 and iNOS is mediated by the same transduction pathway, i.e., the nuclear factor kappa B pathway, here we investigated whether AFMK could block the induction of iNOS in cultured endothelial cells challenged with LPS. **Methods**: Primary cultures of endothelial cells were obtained from cremaster muscle of rats (TAMURA, J Pineal Res, 41:267, 2006). All animal procedures were performed according to protocols approved by the IB-USP CEUA (048/2007). Endothelial cells subcultured for up to two passages were seeded onto glass coverslips (NO measurement) or chamber slide (8 wells − immunofluorescence) and allowed to grow for 48 hr. The cells were incubated with LPS (1 μg/mL), AFMK (1 μM) or LPS+AFMK for 2h. Endothelial cells were loaded for 50 min with DAF-FM (5 μM) for determination of NO by confocal microscopy. The expression of iNOS was determined by immunofluorescence assay using anti-iNOS TRITC-conjugated. All the results are expressed in % relative to control group (100%). **Results**: AFMK inhibited (444.0 ± 47.8%, n=4) LPS-induced NO production (893.3±62.4, n=4). However, AFMK alone increased the production of NO (909.7±78.1, n=3). AFMK was also able to reduce (105.5±7.5%, n=3) the LPS-induced increase in iNOS expression (173.8±12.9%, n=3). However, AFMK alone did not change expression of this enzyme (123.10±10.3%, n=3). **Discussion**: Our results clearly show that in endothelial cells the metabolite of melatonin, AFMK, is also able to inhibit the expression of iNOS and the production of NO induced by LPS. This confirms the anti-inflammatory effect of this kynurenine. However, it was really interesting to note that AFMK per se could lead to an increase in the content of NO, without changing the expression of iNOS. Although it was observed that AFMK did not inhibit nNOS, we cannot exclude the possibility that this kynurenine may modulate the activity of eNOS.

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01.027

Reactivity of endothelial cells in culture is conditioned by nocturnal melatonin surge in donor rats. Marçola M, Tamura EK, Fernandes PACM, Markus RP IB-USP − Fisiologia

Introduction: The endothelial cells are responsible for maintaining the vascular homeostasis and contribute for the mounting of inflammatory responses. They have a privileged location regarding the detection of circulating substances. We have previously shown that these cells, even after long-term cultures, retain information about the condition of the donor animal. Endothelial cells obtained from animals killed during nighttime are less able to adhere to neutrophils and express lower levels of adhesion molecules than those obtained from animals killed during the daytime (Marçola, *41º Cong. Bras. de Farmac. e Terap. Exper*., 2009). Nocturnal melatonin surge is due to activation of beta-adrenoceptors located in the pinealocytes. The phosphorylation of the transcription factor CREB (cyclic AMP regulating binding element) induces the transcription of the key enzyme in melatonin synthesis. In the present work we evaluated whether the inhibition of nocturnal melatonin surge by treating the animals with propranolol, could increase the expression of the adhesion molecules PECAM-1 and ICAM-1, and the ability of cultured endothelial cells to adhere neutrophils. **Methods**: Male adult rats, maintained in 12/12 light/dark cycle, were treated with two injections (one by day just before lights off) of vehicle or propranolol (20mg/kg) and sacrificed after 6 hours of the last injection. All animal procedures were performed according to approved institutional protocols (089/2009). The endothelial cells obtained from the cremaster muscle (TAMURA, J Pineal Res, 41: 267, 2006) were used after confluence (±14 days *in vitro)*. Neutrophil adherence was quantified by colorimetric *in vitro* assay, as described previously (LOTUFO, Eur. J. Pharmacol., 534: 258, 2006). The expression of adhesion molecules (PECAM-1 and ICAM-1) was determined by immunofluorescence. All the results are expressed in % relative to control group (100%). **Results**: The leukocyte-endothelium interaction was higher in animals injected with propranolol (152,61±15,88%, n=3) when compared to saline injected animals. A higher expression of the adhesion molecules was also obtained in propranolol treated animals (ICAM-1: 339,14±84,71%, n=4 and PECAM-1: 798,24±64,64%, n=4). Therefore, propranolol treatment increased the expression of ICAM-1 and PECAM-1 and the ability of endothelial cells to adhere neutrophils, even after 16 days in culture. **Discussion**: Our results, once more show that endothelial cells may be pre-conditioned by the state of the donor animal at the moment of the euthanasia. In addition, they suggest that the difference between day and night is due to the nocturnal melatonin surge, as the ability to adhere neutrophils and the expression of ICAM-1 and PECAM-1 is reduced in animals treated with propranolol and killed at night. The great relevance for understanding the preconditioning of endothelial cells is to obtain cells in a more quiescent state, in order to improve the result of cells obtained from banks of cells.

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01.028

The C-Rel subunit from nuclear factor kappa B (NFKB) family may play a differential role in peripheral melatonin synthesis. Lapa MAPC, Ferreira ZS, Markus RP [1]IB-USP − Fisiologia

