Setor 01. Farmacologia Celular e Molecular/Cellular and Molecular Pharmacology

01.001

Detection of adenosine deaminase from human erythrocytes in slices of agarose from gel electrophoresis. Cavalcante IJM, Osório CBH, Luz PB, Carmo JRF, Sousa PS, Alencar NMN de, Vale MR [1]UFC Fisiologia e Farmacologia

Introduction: The adenosine deaminase (ADA - EC3.5.4.4.) is a key enzyme in the catabolism of purines. It catalyzes the deamination of adenosine or 2´deoxi- adenosine producing ammonium and inosine or 2'deoxy-inosine, respectively. Its activity is expressed by two isoenzymes (three isoforms). ADA1 (36kDa) or ADA1 bound to CD26 (280kDa) is widely distributed in body tissues and its genetic absence causes Severe Combined Immunodeficiency (SCID) which is connected to problems in the maturation and function of lymphocytes and other cells of the immune system. ADA2, 100kDa, is normally found in serum and synthesized only by the monocytic-macrophage system. The biological importance of ADA2 is not yet fully established, mainly due to its kinetic characteristics but its importance comes from its use as a marker for some infectious disease like tuberculosis. The erythrocytes contain only the monomeric form of ADA1. Moreover, high levels of ADA in erythrocytes may be associated with hereditary hemolytic anemia. This study aimed to show a new method for detection of ADA by using electrophoresis in agarose gel. **Methods:** Human erythrocytes were hemolyzed in phosphate buffer, 50mM, pH 6.7 and subjected to an electrophoresis in 1% agarose gel under 100V during 4 hours. The gel was sliced and each slice (3 mm x 5 mm) was incubated for 2h with adenosine 22mM, at 37ºC. The ammonium formed was quantified by Berthelot's reaction. The experimental protocols were submitted and approved by the Research Ethics Committee of the Federal University of Ceará (COMEPE) under the Protocol COMEPE No. 173/08. **Results:** It was observed a single peak of enzyme activity. The interference of hemoglobin color in the ammonium detection was neutralized running a blank which was treated in identical way, except for the incubation with adenosine which was substituted by buffer only. **Discussion:** This study was developed as part of a project that tries to validate an equation based in the kinetics properties of ADA isoenzymes which should be useful for the differential diagnostic of tuberculosis and other diseases. The methodology described here is the initial step in the development of a new method for the detection and discrimination of ADA isoenzymes. The results encourage us to continue since the method was able to detect the activity of the enzyme inside the agarose gel. Additional studies are needed in order to to establish the detection of the other isoforms in other tissues. **Acknowledgments:** CAPES/ Funcap/ CNPq.

01.002

Efeito da desnutrição multifatorial no ducto deferente de rato: homeostasia do Ca^{2+}, análise histológica, transporte de células e capacidade reprodutiva de ratos adultos machos. Muzi-Filho H[1], Souza AM[1], Bezerra CGP[1], Boldrini LC[2], Takiya CM[2], Oliveira FL[2], El-Cheikh MC[2], Einicker-Lamas M[3], Vieyra A[3], Lara Morcillo LS[1], Cunha VMN[1]. [1]ICB-UFRJ - Farmacologia Celular e Molecular, [2]ICB-UFRJ Ciências Morfológicas, [3]IBCCF-UFRJ

Introdução: A desnutrição afeta 174 milhões de crianças menores de 5 anos em todo o mundo, uma vez que resulta em sequelas de implantação silenciosa que podem se manifestar na população adulta. Sabe-se que diversas estruturas moleculares relacionadas à homeostasia do Ca^{2+} encontram-se alteradas pela desnutrição multifatorial. Os objetivos deste trabalho foram: 1- investigar o efeito da desnutrição sobre moléculas reguladoras do íon Ca^{2+} e a composição histológica do DDR; 2- a produção e o transporte de células no trato reprodutor; e 3- a capacidade reprodutiva de ratos adultos jovens machos. **Métodos:** Foram estabelecidos dois modelos de desnutrição multifatorial: 1- ratas Wistar grávidas foram alimentadas com Dieta Básica Regional (DBR), sendo sua prole alimentada com uma dieta convencional (DBR-IU); 2- logo após o desmame, ratos provenientes de mães sadias foram submetidos à dieta DBR por 13 semanas (DBR-CR). Nos grupos controles, os ratos se alimentavam da dieta convencional. Os animais foram sacrificados (CEUA DFBCICB 007) e os órgãos do aparelho reprodutor foram removidos. Com o DDR, foram realizados ensaios bioquímicos para avaliar o conteúdo de Ca^{2+} intravesicular, a atividade e a expressão das Ca^{2+}-ATPases e a expressão da imunofilina FKBP12, assim como ensaios histológicos pelo método da hematoxilina-eosina. Nos ensaios de citometria os órgãos foram homogeneizados para avaliação do conteúdo e da diferenciação celular. Foram também realizados ensaios de acasalamento de ratos machos dos três grupos com fêmeas adultas normais (método de harém). **Resultados:** A expressão de SERCA e a atividade Ca^{2+}-ATPásica estão aumentadas, assim como a expressão de FKBP12 está diminuída nos grupos DBR-IU e DBR-CR. No grupo DBR-IU, estas alterações estão relacionadas à redução do conteúdo de Ca^{2+} intravesicular. As observações histológicas mostram que, na porção epididimal do DDR, ocorrem alterações de mucosa e nas camadas musculares circular e longitudinal em ambos os modelos de desnutrição. Nos ensaios de citometria, observa-se que a produção celular testicular é significativamente menor no grupo DBR-IU (37 %). No grupo DBR-CR ocorre aumento do percentual do número de células em apoptose (320 %) e redução do número de células totais no DDR (25 %). Observa-se também que o número de fêmeas acasaladas e o de filhotes oriundos do mesmo macho são significativamente menores com os machos dos grupos DBR-IU (56 e 58 %, respectivamente) e DBR-CR (32 e 26 %). **Discussão:** Nossos dados indicam que tanto a desnutrição intrauterina quanto a crônica alteram a atividade e a expressão de estruturas moleculares ligadas à homeostasia do Ca^{2+} e comprometem a capacidade reprodutiva de ratos machos. Tais modificações poderiam estar relacionadas com a diminuição da atividade contrátil do DDR, já que é observada a deficiência do transporte de células totais no fluído seminal e alterações histológicas compatíveis com respostas adaptativas a desnutrição. **Apoio Financeiro:** Projeto Casadinho-CNPq; PROCAD-CAPES; FAPERJ Primeiros Projetos, Programa ALV**.**