Introduction: Colostral mononuclear cells stimulated with zimosan in vitro produces melatonin by a mechanism dependent in NFKB, as it is blocked by inhibiting the binding of NFKB dimmers to DNA (Lapa, 41º SBFTE, 2009). In contrast, the activation of pineal NFKB pathway leads to inhibition of melatonin synthesis (Cecon, Chronobiol Int., 27: 52, 2010). The NFKB dimers can be formed by subunits that has (Rel A, Rel B, c-Rel) or not (p52, p50) the transactivating domain. In the first case NFKB leads to gene transcription, while in the second it inhibits the production of mRNA. Regarding the pineal gland, the activation of the dimer p50/p50 is responsible for inhibiting the transcription of the key enzyme for synthesizing melatonin (aryl-alkil-N-acetyltransferase – Aa-nat) (Cecon, Chronobiol Int., 27: 52, 2010). Therefore, here we evaluated the possibility that zimosan leads to nuclear transcription of other subunits of NFKB in the colostral mononuclear cell. **Methods**: This project was approved by the ethical committee of Institute of Bioscience and Universitary Hospital (CEA/IB protocol 076/2007; CEP-HU/USP: 875/08, SISNEP CAAE: 0085.0.198.198-08). Colostrum was obtained from healthy puerperae (24-48 h after delivery) from the *Hospital Universitário* − USP. Isolated mononuclear phagocytes were ressuspended in RPMI 1640 medium (2.10^6 cell/mL) and activated with IgA-opsonized zymosan (opZ, 10 mg/mL, 5 or 90 min) and nuclear proteins were isolated. Determination of the NFKB subunits were done by ELISA commercial kits (Cayman Chemical, USA) for human NFKB subunits p50 and RelA or by electromobility super-shift assay. The nuclear proteins were incubated with 2 µg/mL of rabbit polyclonal affinity-purified antibodies selective for p50, Rel A, p52, c-Rel and Rel B (sc-114x, sc-109x, sc-298x, sc-70x, sc-226x, respectively, Santa Cruz, CA, USA) for 45 min before addition of P^{32}-NFKB probe. **Results**: No difference between noZ and opZ were observed. Five min after stimulation there was an increase in p50 (an increase of 57%, and 137% in noZ and opZ, respectively), and the appearance of Rel A and c-Rel subunits when compared to no stimulated cells. Ninety minutes after stimulation with opZ, p50 amount returned to control levels, while Rel A drops to values bellow control (0.5348 ± 0.1066, n=3 and 0.1853 ± 0.0147, n=3 for control and opZ respectively). The other NFKB subunits (p52 and REL B) were not found in the assays. **Discussion**: Activation of NFKB pathway in the pineal gland result in the inhibition of melatonin production, while in the mononuclear cells of the colostrum NFKB pathway activation triggers the production of this indolamine (Markus, Neuroimmunomodulation, 14: 126, 2007). Here, for the first time we disclose the possible mechanism for this discrepancy. In pinealocytes, nuclear translocation of the dimmers p50/p50, p50/Rel A was observed. The first is responsible for inhibiting the transcription of Aa-nat, while the second leads to the transcription of tumor necrosis factor. Our data show that a third subunit that is able to induce transcription of genes is activated only in mononuclear phagocytes (c-Rel), suggesting that this subunit could be responsible for inducing the transcription of the key enzyme in the melatonin biosynthetic pathway. **Support**: FAPESP (2007/07871-6), CAPES, CNPq.

01.029

NADPH oxidase mediates heme-induced cytoskeletal alterations, endothelial permeability and increased expression of adhesion molecules in HUVEC. Nascimento-Silva V[1], Morandi V[2], Barja Fidalgo TC[1], Arruda MA[3] [1]UERJ − Farmacologia, [2]UERJ − Biologia Celular, [3]FIOCRUZ − Farmanguinhos

Introduction: Heme is an essential molecule for life as we know it. It acts both as the prosthetic group of a number of proteins and as a signaling molecule that controls diverse molecular and cellular processes, from signal transduction to protein complex assembly. On the other hand, free heme can be hazardous when released at the extracellular milieu. In fact, free heme accumulation seems to correlate with the severity of a number of inflammatory/hemolytic conditions, as observed in atherosclerosis, renal failure and sickle-cell disease. Heme released during hemolytic episodes induces expression of endothelial adhesion molecules resulting in adherence of leukocytes and reticulocytes to endothelial cells. Our group has described that free heme acts as a prototypical proinflammatory molecule and this effect seems to rely on the activation of several redox-sensitive signaling routes through NADPH oxidase (NADPHox) activity. In this work, we aim to define the still underappreciated role of heme on vascular biology and the putative role of NADPHox activity on heme-induced endothelial cell activation, evaluating ROS production, changes in the cytoskeleton dynamics and vascular permeability. **Materials and Methods**: HUVEC (CONEP: 0009.0.325.000-09) were cultured on gelatin-coated glass coverslips overnight. Cells were then pre-treated or not with DPI (10μM) and incubated in the absence or in the presence of heme (3-30μM). ROS generation was assessed by dihydrorhodamine 123 (DHR) assay. Confocal and epifluorescence microscopy analysis was employed to detect VCAM-1 expression as well as NFκB and $p47^{phox}$ subcellular localization and cytoskeleton alterations. Protein expression was detected by Western blot analysis. **Results**: Heme (3-30μM) induces NADPH oxidase-dependent ROS production as assessed by DHR assay (Control: 11.6±1,5MFI; Heme30μM: 40.5±3.6MFI) ; Heme is also able to induce actin cytoskeleton alterations as well as focal adhesion kinase (FAK) phosphorylation and its subsequent association with actin. These events are also ruled by NADPHox-derived ROS. Importantly, heme induces VCAM-1 membrane expression and increases endothelial permeability (11±0.98% increase) via NADPHox activation, once DPI abrogated heme effect (90±7% inhibition). Furthermore, heme induced the translocation of the cytosplasmic NADPHox subunit $p47^{phox}$ to cell membrane (a parameter of NADPHox activation) and triggered NFκB nuclear accumulation, a critical step in several events associated with vascular pathologies. **Discussion**: We show, for the first time, a direct link between heme and NADPHox. Free heme, in a concentration range found during hemolytic episodes induces endothelial activation in a NADPHox-dependent manner. A better knowledge about the way heme modulates NADPHox activity may lead to the development of more precise therapeutic strategies designed to counteract endothelial dysfunction associated with intravascular hemolysis. **Financial Support**: FAPERJ, CNPq, SR-2/UERJ

01.030

Testosterone induces VSMC proliferation via P38-COX2-dependent, NFKB-independent pathways. Chignalia AZ[1], Munhoz CD[1], Yogi A[1], Camargo LL[1], Oliveira MA[1], Lopes LR[1], Rossoni LV[2], Carvalho MHC[1], Fortes ZB[1], Tostes RCA[1] [1]ICB-USP − Farmacologia, [2]ICB-USP − Fisiologia e Biofísica

Introduction and Objectives: Testosterone has been related to many cardiovascular diseases, including atherosclerosis. However, the mechanisms whereby testosterone contributes to the alterations observed in atherosclerosis are controversial. Here we investigated testosterone effects on cellular processes involved in atherosclerotic plaque formation, focusing on vascular smooth muscle cells proliferation. **Methods**: Aortic rabbit vascular smooth muscle cells (RASM) were stimulated with testosterone 10^{-8} to 10^{-7}M from 1 minute up to 24 hours. Whenever suitable, cells were stimulated with testosterone conjugated with bovine serum albumin 10^{-7}M or pre-incubated for 30 minutes with flutamide (10^{-5}M, androgen receptor antagonist), SB-203580 (10^{-5}M, p38 MAPK specific inhibitor), sodium salicylate (5x10-3M, inespecific inhibitor of NFkB and cyclooxigenases) and NS389 (10^{-5}M, COX2 specific inhibitor). Activation of p38MAPK was evaluated by immunoblotting. Expression of COX2 and TGFB were investigated by real-time PCR. NFkB activity was assessed by gel-shift mobility assay. Cell proliferation was investigated by the MTT and wound healing assays. **Results**: Testosterone and testosterone-BSA increased p38MAPK activation in a time- and concentration-dependent manner (1 fold, 10^{-7}M, 2 to 60 minutes, n=5). Expression of COX2 and TGF B were augmented by testosterone after 6 hours of stimuli (2.5 fold, 10-7M, n=6). NFkB activation was diminished by testosterone. Cell proliferation was augmented by testosterone after 24 hours of stimuli, an effect inhibited by flutamide, SB-203580, sodium salicylate and NS-398. **Discussion**: Our data indicate that testosterone induces RASM proliferation, which contributes to atherosclerotic plate formation. Testosterone-induced short-term effects (P38MAPK activation) occur via membrane-localized androgen receptor. Long-term effects induced by testosterone (expression of COX2 and TGFB; cell proliferation) are mediated by the classical androgen receptor. Considering that P38MAPK is necessary to testosterone-induced RASM proliferation, short-term effects are a key element to the development of cell proliferation (long-term effect). The decrease in NFkB activation suggests that there is a compensatory mechanism in response to the pro-inflammatory effects, which could involve the expression of non-inflammatory genes or the decrease of translation of pro-inflammatory genes. **Support**: FAPESP/CNPQ. **License Number (Ethics Committee on Animal experimentation)**: 036, page 15, book 2/2005.

01.031

Modulation of gastrointestinal epithelial cells activation by heme. Barcellos-de-Souza P[1], Nasciutti LE[2], Barja Fidalgo TC[1], Arruda MA[3] [1]UERJ − Farmacologia, [2]UFRJ − Histologia e Embriologia, [3]FIOCRUZ − Farmanguinhos

Introduction: Gastrointestinal epithelium (GE) works as an intrinsic barrier against microbial invaders. However, the role of GE in immunity is beyond physical intervention, since it is recognized that GE is able to regulate cellular mechanisms that can distinguish potentially pathogenic microorganisms from endogenous bacterial flora, as well as coordinate the proper biological response against them.
In many pathological situations arising from chronic inflammatory conditions such as gastritis and inflammatory bowel disease, GE epithelial cells are deprived of the protection of the mucus secreted by GE-specialized cells. In these circumstances, notably in gastric and duodenal ulcers, disruption of blood vessels and subsequent lysis of erythrocytes are common. This may lead to the release of high amounts of heme, which interacts with GE. Previous works from our group have shown that heme itself is a proinflammatory molecule, activating a number of phlogistic signaling events in a nicotinamide adenine dinucleotide phosphate oxidase (NADPHox)-dependent manner. In this study we aim to evaluate the effects of heme upon GE epithelial cells. **Methods**: A well established non-transformed rat small intestine epithelial cell lineage (IEC6) and a human gastric epithelial cell lineage (HGE3) were used in this study. Intracellular reactive oxygen species (ROS) generation was measured using a cell permeant, oxidation-sensitive dye CM-H_2DCFDA (10 μM). Cell proliferation was evaluated by tritiated thymidine (10 μM) incorporation to cell DNA. Wound healing assay was performed by scratching confluent cultures and then the number of cells that migrated to the injured area was counted. Total cell extracts were obtained for immunoblotting. **Results and Discussion**: Our results show that free heme, in concentrations easily found at hemorrhagic sites (about 20 μM), evokes intracellular ROS production by IEC6 and HGE3 cells, which is inhibited when cells are pretreated with diphenyleneiodonium (DPI, 10 μM), a NADPHox inhibitor. Heme, in NADPHox-activating concentrations, is involved with IEC6 and HGE3 proliferation and wound healing. FAK phosphorylation, which is related to several cellular responses, is increased by heme (for up to 2 hours) in a NADPHox activity dependent manner. These data indicate a prominent role for heme-derived signaling in the pathophysiology of gastrointestinal mucosa dysfunction. **Financial Support**: CNPq, FAPERJ, SR-2/UERJ, ABC/UNESCO/L'Oreal.