01.003

Reexamining the kinin B_1 receptor function and the concept of *de novo* synthesis. Bravo JCR, Farias NC, Fonseca RG, Feres T, Pesquero JB, Paiva TB UNIFESP - Biofísica

Introduction: In rabbit aorta, kinins mediate their effects via kinin B_1 (KB_1R) and kinin B_2 receptors (KB_2R). In normal tissues, KB_2Rs are constitutively expressed and kinin B_1 receptors (KB_1Rs) are apparently constitutively expressed. However, KB_1Rs become functional only when Ca^{2+}-dependent K^+ channels ($K^+_{Ca}{}^{2+}$) are in open state, as happens under inflammatory processes, or when ATP-dependent K^+ channels are activated, as happens during fasting. The presence of KB_1R was investigated in non-responsive preparations. **Methods:** Smooth muscle cells in rabbit aortic rings were impaled by microelectrodes to measure membrane potentials (MP). The expression of KB_1R in these preparations was investigated through the effect of kinin B_1 agonists and antagonists in the MP and by KB_1R mRNA evaluation. **Results :** Hyperpolarizing responses (-70 ± 1,5 mV) to KB_1R stimulation by the agonist des-Arg^9-BK [10^{-6} M] were observed in 59% of recently set-up aortic rings from fed rabbits (n= 3) and were inhibited (-52 ± 1,3 mV) by iberiotoxin [10^{-8} M] n= 3 , a specific inhibitor of $K^+_{Ca}{}^{2+}$. These responses also were observed in all aortic rings from fasted animals (-73 ± 1,8 mV; n= 7) and were inhibited (-56 ± 1,5 mV) by glybenclamide [10^{-6} M], an inihibitor of ATP-dependent K^+ channels. All hyperpolarizing responses were inhibited by the specific KB_1R antagonist Lys-(Leu^8)-des-Arg^9-BK [10^{-5} M]. In the preparations from fed animals (n= 3), after 6-h of stretching, a depolarizing effect (-33 ± 1,6 mV)caused by the KB_1R agonist des-Arg^9-BK was observed in all preparations (n= 3). This effect was also inhibited by a KB_1R antagonist. In non-responsive rings, the presence of mRNA of this receptor was confirmed by real time-RT-PCR . Moreover, in these non-functional rings, the KB_1R was shown to be responsive when $K^+_{Ca}{}^{2+}$ channels were activated by increasing intracellular Ca^{2+} concentration. **Discussion:** These findings show that KB_1Rs are present and functional in 50% of recently mounted aortic rings and that in the other 50% they are expressed but non-functional. As KB_1Rs could be turned responsive after K^+_{Ca2+} and K^+_{ATP} activation, we propose the new concept that the functionality of KB_1R might be dependent on the activation of Ca^{2+} and ATP dependent K^+ channels. (**Support:** FAPESP, CNPq, CAPES).

01.004

Characterization of a novel cannabinoid-1 (CB1) inverse agonist: interaction with receptors expressed in xenopus oocytes, synaptic modulation and hypophagic effect.

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Introduction: The CB1 cannabinoid receptor is the most abundant G-protein-coupled receptor in the CNS and its activation promotes analgesia and stimulates the appetite, among other actions. This receptor and its endocannabinoid ligands may also have a role in ischemic neural damage, but this is still controversial. Some studies show a neuroprotective effect of CB1 agonists in ischemia models (e.g., Zani et al., Br. J. Pharmacol. 152:1301, 2007). However, others show that CB1 antagonists like rimonabant (SR141716A) are neuroprotective (e.g., Muthian et al., Neuroscience 129:743, 2004). LASSBio-881, a compound developed at UFRJ, has antinociceptive, anti-inflammatory and antioxidant effects *in vivo*, and also binds to CB1 receptors (Duarte et al. Bioorg. Med. Chem. 15:241, 2007). The compound has significant neuroprotective effects as well (Balassiano *et al*., this meeting). However, the type of interaction with the CB1 receptor is unknown. Our aim was to characterize the actions of LASSBio-881 on CB1 receptors and to further explore effects that may be related to these receptors. **Methods:** LASSBio-881 and the CB1 agonist, WIN 55212-2 (WIN) were assayed in *Xenopus* oocytes expressing human CB1 and two G-protein-gated K+ channels (Kir3.1 and Kir3.4) by current recordings. Effects on spontaneous inhibitory postsynaptic currents (sIPSC) were evaluated in cultured rat hippocampal neurons through whole-cell patch-clamp recordings at 0 mV. To evaluate the effect on feeding behavior, mice receiving LASSBio-881 or vehicle i.p. were observed after fasting for 24 h. Chow consumption and body weight changes were observed for 24 h. Animal procedures were approved by the Ethics Committee on Animals Research of CCS/UFRJ, protocols DFBICB 009 and 029. **Results and Discussion:** At near-saturating concentration (20 microM) LASSBio-881 did not activate K+ currents in oocytes expressing CB1 receptors. To the contrary, the compound reduced the baseline current, which changed by -30.7±2.6% (mean±s.e.m., n=7, $P<0.001$) relative to the response to the CB1 agonist WIN (500 nM). When both were co-applied, the response was 12.6±1.6% of that induced by WIN alone (n=7, $P<0.001$). In hippocampal neurons, WIN (100 nM) reduced the frequency of sIPSC, but subsequent application of LASSBio-881 (20 microM) partially reversed these changes. In some cells, LASSBio-881 alone led to a reduced sIPSC frequency with increased amplitude. In fasted mice, a single dose of LASSBio-881 (50 mg/kg) one hour before reintroducing chow pellets reduced consumption by 59% in the first hour. The cumulative consumption compared to the group receiving vehicle remained significantly smaller after 24 h (n=6, $P<0.05$). All animals regained the weight lost upon fasting, but the control group tended to overshoot, unlike that pre-treated with LASSBio-881. Our data suggest that LASSBio-881 is a CB1 inverse agonist/antagonist. Accordingly, LASSBio-881 inhibited post-fasting hyperphagia. We can also conclude that the antinociceptive effect of LASSBio-881 is not due to activation of CB1 receptors. Support: PRONEX, FAPERJ, CNPq.