01.032

Schild analysis of the self cancelling effects of tricyclic antidepressants on alpha-1 adrenoceptor mediated responses. Nojimoto FD, Pupo AS UNESP − Farmacologia

Introduction and Rationale: The Schild analysis provides the equilibrium constant for binding (pK_B) of drugs which behave as competitive antagonists and is a valuable tool for the systematic study of drugs and receptors. A paramount condition that must be obeyed when using Schild analysis is that the slope parameter of the Schild plot should not be different from the theoretical unity, albeit slope not different from unity is not a guarantee that the equilibrium dissociation constant for the antagonist is valid (Kenakin & Beek, *JPET*, 232:732, 1985). This is best illustrated for drugs that inhibit a saturable agonist removal process and antagonise the receptor activated by the agonist (Pupo et al., *BJP*, 127:1832, 1999). Examples of such drugs include the tricyclic antidepressants (TCAs) amitriptyline (AMI) nortriptyline (NORT) and imipramine (IMI), which therapeutic effects result from increases in the synaptic levels of norepinephrine (NE) and 5-HT due to the inhibition of the transporters NET and SERT. These TCAs are competitive antagonists of α_{1A} and α_{1D} adrenoceptors (ARs) in the same concentration range necessary for the inhibition of NET, (Nojimoto et al., *Neuropharmacology*, *in press*, 2010). These combined properties may complicate the Schild analysis of antagonism, because there is a self cancellation of receptor antagonism by the sensitizing influence of the inhibition of agonist removal. As a consequence, it is predicted that the pK_B for these TCAs will be underestimated. **Objective**: This study was designed to test this hypothesis by determining the antagonism of α_{1A}-ARs of the rat vas deferens (VD) and α_{1D}-ARs of the rat thoracic aorta (TA) by the TCAs AMI, NORT and IMI in the absence and presence of cocaine, an inhibitor of neuronal NE uptake. **Methods**: All proceedings were approved by the Bioscience Institute/UNESP Ethical Committee for Animal Research (CEEA) (61/08). The competitive antagonist behaviour of AMI, NORT and IMI was determined by Schild analysis in NE concentration-response curves in the VD and TA in absence and presence of cocaine (6 μM). Additionally, the competitive antagonist behaviour of AMI, NORT and IMI was also determined against methoxamine, a selective α_1-AR agonist which is a poor substrate for neuronal uptake. **Results**: In absence of cocaine, AMI, NORT and IMI antagonized the contractions induced by NE in the VD (pK_B 5.7 to 6.5) and TA (pK_B 6.7 to 7.3) showing slopes in the Schild plots not different from 1.0 (0.9-10), but all three TCAs were ~10 more potent in the TA, a tissue where neuronal uptake is less active. In presence of cocaine, AMI, NORT and IMI antagonized the contractions induced by NE in the VD and TA showing slopes in the Schild plots not different from 1.0, but showing similar potencies in the VD and TA (pK_B= 6.5 to 7.5). The potencies estimated for AMI, NORT and IMI against methoxamine were similar to its respective potencies estimated against NE in presence of cocaine. **Conclusion** : When agonist removal process is active (ie neuronal uptake), the self cancelling effects of the TCAs AMI, NORT and IMI result in severe underestimation of the equilibrium constant for binding of these drugs at α_1-ARs and may lead to wrong conclusions on drug selectivity and receptor type involved in the pharmacological response. **Supported by** FAPESP (06/58828-0)

01.033

MicroRNA *let-7b* targets AKT-1 and regulates skeletal muscle atrophy in diabetic rats. Sousa TA[1], Kato M[2], Paula-Gomes, S.[1], Silva VAO[1], Tragante V[1], Zanon NM[1], Wang M[2], Kettelhut IC[1], Natarajan R[2], De Lucca FL[1] [1]FMRP-USP − Biochemistry and Immunology, [2]Beckman Research Institute − Gonda Diabetes Center

Introduction: The discovery of microRNA (miRNA) added a new layer of complexity in the regulation of gene expression. MiRNAs are endogenous non-coding RNAs (21-25 nucleotides) that regulate gene expression via specific sites at the 3'-untranslated region (3'-UTR) of target mRNAs, causing translational repression or mRNA degradation. It has been estimated that miRNAs regulate approximately 30% of human genes. During recent years, miRNAs have emerged as important regulators of a variety of biological processes as well as human diseases. Skeletal muscle atrophy is one of complications of diabetes, but the molecular mechanisms responsible for the atrophy process are still unclear. It is known that the IGF-1/Akt signaling pathway is an important survival pathway for skeletal muscle. Here, we hypothesize that Akt-1 could be negatively regulated by miRNAs in skeletal muscles of steptozotocin(STZ)-induced diabetic rats. Our *in silico* analysis indicated that Akt-1 is a potential target of *let-7b*. Thus, we investigate the expression of Akt-1 and *let-7b* in the *soleus* muscle of normal and diabetic rats, and the luciferase assay and the endogenous inhibition of Akt-1 expression by mimic *let-7b* were also performed to validate Akt-1 as a target of *let-7b*. **Methods**: *Soleus* muscles were obtained from normal and diabetic rats (*n*=6) at 1, 3, 5 and 10 days after intravenous injection of STZ (45mg/kg b.w.) (Approval Ethic Committee Nº 095/2009). Expression of mature *let-7b* was evaluated by real-time RT-PCR. Rat myoblast cells (L6) were used for transfection experiments. The inhibition of Akt by *let-7b* was determinated by luciferase assay using a Psichek-2 vector with the Akt-1 3´UTR (or Akt-1 3'UTR mutated in the region complementary to *seed* region of *let-7b)* cloned downstream *Renilla luciferase* gene before poly A site. The endogenous expression of Akt was evaluated by Western blot and real time PCR of L6 cells transfected with mimic *let-7b.* **Results**: We found that *let-7b* is overexpressed in *soleus* at 3 days (> 6-fold; $p < 0.05$) after injection of STZ compared with control animals. We also observed an inverse correlation of expression between *let-7b* and Akt-1 protein in *soleus* of diabetic animals. The results of luciferase assay indicate that *let-7b* interacts directly with the 3'UTR of Akt-1 mRNA, demonstrating that Akt-1 is a direct target of *let-7b*. This relevant finding was confirmed by using the assay of inhibition of endogenous expression of Akt-1 with mimic *let-7b*. **Discussion**: This work is the first demonstration of the involvement of a miRNA in skeletal muscle atrophy. The over-expression of *let-7b* in *soleus* muscle of diabetic rats results in down-regulation of Akt-1 which could result in subsequent inhibition of protein synthesis through reduction of mTOR activation and increase in proteolysis due to nuclear translocation of the transcriptional factor FOXO-3a. Since no effective treatment has not been established for skeletal muscle atrophy, the local injection of anti-*let-7b* (antagomir) could be a novel therapeutic strategy for this complication of diabetes. **Supported**: FAPESP.

01.034

Expression of MicroRNAS in skeletal muscle atrophy induced by fasting in rats. Tragante V, Sousa TA, Silva VAO, Zanon NM, Kettelhut IC, De Lucca FL FMRP-USP – Biochemistry and Immunology

Introduction: Muscle atrophy results from an increase in proteolysis associated with a decrease in protein synthesis. The IGF-1/Akt signaling pathway is essential for the maintenance of skeletal muscle mass. MicroRNAs (miRNAs) are non-coding RNAs (21-25 nt) that control gene expression through repression of translation or degradation of messenger RNA (mRNA) target. Current computational predictions suggest that miRNAs regulate the expression of 30% of human protein-coding genes. Recently, changes in expression of miRNAs have been described in several diseases. Here, we were interested in identifying miRNAs involved in the regulation of IGF-1 and Akt expression during the *soleus* muscle atrophy induced by fasting in rats. **Methods**: *Soleus* muscles were obtained from male Wistar rats (n = 6, 100-120g) that have undergone fasting for 48 hours with water *ad libitum* and rats that have been fed during this period (control group). The total RNA was extracted from muscle tissue with Trizol® (Invitrogen). The computational prediction of miRNAs that have IGF-1 and Akt as potential targets was performed using the program TargetScan (http://genes.mit.edu/targetscan). The expression of miRNAs was assessed by real time PCR. Statistical analysis was done using GraphPad Prism for *Student t* test unpaired. The value of $p = 0.05$ was considered significant. Authorization License from Animal Ethics Committee: No. 095/2009. **Results**: We observed a reduction of 29% in *soleus* weight of rats fasted when compared to control animals. Computational analysis revealed that IGF-1 and Akt are potential targets of miRNAs *let-7b*, *miR-101b* and *miR-150*. An interesting finding was that the expression of these three miRNAs increased 3-5-fold ($p < 0.05$) in *soleus* muscle of rats subjected to fasting (48h) when compared with the control group. **Discussion**: Results of this study suggest that increased expression of miRNAs *let-7b*, *miR-101b* and *miR-150* could inhibit the expression of IGF-1 and Akt, resulting in *soleus* atrophy in rats subjected to fasting. Additional studies are needed to elucidate the involvement of *let-7b*, *miR-101b* and *miR-150* in muscle atrophy induced by fasting, particularly the validation of IGF-1 and Akt as targets of these miRNAs. Recently, local injection of miRNAs in muscle was used as a therapeutic tool, accelerating muscle regeneration in models of muscle injury in rats. Thus, both miRNAs and their potential target genes (Akt-1 and IGF-1), could also be used as therapeutic targets in skeletal muscle atrophy which may be an alternative treatment for muscle atrophy resulting from catabolic diseases such as fasting, diabetes and cancer. **Supported**: FAPESP.

01.035

Evidence of a regulatory role of dystrophin on the release of acetylcholine (ACh) in the mouse brain. Della Colleta E, Nogueira FM, Campos DV, Lima-Landman MTR, Lapa AJ, Souccar C UNIFESP − Farmacologia