01.005

Pharmacologic evaluation of new alpha-adrenoceptor antagonists. Chagas-Silva F[1], Vieira RO[1], Nascimento JB[1], Romeiro LAS[2], Barberato LC[2], Oliveira MS[2], Noel F[3], Silva CLM[1] [1]UFRJ - Farmacologia Celular e Molecular, [2]UCB - Gerontologia, [3]UFRJ - Desenvolvimento de Fármacos

Introduction: Some clinical used α_1 adrenoceptor (AR) antagonists (for instance, tamsulosin) also bind to other G protein-coupled receptors (GPCR) of biogenic amines (5-HT receptors) due to the sharing of some structural features at ligand binding region of the GPCR. Previously, it was synthesized a new α_1-adrenoceptor antagonist, an hybrid of N-phenylpiperazine and aryl sulfonamide moiety (LASSBio772), selective for α_{1A} and α_{1D} AR subtypes (*Romeiro et al, J. Med. Chem. submitted*). However, it also has affinity for 5-HT receptors. The objective of this study was to assess the affinity of new analogues (LDT series), with substitutions at the nitrogen 4 (N4) of phenylpiperazine or at position 2 of the aromatic ring, for α_1 AR and serotonin (5-HT) receptors, and also to determine a structure-activity relationship. **Methodology:** *Binding assays***:** 150 ug of protein obtained from rat (α_{1B}) and rabbit (α_{1A}) liver, rat cortex (5-HT_{2A}) and hippocampus (5-HT_{1A}) were incubated with 0, 1 nM [3H]-prazosin (α_1 AR), 1 nM [3H]-ketanserin or 1 nM [3H] 8-OH-DPAT, respectively, for 15-40 min at 30-37^oC in the absence and presence of LDTs (LDT 39, LDT 62 -70 or 2-OH-FPZ) (1-50 uM). The reaction was stopped by 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. 2002. Br. J. Pharmacol. 135:293*), where rat aorta (mainly α_{1D} AR and 5-HT_{2A} receptors) were contracted with phenylephrine (PE) or 5-HT (1 to 10000 nM) before and after incubation with LDTs (10 uM; screening concentration) to assess the vasodilator effect. The results were analyzed by non-linear regression to calculate the parameters IC_{50} and maximum contraction (Emax). **Results and discussion**: The analysis of the competition curves showed a concentration-dependent inhibition with all LDTs. Compared with the prototype (LASSBio772), which showed a nM affinity for 5-HT and α_{1A}/α_{1D} AR, and uM affinity for α_{1B} AR, there was a reduction of 10-100 fold of IC_{50} values for 5-HT_{2A} and 5-HT_{1A} receptors, and 1000 fold for α_{1A} AR. However, the low affinity (uM) for α_{1B} AR was maintained (n=3). For instance, the IC_{50} values of LDT63 for 5-HT_{1A}, 5-HT_{2A} and α_{1A} receptors were respectively (uM): 0.39, 0.17, and 0.33 (n=2), and for LDT64: 9.1, 9.6, 0.9 (n=2), indicating that the replacement of nitrogen in position 4 of piperazine reduced the affinity for 5-HT and α_{1A} receptors, without affecting affinity for α_{1B} AR. The contraction induced by PE, after incubation with LDTs, showed that all substances but 2-0H-FPZ reduced differently the Emax values. For example, LDT64 reduced the Emax from 15.0 ± 1.1 to 1.0 ± 0.7 mN (n = 6, $p < 0.001$), however it did not change the contraction induced by 5-HT (Emax = 18.0 ± 2.2 and 19 ± 1.89 mN, n = 4, before and after, respectively). In conclusion, the data suggest that the substitution in N4 and at position 2 of the aromatic ring of phenylpiperazine maintains the effect at a_{1D} AR, reduces the affinity for α_{1A}, 5-HT_{2A} and 5-HT_{1A}, without altering the affinity for α_{1B} adrenoceptors. **Support**: CNPq, FAPERJ.

01.006

Cloning and expression of tissue inhibitor of matrix metalloproteinases (TIMP)-1 for use as a therapeutic agent. Sturaro RH[1], Neves-Rodrigues A[2], Peronni KC[3], Alves CP[3], Marin JM[1], Espreafico EM[3], Gerlach RF[1] [1]USP - Morfologia, Estomatologia e Fisiologia, [2]UNESP-Jaboticabal - Biologia, [3]USP - Biologia Celular e Molecular e Bioagentes Patogênicos

Introduction: Matrix Metalloproteinases (MMPs) are a family of zinc and calcium-dependent endopeptidases that promote extracellular matrix degradation, being increased in many diseases, such as cardiovascular diseases. The importance of the MMPs has lead to the development of inhibitors for therapeutic applications. The natural inhibitors of the MMPs are the Tissue Inhibitors of MMPs (TIMPs), a group of 4 glycoproteins with molecular weight between 21-34 kDa. The TIMPs have been shown to be valuable tools and also possible therapeutic agents for many diseases characterized by an imbalance between MMPs/TIMPs. To test the therapeutic potential of inhibition of MMPs, large amounts of correctly folded TIMPs are necessary. Therefore, the aim of this study was to clone and express the human recombinant TIMP-1 in E Coli to use it in cardiovascular studies. **Materials and Methods:** Human mRNA from gingival fibroblasts was extracted to construct the cDNA, which served as template for polymerase chain reaction with the TIMP-1 primers. The fragment was inserted in a cloning vector to transform DH5 α bacteria, followed by extraction of the plasmid for homology confirmation by sequencing. The sequence was then inserted into an expression vector (pET28Aa). The human TIMP-1 was expressed in BL21plys and the protein purified in nickel columns. The protein was analyzed by SDS-PAGE, Western Blotting and reverse Zymography. **Results:** Results confirmed the successful expression and purification of the rhTIMP-1, which shows a slight difference with the human native protein due to the lack of glycosilations. However, reverse zymography and fluorimetric assays confirmed the MMP inhibitory activity of the purified rhTIMP-1. **Discussion:** The high inter species homology enables the use of the rhTIMP-1 in assays with rat tissues. Functional studies on the inhibition of MMPs in rodent vessels will now be possible, since these preliminary and very time consuming steps were done. **Citations:** Lambert,E., Dasse,E., Haye,B., Petitfrere,E., 2004. TIMPs as multifacial proteins. *Crit Rev Oncol Hematol* 49, 187-198. **Financial support:** FAPESP e CNPq

01.007

Cellular mechanisms involved in vascular alterations in schistosomiasis. Oliveira SDS[1], Amaral LS[1], Quintas LEM[1], Noel F[2], Silva CLM[1] [1]UFRJ - Farmacologia Celular e Molecular, [2]UFRJ - Desenvolvimento de fármacos