Introduction: Lack of dystrophin expression in Duchenne muscle dystrophy (DMD) causes progressive muscle wasting, reduction of postjunctional folds, and declustering of acetylcholine receptors (nAChRs) at the neuromuscular junction. Morphological changes like cerebral atrophy and neuronal loss have been also reported in DMD. In a previous study we reported alterations in the number of neuronal nAChRs in brain membranes from dystrophic (*mdx*) mice (Souccar et al., 2007, 37th Ann. Meeting Soc. Neuroscience, Abst. 573.16). Because of the involvement of neuronal nAChRs in cognitive functions, learning and memory, this work was aimed to examine the release of ACh and its presynaptic nicotinic modulation in synaptosomes from brain regions rich in dystrophin, in control and *mdx* mice, seeking a possible relationship with the cognitive impairment reported in DMD boys. **Methods**: Synaptosomes were prepared from cortex (Cx), hippocampus (H) and cerebellum (Cb) from male control and *mdx* mice at 4 (young) and 12 (adult) months of age. The synaptosomes were loaded with [^{3}H]Choline (0.08 µM) for 15 min, and perfused with Krebs-bicarbonate buffer (0.5 mL/min) at 37°C, in the presence and absence of nicotinic agonists and antagonists. Two min fractions were collected and counted for radioactivity. The amount of [^{3}H]ACh released in each fraction was expressed as percentage of the total synaptossomal radioactivity (fractional release). All experimental procedures were approved by the Institutional Animal Investigation Ethics Committee (CEP 1583/07). **Results and Discussion**: Perfusion of synaptosomes from Cx of control mice with the selective muscarinic agonist oxotremorine (Oxo, 1-30 µM) decreased [^{3}H]ACh overflow by 20 to 40% of basal values (21.7 ± 3.4 fmoles/mg protein, n=17). Similar effects were obtained in brain regions from *mdx* mice. Atropine (ATR, 0.01-1.0 µM) reduced [^{3}H]ACh overflow in both control (by 27%) and *mdx* (by 20%) preparations. Nicotinic stimulation by 10 µM ACh in the presence of 0.1 µM ATR increased [^{3}H]ACh overflow by 33% in Cx, 52% in H, and 34% in Cb synaptosomes from young control mice. In young *mdx* synaptosomes nicotinic stimulation did not alter [^{3}H]ACh overflow in Cx, while it induced significant decrease of transmitter release in H (20%) and Cb (18%) compared to control values. Nicotinic stimulation of adult *mdx* synaptosomes did not affect [^{3}H]ACh overflow in Cx or Cb, but it increased the transmitter release in H (87%) compared to control. The observed changes in ACh release in H appear to occur in response to the reported altered number of nAChRs (Souccar et al., 2007), reflecting a probable compensatory mechanism to preserve cholinergic neurotransmission. These alterations may be involved in the cognitive deficit reported in some DMD patients. The results favor a role of dystrophin in stabilization of nAChRs and regulation of the neurotransmitter release in the brain. **Financial support**: FAPESP, CAPES, CNPq.

01.036

Facilitatory action of a quaternary derivate of l-hyoscyamine on acetylcholine (ACh) release in rat cortex synaptosomes. Analysis of the mechanisms involved. Nogueira FM, Della Colleta E, Lima-Landman MTR, Lapa AJ, Souccar C UNIFESP/EPM − Farmacologia

Introduction: Presynaptic regulation of ACh release and other neurotransmitters by nicotinic ACh receptors (nAChRs) in cholinergic synapses of the central nervous system has been considered a potential target for the treatment of neurodegenerative diseases like Alzheimer's and Parkinson diseases. Recent studies have shown that acetylcholinesterase inhibitors and allosteric modulators of nAChRs activation like galantamine, are beneficial for the cognitive and neuropsychiatric symptoms in Alzheimer's and Parkinson's diseases (Albuquerque et al., Physiol. Rev. 89:73, 2009). Phenthonium (Phen) is a quaternary derivate of l-hyoscyamine and a competitive muscarinic antagonist 100 times less potent than atropine. At the rat neuromuscular junction, Phen enhanced the spontaneous ACh release without affecting the nerve-evoked transmitter release, and interacted noncompetitively with the nAChRs (Souccar et al., *Br. J. Pharm*. 124:1270, 1998). Seeking new compounds enhancers of the cholinergic function in central synapses with fewer unwanted effects, this work was aimed to evaluate the mechanisms involved in the prejunctional facilitatory action of Phen in synaptosome preparations of rat cortex. **Methods**: Synaptosomes were prepared from the cortex region of male rats (200-250 g) according the procedures approved by the local Animal Investigation Ethics Committee (CEP 341/09). The synaptosomes were loaded with [^{3}H]Choline (66.7 Ci/mmol; 0.08 μM) for 15 min, and perfused with Krebs-bicarbonate buffer (0.5 mL/min) at 37°C, in the presence and absence of agonists and antagonists. Two min fractions were collected and the radioactivity was counted in a β counter. The amount of [^{3}H]ACh released in each fraction was expressed as percentage of the total synaptossomal radioactivity (fractional release). **Results and Discussion**: Perfusion of synaptosomes samples with Phen (10-50 μM) increased [^{3}H]ACh overflow by 10 to 99% above basal values (15.7 ± 1.6 fmoles/mg protein/2 min, n=21). Perfusion of atropine (ATR, 0.1 μM) alone reduced [^{3}H]ACh overflow by 78% of control. Nicotinic activation by ACh (10 μM) in presence of ATR (0.1μM) increased [^{3}H]ACh overflow by 43%, but it did not affect the facilitatory effect of Phen. In presence of the selective muscarinic agonist oxotremorine (10 μM), ATR (0.1 μM) increased [^{3}H]ACh overflow by 28%, whereas the effect of Phen (50 μM) was significantly reduced (35%). [^{3}H]ACh overflow was increased in the presence of 15 mM KCl (172%) and by blockade of K channels with 3-100 μM 4-aminopyridine (48- 156%), or with 1-10 mM tetraethylammonium (53-76%). Neither of these compounds, however, altered the facilitatory effect of Phen. Nevertheless, blockade of voltage-dependent Ca^{2+} channels with $CdCl_2$ (100 μM) potentiated the effect of Phen (by 157%) at a concentration that reduced the K^+-induced transmitter release. The results indicate that Phen-induced basal ACh release involves its muscarinic antagonist action, but is unrelated to its antinicotinic activity or to an increased Ca^{2+} influx. A possible involvement of K^+ channels regulated by the neurotransmitter (M channels) is currently being investigated. **Financial support**: FAPESP, CAPES, CNPq.