Introduction: Schistosomiasis caused by the intravascular parasite *Schistosoma mansoni* leads to morphological and functional changes that compromise vascular physiology. The objective of this study is to evaluate the myocyte- and endothelial-dependent functions, namely contraction, NO production, cellular migration and vascular permeability. **Methods:** All procedures were approved by local (URFJ) ethical committee (DFBC-ICB011). *Measurement of isometric tension***:** Aortic rings of control (Co) and *S. mansoni* infected (Inf) mice were fixed to an isometric tension transducer and immersed in aerated physiological solution (37^{o}C). Vascular contractions were induced with 5-HT (10^{-9}-10^{-5}M) in the presence of 250 μM L-NNA. *Cellular migration and vascular permeability***:** Mice were treated either with Evans blue dye (1h, i.v.) or lipopolysaccharide (LPS, 3h, 0.01 mg/kg, i.p.). The animals were sacrificed and 5 ml of steril saline (PBS) were injected i.p. The exsudate was removed, centrifuged and the pellet resuspended in PBS (1 ml) for total and differential cell counting. The supernatant, which contains the dye, was used as an marker of vascular permeability (405 nm). *Culture of endothelial cells:* Vessels from mesenteric microcirculation were used for primary cell culture. Nitric oxide (NO) production was measured in 1st passage cells using the fluorescent dye DAF-FM (2.5 μM, 45 min) in the absence or presence of pharmacological stimulation (100 μM ATP). *Western blotting:* The endothelial cells were lysed, 20 μg of protein were loaded on SDS-PAGE gel (7.5% or 12%), transferred to nitrocellulose and probed with antibodies against endothelial nitric oxide synthase (eNOS) or caveolin-1 (CAV-1). **Results and Discussion:** The contraction induced by 5-HT was higher in the Inf (Emax=16.4 ± 0.7 mN, n = 11) than in group Co (11.3 ± 1.0 mN, n = 9), which could be due to the fact that interleukin-13, which is enhanced in schistosomiasis, increases the expression of 5-HT_{2A} receptors in smooth muscle cells (Zhao et al., 2006. Gastroenterology 131: 568). The ATP-induced NO production is smaller in infected (7.1 ± 3.1%) than in control mice (24.6 ± 5.4, n=5, $P < 0.05$). The expression of eNOS is also reduced in the former group, which corroborates previous data showing a reduction of endothelium-dependent relaxation in schistossomiasis (Silva et al., 2007. Vasc. Pharmacol. 46: 122). No alteration of CAV-1 expression was observed, suggesting that the reduced NO production is due to a reduced expression of eNOS. When comparing Co to Inf animals we observed an increase in the total number of cells in the peritoneum both in the absence (4.02 ± 0.44 and 29.3 ± 3.22 x 10^6 cells, respectively, n=9, $p< 0.05$) and presence of LPS stimulus (15.3 ± 1.3 and 44.6 ± 1.5 x 10^6 cells, respectively, n=5, $p< 0.05$), accompanied by an increased vascular permeability, suggesting that the endothelial dysfunction contributes to the inflammatory process. **Conclusion:** The chronic inflammatory condition associated to schistosomiasis increases aorta contraction in response to 5-HT and reduces eNOS expression leading to a diminished NO production. Moreover, endothelial barrier is probably disrupted by the disease allowing an increased cellular migration and vascular permeability. **Apoio:** CAPES, FAPERJ.

01.008

Recombinant MMP-2 inhibition by zinc and lead acetate. Antonio RC[1], Rizzi E[1], Faria MCF[2], Gerlach RF[2], Tanus-Santos JE[1] [1]FMRP-USP - Farmacologia, [2]FORP-USP - Morfologia, Estomatologia e Fisiologia

Introduction: Matrix Metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases that promote extracellular matrix degradation. MMPs are widely distributed in the body and are involved both in physiological and pathological processes. The importance of the MMPs has lead to the development of inhibitors, and, in recent years, many inhibitors have been synthesized for therapeutic applications. Metals are widely distributed in nature and are widely used by humans, even as medications. While the optimal zinc concentration in MMP activity buffers is 1 uM, it has been shown that zinc at 15 uM inhibits MMP activity. However, to date, those assays were not carried out with both the proteases and their substrates in solution, and were not done using a recombinant MMP. Therefore, this study aimed at testing the effect of Zn, and Pb on the activity of the recombinant human MMP-2 (rhMMP-2). Methods: The assays were carried out with $ZnCl_2$ (600 uM to 0,25 uM) and $Pb(CH_3COO)_2{\cdot}3H_2O$ (800uM to 0,39 uM). Solutions pH was carefully monitored, and only metal-free tubes were used in this study. The substrate used was DQ-Gelatin (Molecular Probes), and reactions were read in a fluorimeter, assays were done in triplicate, and each metal was tested at three independent times. Results: $ZnCl_2$ strongly inhibited the rhMMP-2 (I50= 18,75uM), the same happening with Pb acetate (I50= 12,5 uM). Conclusion: This findings show that rhMMP-2 activity is altered by increasing metal concentrations, suggesting that at least part of the effects of metals in the body may be due to their effect on MMPs. **Entidades Finaciadoras**: Capes, CNPq, FAPESP

01.009

New diselenoamino acid derivatives with pharmacological properties *in vitro***.** Sudati, JH, Alberto EE, Fachinetto, R, Braga AL, Rocha JBT UFSM - Química

Introduction: Reactive oxygen species (ROS) primarily arise from products of normal metabolic activities and are thought to be the etiology of many diseases. The synthesis of compounds which can mimic the properties of the selenoenzyme glutathione peroxidase (GPx), a selenium-containing antioxidant enzyme that normally removes ROS, has inspired great interest. In this study, a variety of diselenides derivated from L-phenylalanine (**A**-**C**) (differing in the number of carbon between amino acid residue and selenium atom; compound **A** (1 carbon), **B** (2 carbons, **C** (3 carbons)) and L-valine (**D**) was synthesized to be a functional mimic of the GPx, kinetic analyses according to the model reaction (H_2O_2 + 2PhSH à $2H_2O$ + PhSSPh) have been performed. **Methods:** The catalytic behavior of these compounds, as GPx model enzyme, was evaluated according to Tomoda's method using benzenethiol as a glutathione alternative according to the reaction: H_2O_2 + 2PhSH à $2H_2O$ + PhSSPh. The reduction of H2O2 was monitored through the UV absorption increase at 305 nm, due to diphenyl disulfide formation. Reaction was initiated by the addition of an amount of H_2O_2 (5 mM) to a methanol solution containing PhSH (1.9 mM) and catalysts **A**-**D** (0.025 mM) at 30ºC during 6 min, reactions was repeated at least more than three times under the same conditions. T_{50} values (time required, in minutes, to reduce the thiol concentration with 50% after the addition of H_2O_2) were calculated. Diphenyl diselenide (PhSeSePh), a GPx mimic, was used as a positive control. **Results**: Taking advantage of the modular characteristic of our catalysts, we evaluated the influence of the chain length between the diselenide moiety and amino acid residue in the reduction of hydrogen peroxide. Diselenide **D**, derived from L-valine, showed the best GPx like activity (T_{50} = 42.18 (± 2.11) min) followed by compounds **C** and **B** derived from L-Phenylalanine with longer chain length (T_{50} = 45.15 (± 3.17) min and 51.38 (± 2.45) min, respectively. These three diselenides showed better T_{50} values than PhSeSePh (T_{50} = 51.80 (± 2.83) min). Compound **A**, with a shorter chain length, was the less effective catalyst in this screening (T_{50} = 93.03 (± 5.77) min). **Discussion:** Our study showed that there are two key factors for improving catalytic efficiency of GPx mimics. First, diselenides are influenced by steric effect of amino acid residue (best catalyst: **D**), and second, the number of carbon between the amino acid residue and the Se atom in diselenide structure (best catalyst: **C**). We conclude that these conditions are significant considering the design mimics with high catalytic efficiency. This investigation of selenium-containing enzyme models may yield useful as artificial catalysts for medical applications. **Agradecimentos:** The financial support by CAPES/CNPq is gratefully acknowledged.

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01.010

Comparison of flow cytometric immunophenotyping between bone marrow mesenchymal stem cells and periodontal ligament cells: a study in dogs. Colenci R[1], Assunção LRS[2], Golim MA[3], Bomfim SRM[4], Oliveira SHP[1] [1]UNESP-Araçatuba - Ciências Básicas, [2]UNESP - Odontologia Infantil e Social, [3]UNESP - Hemocentro, [4]UNESP - Cirurgia, Clínica e Saúde Animal

Introduction: Bone marrow mesenchymal stem cells (BMMSC) are capable of regenerating bone, cartilage, muscle, connective and adipose tissue. These cells express surface proteins that can be recognized by specific antibodies. Periodontal ligament involve progenitor cells which have the capacity to generate other cells, such as fibroblasts. The purpose of this study was (1) to identify, by means of cells markers, the presence of BMMSC within bone marrow aspirate (BMA) and after culture, and (2) to compare these cells with periodontal ligament cells (PLC). **Methods:** BMMSC were isolated from dog's iliac crest marrow and purified by density gradient centrifugation method, and then cultured *in vitro*. PLC were obtained from extracted incisive of dog and were used as control. **Results:** Flow Cytometry Analysis (FCA) was performed after BMA and before passages 1, 2 and after passage 3, using CD34 and CD90 cells markers. FCA showed 55,98% of CD34+ and 32,67% of CD90+ after BMA; 3,33% of CD34+ and 33.0% of CD90+ before 1st passage. Before the second passage, we obtained 10,54% of the cells expressing CD90+, whereas after the third passage, this number declined to 1,58%. PLC presented 4,04% of CD90+ and 1,05% of CD34+ after third passage. **Discussion:** According to immunophenotyping and fotomicroscopy analysis we conclude that BMMSC are very similar to PLC after the third passage of culture. **Financial support:** FAPESP: 06/59420-5 Ethics committee: 57/06

01.011

Insights towards a pharmacophoric model for schistosomicidal benzodiazepines. Thibaut JPB[1], Menezes CMS[2], Sánchez GR[2], Vieira RO[1], Barreiro EJ[2], Lima LM[2], Noel F[1] [1]ICB-UFRJ - Farmacologia Bioquímica e Molecular, [2]LASSBio-UFRJ - Farmácia

Introduction: Clonazepam (CLO) and 3-methylclonazepam (MeCLO) induce contraction of adult male *Schistosoma mansoni in vitro* and have a proven schistosomicidal activity in humans, but their mechanism of action is unknown. We previously showed that the intense and immediate contraction of these worms induced by CLO and MeCLO is not mediated by the parasite benzodiazepine binding sites (Thibaut *et al*., *Eur J Pharmacol* 606: 9, 2009). Here we decided to investigate the structural requirements for the *in vitro* schistosomicidal effect of meclonazepam and benzodiazepines as a whole. **Methods:** Six new N1-modified clonazepam analogues were synthesized using clonazepam as starting material and their schistosomicidal activity tested *in vitro* using the screening assay recommended by the WHO. In accordance with our Institutional Ethical Committee for animal care (CEUA, Nr DFBCICB011) adult worms were surgically removed from portal veins and mesenteric of infected mice and distributed in tissue culture dishes in DMEM. The worms were kept at 37°C in an atmosphere of 5% CO_2 in air and were observed daily for 5 days under a Nikon Eclipse TC-100 inverted microscope. The measurement of worm "motility" is scored on an arbitrary scale: 4 = hyperactivity; 3 = normal activity; 2 = low activity, i.e. occasional movement of head and/or tail; 1 = minimal activity, i.e. only gut movements; 0 = total absence of motility even at the gut extremities. Worms were defined as 'dead' if they remained without any movement of body (scale 0) during 1 minute. The molecular modeling study based on stereoelectronic features was performed with the Spartan'08 software, using the chemical structures constructed using the MMFF94 force field software. The elected compounds for this study were: *R*-(-)- and *S*-(+)-meclonazepam, clonazepam, diazepam, flunitrazepam, our N1-modified clonazepam analogues and the meclonazepam analogues reported by Mahajan et al. (Bioorg & Med Chem Lett 18: 2333, 2008). **Results and Discussion:** The *In vitro* culture experiments showed that the six N1-modified clonazepam analogues were not schistosomicidal nor disturbed the worm motility and morphology. Based on these results and the molecular modelling study, we were able to propose a pharmacophoric model for the schistosomicidal activity of meclonazepam and other benzodiazepine compounds. According to our findings, the schistosomicidal activity seems to be strictly related to stereolectronic features: the presence of an oxo (preferential) or thienylic carbon (C2), the lack of substitution of the amide nitrogen atom (N1), the presence of the imine bond (4- and 5-positions) and the presence of an electron-withdrawal group at C7 seem to be essential features for the schistosomicidal activity.