01.037

Treatment effect of acute *in vivo* ethanol on adrenergic neurotransmission in the smooth muscle of periadolescent rats vas deferens. Silva Junior ED, Jurkiewicz A, Jurkiewicz NH UNIFESP − Farmacologia

Introduction: Ethanol is a hydrophilic molecule and low molecular weight that exerts its biological actions by multiple receptors including ion channels. The actions of ethanol are well studied in the central nervous system. However, few studies were conducted in autonomic nervous system. Changes in purinergic and noradrenergic transmission in the vas deferens are expected in animals that have not reached sexual maturity. Thus, the objective of this work was to study the effect of ethanol, using two doses, on the adrenergic transmission of the vas deferens of rats periadolescents. **Methods**: We used Wistar rats of 35 days old, were divided into two groups, the first group (group A) were treated orally with ethanol (40% v / v) 4 mL/kg, and the second group (group B) with 8 mL/kg. Four hours after treatment, animals were sacrificed, the vas deferens removed and mounted in isolated organ baths, were realized concentration-response curves for noradrenaline, barium, dopamine and phenylephrine (10-10 − 10-3 M), dose single tyramine (10-4 M) and frequency-response curves (0.1 − 20 Hz, 1ms, 60V) by Electrical Field Stimulation (EFS). The content of norepinephrine was also measured by high performance liquid chromatography (HPLC). For each treatment group was a control group. The results were expressed as mean ± SEM and tissue response was given in grams tension/grams tissue and tested with HPLC in picograms of norepinephrine/milligrams tissue. **Results**: In Group A there was a decrease in the maximum effect (Emax) to norepinephrine and barium (52.07 ± 3.78, n = 6; 72.18 ± 4.24, n = 6, respectively) when compared to their controls (64.26 ± 3.74, n = 7; 88.89 ± 5.76, n = 7, respectively), the same not occurred in group B. Phenylephrine and dopamine for both groups, showed no change when compared with their respective controls. There was no statistically significant changes between groups for the other pharmacological parameters studied (pD2 and ρ). The indirect release of norepinephrine by tyramine was not affected by any of the treatments studied. To EFS, the group A showed a decrease in phasic and tonic response when compared to their controls. The group B showed no changes. The measurement of norepinephrine stored in peripheral adrenergic neurons by HPLC showed that the group A showed a lower content (38.24 ± 5.51, n = 6) than control (74.82 ± 6.89, n = 25). GROUP B shows a smaller decrease in content, but without statistical significance. **Discussion**: From our results, we observed that in rats periadolescents, the effects of ethanol on adrenergic transmission, decreased with increasing dose and may be associated with unclear actions in the membrane pre and post-junctional of adrenergic transmission. **References**: Boselli, C., Govoni, S. Alcohol, 2000; Boselli, C., Govoni, S. *Journal of Autonomic Pharmacology*, 2001. Approved by CEP-UNIFESP: Protocol No. 1650/04. **Financial Support**: Capes.

01.038

Characterization of signaling pathways of Angiotensin I-converting enzyme in mesangial cells of spontaneously hypertensive rats (SHR). Reis RI[1], Parreiras-e-Silva LT[2], Becari C[3], De Andrade MCC[4], Salgado MCO[3], Costa-Neto CM[2], Casarini DE[5]
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Introduction: Angiotensin I converting enzyme (ACE) is a glycoprotein composed of a single large polypeptide chain, containing two homologous domains, called N- and C-domains, each one with a catalytic site. ACE plays an important function in vascular and electrolyte homeostasis, being responsible for the generation of the vasoactive peptide angiotensin II (AngII) and degradation of other bioactive peptides. The bound form of this enzyme has been found in epithelial, neuroepithelial, endothelial and mesangial cells, and also in tissue homogenates of epididymis, kidney, testis and aorta. Also, the soluble form of ACE has been found in body fluids, urine and plasma. Recently, signaling pathways triggered by this enzyme have been reported in endothelial cells, showing a new possible role as a transducer molecule. **Methods**: Aiming to explore this hypothesis, we analyzed the activation of JNK and ERK1/2 intracellular signaling pathways after stimulation of CHO cells stably expressing ACE (CHO-ACE) with angiotensin I (AngI, 10^{-6} M) in the presence and absence of captopril (10^{-6} M) during different times. **Results**: Our results show that AngI is able to trigger both signaling pathways activation, and that captopril is unable to impair it. We also performed the same experimental procedure in mesangial cells collected from SHR and Wistar rats. Surprisingly, ERK1/2 activation in mesangial cells from SHR was significantly higher as compared to those from Wistar rats. **Conclusion**: In summary, our results evidence that ACE is able to activate JNK and ERK 1/2 signaling pathways in both a heterologous (ACE-transfected CHO cells) and endogenous (mesangial cells). Besides that, the higher response found for ERK1/2 in mesangial cells from SHR may shed some light in the signaling pathways regulated by ACE and its possible correlation with hypertension and associated pathological states.