Supported by: CAPES, FAPERJ, CNPq

01.012

Isolation of bioactive peptides by the action of serinoproteases the venom of *Bothrops jararaca* on endogenous substrates and actions in cell culture and bioassays. Auada AVV, Maria DA, Kleber FA, Azevedo RA, Lebrun I Instituto Butantan - Bioquímica e Biofísica

Introduction: Venoms are a rich source of proteolityc enzymes, from the *Bothrops jararaca* venom serinoproteases and metalloproteases are the main enzymes that act in various tissues and proteins present in the victim. Besides the action on tissues these proteases could generate some compounds that could have specific actions in cells or other mechanisms towards generation of bioactive peptides. There are protein precursor of bioactive peptides, but today it was described a new class of proteins that in some conditions may generate bioactive peptides they were named cripteins. **Objective:** Identification of bioactive peptides by the action of the serinoproteases, trypsin and from the venom of *Bothrops jararaca* on endogenous substrates. Biochemically isolate and characterize the obtained peptides and verify the possible effects and biological properties of these peptides through several biological tests "in vitro" and "*in vivo*". **Methods:** Serinoprotease from *Bothrops jararaca* venom was separated from whole venom using a exclusion HPLC column. The serinoproteases were incubated with the endogenous substrates chosen by a preset time. The endogenous substrates were also incubated with trypsin, as well the serinoproteases from the venom of *Bothrops jararaca*. Directly from the incubated, the remaining proteins and resulting products were observed by gel electrophoresis SDS-PAGE silver stained and also by means of HPLC profile. The hydrolysates were tested in cell cultures of fibroblasts. After the test in cell cultures the active hydrolisates were purified by HPLC and then the peaks will be tested by the same methods in order to identify the active peptides. Approved by the Ethical Committee for Animal, protocol 557/09. **Results:** In fibroblast cell culture a small cell proliferation was obtained with hemoglobin and collagen hydrolysates using trypsin as proteolytic enzyme. Results obtained in HPLC showed us where profiles of hidrolises different for each type of substrate used. **Discussion:** The study suggests that these serinoproteases were able to generate peptides with relevant biological activity, specifically with the snake venom it could be another component to the bite response. **Financial support:** Fundap, Butantan Foundation, FAPESP, CNPQ, INCTOX.

01.013

Effects of ovariectomy and 17b-estradiol replacement on ERK1/2 activation in rat hippocampus. Pereira RTS[1], Porto CS[1], Abdalla FMF[2] [1]UNIFESP - Farmacologia, [2]Instituto Butantan - Farmacologia

Introduction: 17b-estradiol plays a potent neurotrophic and neuroprotective role in brain (reviewed in Brann et al., Steroids 72:381, 2007). The mechanisms underlying estrogen neuroprotection are not fully understood, however, several candidate targets have been identified, for exemple, members of the Bcl-2 family (Nilsen and Brinton, Proc. Nat. Acad. Sci. USA, 100: 2842, 2003). Recent studies from our laboratory have shown that 17b-estradiol may help maintain long-term neuronal viability in hippocampus by regulating the expression of Bcl-2 family members if initiated immediately after ovariectomy (Sayuri et al., *Anais da XXIII Reunião Anual da FeSBE*, pp. 89, 2008). 17b-estradiol has also been shown to activate extracellular signal-regulated kinase (ERK) that mediates neuroprotection in the hippocampal CA1 after global ischemia (Jover-Mengual et al., Endocrinology 148:1131, 2007). Whether ERK signaling cascade is involved in estrogen-induced expression of Bcl-2 after ovariectomy remains to be explored. In the present study was examined the effects of ovariectomy and 17b-estradiol replacement on ERK1/2 activation in adult rat hippocampus. **Methods:** The experimental procedures were approved by the Research Ethical Committee from Instituto Butantan (number 569/09). Hippocampi were obtained from rats in proestrus (control), rats ovariectomized for 15 days (C15) and 21 days (C21), rats ovariectomized for 15 days and then treated with 17b-estradiol benzoate for 7 days (10 mg/rat, s.c., every other day) (E7) and rats ovariectomized and immediately treated with 17b-estradiol benzoate for 21 days (10 mg/rat, s.c., every other day) (E21). Western blot for detection of ERK1/2 and phospho-ERK1/2 was performed as previously described (Lucas et al., Biol Reprod., 78: 101, 2008). **Results:** Ovariectomy for 15 and 21 days (C15 and C21) did not have any effect on ERK1/2 phosphorylation compared to values obtained from control animals. Similar results were obtained with 17b-estradiol replacement limited to the last week of hormonal deprivation (E7). On the other hand, 17b-estradiol replacement throughout the post-ovariectomy period (E21) induced a rapid increase in the phosphorylation state of ERK1/2 (pERK1, 199.56 ± 14.81, n=3; pERK2, 139.03 ± 19.27, n=3) ($P<0.05$) compared to values obtained from control (100%) or ovariectomized rats (pERK1: 128.59 ± 21.19%, n=3; pERK2: 105.93 ± 5.93:, n=3 and pERK1: 104.38 ± 4.38%, n=3; pERK2: 106.76 ± 4.35%, n=3, respectively, C15 and C21). **Discussion:** These results suggest that 17b-estradiol is involved in the regulation of ERK1/2 phosphorylation in rat hippocampus if initiated immediately after ovariectomy (E21). The neuroprotection by estrogen might be mediated in part by expression of Bcl-2 through ERK1/2 phosphorylation. Further experimental approaches will be important to clarify these events. Supported by PRONEX/CNPq/FAPESP.

01.014

Efeito de antagonistas do receptor da AT1 sobre a proliferação de células de músculo liso vascular. Tambellini N[1], Oliveira KA[1], Favero GM[1], Otuki MF[2], Fernandes D[2]
[1]UEPG - Ciências Biológicas e da Saúde, [2]UEPG - Ciências Farmacêuticas

Introdução e objetivo: A proliferação excessiva de células de músculo liso vascular (CMLV) e consequente remodelamento vascular é um importante processo no desenvolvimento da aterosclerose e da reestenose após a angioplastia. Recentemente tem sido demonstrado que ativação do receptor AT1 pela angiontensina II (ANGII) pode estimular a proliferação de CMLV. Assim o objetivo deste trabalho é de investigar o efeito de antagonistas do receptor AT1 sobre a proliferação de CMLV. **Material e métodos:** Foi utilizada a linhagem celular CMLV de rato, a A7r5. A proliferação celular foi avaliada pela contagem de células em câmara de neubauer pelo método do Azul de Tripan. As células foram mantidas em condições de cultura em placas de 12 poços e foi adicionado angiotensina II nas concentrações de 1μM, 10 μM e 100 μM. Outro grupo de células recebeu losartan na concentração de 100 μM 30 min antes da angiotensina II (100 μM). As células foram incubadas por 24, 48 ou 72 horas. Em cada um dos tempos elas foram removidas da placa por ação da tripsina e coradas com azul de Tripan, sendo então contadas em hemocitômetro. **Resultados:** Os resultados no período de 48 horas após o tratamento com as drogas mostram que a concentração de 1μM de angiotensina não aumenta a proliferação celular, já as concentrações de 10 μM e 100 μM da mesma droga geram um aumento de 30% na proliferação celular quando comparadas ao controle. O losartan na concentração de 100μM preveniu o aumento da proliferação celular induzido pela angiotensina (CNT 40±2,1; ANGII 100μM 57±1,7; ANGII + LOS 26±7.2). Em todos os grupos estudados a viabilidade celular foi maior que 95%. **Discussão:** Estes dados mostram que o bloqueio de receptores AT1 é capaz de inibir a proliferação de CMLV. Assim, o tratamento com estes compostos pode representar uma abordagem terapêutica viável para controlar a hiperplasia da neo-intima. **Entidade Financiadora:** CNPq

01.015

Mutagenesis in angiotensin II AT_1 receptor reveals structural requisites for activation of different signaling pathways and the contribution of the ligands to stabilize distinct receptor conformations. Reis RI[1], Restini CA[2], Santos EL[3], Pecher C[4], Schanstra J[4], Bascands JL[4], Bendhack LM[2], Casarini DE[5], Oliveira EB[1], Pesquero JB[3], Costa-Neto CM[1] [1]FMRP-USP - Bioquímica e Imunologia, [2]FCFRP-USP - Física e Química, [3]UNIFESP - Biofísica, [4]Universté Paul Sabatier, [5]UNIFESP - Medicina

Introduction: The AT_1 receptor has been extensively studied concerning to its amino acid residues involved in agonist/antagonist binding, as well as those involved in G-protein activation. Studies about mitogen activated kinases (MAPK) activation by the AT_1 receptor have also been described, and recently some attention has been given to G-protein-independent activation of MAPKs. In addition to that, a novel panel of GPCR mutations suggests the possible existence of GPCRs activated by conformational selection rather than by conformational induction mechanisms, due to a broad spectrum of agonist specificities. **Methods:** Aiming to test this hypothesis, we stimulated some AT_1 receptor mutants with Sar^1-Ile^4-Ile^8-AngII, an analog of Angiotensin II which is known to not trigger G-protein activation. **Results and Discussion:** Our results showed that despite the total absence of calcium mobilization response after Angiotensin II stimulation, the analog peptide was able to trigger intracellular calcium mobilization in a similar pattern to that of the AT_1 receptor when stimulated with Angiotensin II. Moreover, concerning to ERK1/2 activation while the mutants demonstrated ERK1/2 phosphorylation in the early stage when stimulated with Angiotensin II, these mutants were able to activate ERK1/2 only after 30min of stimulus with Sar^1-Ile^4-Ile^8-AngII. Concluding, we have demonstrated that the AT_1 receptor bears different structural requisites to achieve different active conformations, and also that different ligands stabilize the receptor in distinct conformations, that ultimately will result in activation of different signaling pathways. **Financial support:** FAPESP, CAPES, CNPq

01.016

Effects of vigabatrin on the activity of nicotinic receptors from the rat hippocampus. Oliveira M[1], Setti-Perdigão P[2], Castro NG[2] [1]UFSJ - Engenharia Biomédica, [2]ICB-UFRJ - Farmacologia Molecular

Introduction: Vigabatrin (VGB) is an irreversible inhibitor of GABA - transaminase which increases GABA levels in the CNS. Due to this action, VGB is widely used clinically as an antiepiletic agent. Recent studies have demonstrated that GABAergic substances, including, midazolam, NO - 711 (GABA reuptake inhibitor), and GABA itself, increase the amplitude of α7 currents (IA currents) in cultured dissociated rat hippocampal neurons (Santos et al., *J. Pharmacol. Exp. Ther*. 319:376, 2006). **Methods:** The current work was designed to verify if pre-treatment with VGB also potentiate α7 currents and to determine the duration window of the potentiating effect. Whole-cell currents were recorded by means of the patch-clamp technique And elicited by a 500 ms pulse of choline 10 mM. We have analyzed different concentrations of VGB (1, 10 and 100 μM) and different periods of treatment (24 hours, 48 hours and 7 days) also in rat hippocampal neurons. Additionally we verified toxicity of the treatments by cell counting and LDH release assay. **Results and Discussion:** short - term treatment (24 h) with 1 and 10 μM of VGB increased the IA current amplitude in 56 % and 70 % respectively. On the other hand, long term treatment (48 h and 7 days) with VGB (1, 10 and 100 μM) had the opposite effect, leading to decreased α7 current amplitudes. The potentiating effect of 1 and 10 μM VGB on nicotinic responses was consistent with that previously described for other GABAergic agents, except that it was transient. The potentiating effect was dependent of $GABA_A$ activation since muscimol showed the same potentiating effect on α7 current amplitude. The switch to an inhibitory effect on IA current after 48-h treatment was not seen with other GABAergic compounds. VGB inhibitory effect does not depend on $GABA_B$ receptors activation since faclofen was unable to reverse this inhibitory effect. Surprisingly, all GABAergic substances used in this work showed neurotoxicity after long - term treatment. The data suggest that prolonged activation of $GABA_A$ receptors potentiates in a prolonged manner the IA current amplitude and VGB inhibitory effect could be result of its great toxicity or another mechanism of action independent of GABA. **Support:** CNPq grant 478428/2007-3 and CAPES

01.017

JMF2-1, a non-anesthetic analog of lidocaine, inhibits T cell proliferation and survival by increasing intracellular cAMP levels. Olsen PC[1], Coelho LP[1], Jurgilas PB[1], Costa JCS[2], Viola JP[3], Cordeiro RSB[1], Silva PMR[1], Martins MA[1] [1]FIOCRUZ - Inflammation, [2]Farmanguinhos-FIOCRUZ, [3]INCa - Cellular Biology

Introduction: Our previous findings showed that inhalation of JMF2-1, an analog of lidocaine with reduced anesthetic activity, prevents cardinal features of asthma. We found that JMF2-1 reduces airway hyperresponsiveness, T_H2 cytokine generation and lung eosinophilic inflammatory infiltrate. These effects were likely due to an inhibition of T cell function and survival. In the current study, we elucidated the molecular mechanisms of these effects. **Methods:** Lymphocytes obtained from lymph nodes of DO11.10 or BALB/c mice were stimulated with OVA allergen (0,5 mg/mL) or anti-CD3 mAb (5 μg/mL) for 72 or 48 hours, respectively, in the presence or absence of JMF2-1 (300 μM). Apoptosis and proliferation were confirmed by staining DNA with propidium iodide and by analyzing Sub-G_0 and S+G_2 population through flow cytometry. A pan-caspase inhibitor and a specific inhibitor of caspase-8, ZVAD and ZIETD (50 μM) respectively, were used as a pre-treatment to confirm the apoptosis pathway. ELISA technique was used to quantify cytokine production in supernatants of DO11.10 stimulated cells pre-treated with caspase inhibitor and treated consecutively with JMF2-1. Western blotting with GATA-3 monoclonal antibody was performed to determine expression of this protein in stimulated lymphocytes treated with JMF2-1. Radioimmunoassay was used to quantify cAMP in lymphocytes 20 minutes after JMF2-1 treatment. (Protocol number of Animal Ethics Committee approval 00085-02). **Results:** We found that JMF2-1 increased the apoptosis rate of T cells activated either by OVA allergen (59,62%) or anti-CD3 mAb (44,75%) without modifying the survival rate of unstimulated lymphocytes *in vitro*. Apoptosis induced by JMF2-1 was partially reversed by both caspase inhibitors (ZVAD prevented 46,53% and ZIETD 19,65% of cell death induced by JMF2-1). Caspase blockade prevented cell death caused by JMF2-1 but it did not affect the inhibition of cytokine production. Furthermore, JMF2-1 increased cAMP intracellular concentrations and inhibited GATA-3 expression in these cells. **Discussion:** These results show that JMF2-1 acts by increasing cAMP concentrations in lymphocytes leading to two separate mechanisms: (i) inhibition of proliferation and cytokine production, by inactivating GATA-3, as well as (ii) stimulating T cell apoptosis, via activation of caspase-8 pathway. Altogether, these findings help to explain the anti-inflammatory effects of JMF2-1 observed in *in vivo* systems of experimental asthma. **Financial Support:** CNPq, CAPES, FAPERJ, PDTIS.