01.039

Estrogen attenuates cellular death induced by H_2O_2 in C6 cells: a role for ESR and GPER receptors. Franco LAM, Yshii LM, Lopes DCF, Sá Lima L, Scavone C, Munhoz CD ICB-USP − Farmacologia

Introduction: Chronic inflammatory responses as well as glial cells activation are associated with neurodegenerative diseases, such as Parkinson´s and Alzheimer´s (Block ML, *Nat Rev Neurosci*. 8(1):57-69. 2007). This chronic inflammatory stimulus can be modulated by high concentrations of reactive oxygen species (ROS) that potentially leads to a vicious cycle, inducing more cellular damage (Ryan KA, Infect Immun. 72(4):2123-30. 2004.). Despite a large number of evidence suggesting that glial cells play a major role in neuronal signaling and inflammatory responses in the brain, little is known about their role in neuroprotection. Estrogens (E2) are well known by its protective actions and the estrogen receptors ESR1, ESR2 and GPER are very important for the manifestation of these effects in the brain (Morale MC, *Neuroscience*, 138(3):869-78. 2006.). This work investigated whether E2 protects C6 cell line of rat glioma from damages induced by hydrogen peroxide (H2O2). **Methods**: PCR, Western blotting and immunofluorecence assays were performed to confirm the presence of estrogen receptors ESR1 and GPER in C6 cells. Cellular viability was measured by Lactate Desidrogenase assay (Promega). All results are expressed as mean±S.E.M. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Student Newmam-Keuls posthoc-test (GraphPad Prism5 software package, GraphPad Software, San Diego, CA, USA). Significance was set at $p<0.05$. **Results**: Our results confirmed the presence of ESR1 (mRNA and protein) and GPER (mRNA) receptors in C6 cells. In addition, we confirmed H_2O_2 toxicity in this cell lineage ($n = 24$, $p < 0.001$). The pre-treatment with E2 (24 hours) ($n = 10$, $p < 0,01$), or with G1 (20 minutes)($n = 30$ $P < 0.001$), the agonist for GPER, attenuated the toxic effects of the H2O2, while short-term (5, 15 or 30 minutes) E2 were toxic and even potentiated H2O2 toxicity in C6($n = 24$ $p < 0.001$). **Conclusion** : Our results show that E2 protects C6 from H_2O_2 toxicity and suggest the involvement ESR and GPER receptors in this protective effect. Our results also suggest a biphasic effect of the E2 in C6 cells, where long-term E2 treatment is protective and attenuates H_2O_2 toxicity and short-term E2 potentiates it. License number of the ethics committee: 61/71-02 **Financial Support**: FAPESP, CNPq and CAPES.

01.040

P2Y1 receptors stimulation on rat pineal glands: effects on nuclear factor kappa B pathway (NFKB) and inducible nitric oxide synthase (iNOS). Petrilli CL, Carvalho-Sousa CE, Muxel SM, Markus RP, Ferreira ZS IB-USP − Fisiologia

Introduction: We have previously characterized the P2Y1 receptor on rat pineals where it potentiates noradrenaline-induced melatonin synthesis (Ferreira, *Eur. J. Pharmacol*. 415:151, 2001). The functional responses of P2Y receptors may be regulated by a cross-talk of second-messenger pathways, such as the activation of phospholipase A2, phospholipase D, protein kinase C, adenylyl cyclase, phospholipase C, nitric oxide synthase isoforms (NOS), phosphoinositide 3-kinase /serine-threonine kinase, mitogen-activated protein kinase, extracellular signal-regulated kinases 1 and 2, c-Jun N-terminal kinases, cGMP and NFkB (Franke, *Pharmacol Ther*. 109:297, 2006). In the pineal, P2Y1-coupling mechanisms involve PLC activation, intracellular calcium mobilization and increase in extracellular pH (Ferreira, *Pharmacology* 69:33, 2003). Recently, we also demonstrated that pinealocyes P2Y1 receptor stimulation increased NO formation in a concentration-dependent manner, an effect fully abolished by the pre-treatment with the antagonist A3P5P or the non-selective NOS inhibitor L-NAME. Even more, increased content of cGMP was also observed. Considering the diversity in transducing systems operated by purinergic receptors, we investigate the effects of P2Y1 receptors stimulation on the NFkB pathway and inducible nitric oxide synthase (iNOS) in the pineal. **Methods**: Ethical Committee approved protocol (CEA/IB 106/2010). Rat pineal glands (Wistar, females, 1 month-old) were cultivated for 48h or dispersed pinealocytes were prepared by trypsinization (0.25%, 37°C, 15min) followed by mechanical dispersion. The NFkB pathway was analysed in nuclear extracts of cultured ADP-stimulated pineals by EMSA. The iNOS isoform was analyzed in dispersed pinelocytes by immunohistochemistry. The cells plated in an 8-well culture plate (10^5 cells/well) were stimulated in the absence or presence of ADP. Pinealocytes (fixed on acetone/methanol) were incubated by the primary antibody anti-NOS2 conjugated to TRITC (sc-7271 ; dilution 1/50, 18h, 4°C) and analyzed by confocal microscopy. The data are expressed as % relative to the non-stimulated group (100%). **Results**: Stimulation of pineal P2Y1 receptors by ADP (0.3mM, 1min) leads to an increase in nuclear translocation of the transcription factor NFkB as compared to non-stimulated glands (75,61 ± 14,59%, n=4). On the protein expression levels of the iNOS isoform, the stimulation of pinealocytes with ADP (0.3 mM, 30 to 120 min) leads to a time-dependent manner increase on the isoform expression (from 32.7±17.4 to 173.4±31.3%, n=23 cells per treatment) with the maximal increase observed with 60 min ADP-stimulation (220.9±41.0 %, n=19 cells). **Discussion**: The activity of two constitutive NOS isoforms in the pineal is mainly controlled by noradrenergic/purinergic mechanisms via activation of a1/P2Y1 receptors with calcium mobilization. The data presented here point to the pineal purinergic regulation of iNOS, transcriptionally induced by NFkB, disclosing the regulation of this metabolically active isoform in the pineal gland, which accounts to the increased levels of NO. Several interactions involving purinergic and nitrergic systems has been proposed being strengthened by the co-localization of P2Y1 receptor and iNOS in several brain areas. **Acknowledgments**: The technical support of Debora Aparecida de Moura is gratefully acknowledged. **Financial Support**: FAPESP, CNPq, CAPES.