01.018

AlphaB-crystalline regulates FAK activation by mechanical stress in cardiomyocytes. Pereira MBM, Santos AM, Franchini KG UNICAMP - Clínica Médica

Introduction*:* Cardiomyocytes respond to increases in functional demand by hypertrophic growth. This reactive hypertrophy involves concerted gene expression and the accumulation of myocyte proteins and organelles that are coordinated by signaling cascades activated by mechanical stress and a variety of soluble endocrine, paracrine, and autocrine factors (SADOSHIMA et al., *Annu Rev Physiol*, 59: 551, 1997). Focal adhesion kinase (FAK) is activated and plays a role in cardiomyocyte hypertrophy induced by mechanical stress (SEKO et al., *Bioch. Biophys. Res. Comm.,* 259: 8, 1999). Typically, mechanical stimulation induces a prolonged activation of FAK in cardiomyocytes. This may be explaned, in part, by the reduction in FAK/Shp2 association in stimulated cells (MARIN, et al., *Circ. Res*. 103: 113, 2008), however, it is possible that additional mechanisms exists to maintain FAK in its phosphorylated and active state. In the present study we examined whether association with the chaperone, alphaB Crystalline (CryAB), contributes to prolong FAK activity in stretched cardiomyocytes. **Methods***:* alphaB Crystalline and FAK expression and phosphorylation at Tyr397 (pFAK) were quantified in neonatal rat ventricular myocytes (NRVMs). FAK/alphaB Crystalline interaction was investigated by co-immunoprecipitation and pulldown assays. alphaB Crystalline depletion by RNAi was used to examine its effect on FAK activation. Data are presented as means ± SEM. **Results***:* Cyclic stretch (10%, 2 hs) increased pFAK (2-fold) and enhanced alphaB Crystalline expression (4-fold) and phosphorylation at Ser59 (3-fold). Stretch also induced FAK/alphaB Crystalline association. Pulldown assays with recombinant FAK domains indicated that the interaction with alphaB Crystalline is mediated by FAK C-terminal domain. FAK immunostaining was coincident with myofilaments in nonstretched while in stretched cells it was predominantly located in the nuclei. alphaB Crystalline was predominantly located at the nuclei of stretched NRVMs, coincident with FAK staining. Depletion of alphaB Crystalline markedly reduced FAK activation and re-location to the nucleus. **Discussion***:* This study demonstrates that alphaB Crystalline associates and regulates FAK activation in cardiomyocytes in response to mechanical stress. **Apoio Financeiro**: FAPESP

01.019

Differential mediation of neutrophil accumulation induced by *Mycobacterium bovis* BCG in mouse pleurisy: role of inflammatory mediators and cytokines. Candea ALP[1], Menezes de Lima Jr O[1], Rosas EC[2], Henriques MGMO[2] [1]UFSC - Farmacologia, [2]FIOCRUZ - Farmacologia Aplicada

Introduction: Mycobacterial infections are among the main health problems worldwide. However, the initial inflammatory events during pulmonary mycobacterial infection are still unclear. There are increasing evidences that neutrophils are important in the control of mycobacterial infections, but few studies focused on the investigation of the inflammatory mediators and cytokines involved in mycobacteria-induced neutrophilia. In the present study we performed a pharmacological characterization of several mediators on the leukocyte influx in a murine model of *Mycobacterium Bovis*-BCG-induced pleurisy, analyzing three different time points of the reaction. **Methods and Results:** Male C57Bl/6 mice were injected with *Mycobacterium bovis*-BCG (4 x 10^5 CFU/cavity) diluted in 100 mL of sterile saline. After 4 h, 24 h or 15 days the mice were sacrificed by CO_2 inhalation and the thoracic cavity was washed with 1 mL of heparinized PBS, to analyze total and differential leukocyte accumulation. Drug pretreatments with: WEB 2170 (2-4 mg kg^{-1}, i.p.), diacerein (30-100 mg kg^{-1}, p.o.), CP-105696 (1-4 mg kg^{-1}, i.p.) L-NAME (5-25 mg kg^{-1}, i.p.) or thalidomide (6-30 mg kg^{-1}, s.c.) were performed 1h before and, in some groups, 13 and 14 days after BCG administration. All procedures were approved by the Committee for Animal Care and Use (CEUA-FIOCRUZ) under L0052-2008 license. After 4 h only the PAF antagonist WEB2170 inhibited neutrophil accumulation. The 24 h neutrophilia was inhibited by the LTB_4 antagonist CP-105696, the NO inhibitor L-NAME, the TNFα inhibitor thalidomide and the IL-1 inhibitor diacerein. After 15 days only L-NAME failed to inhibit neutrophil accumulation. **Conclusion**: Our data indicate that BCG-induced neutrophil accumulation is differentially mediated along the several phases of the inflammatory reaction. **Financial support** CNPq/FIOCRUZ

01.020

Biochemical and pharmacological characterization of bradykinin-related peptides from a solitary wasp venom. Moura AB[1], Picolo G[2], Hisada, M.[3], Sciani, J.[4], Conceição, I.[5], Lima-Landman, M. T. R.[1], Oliveira, V.[4], Melo RL[6], Cury Y[2], Konno K[6], Hayashi, M. A. F.[1] [1]UNIFESP - Farmacologia, [2]Instituto Butantan - Fisiopatologia, [3]Suntory Bioorganic Research, [4]UNIFESP - Biofísica, [5]Instituto Butantan - Farmacologia, [6]LETA-CAT-CEPID Instituto Butantan -

Introduction: Bradykinin (BK) is one of the most important compound generated following tissue injury. This nonapeptide is released by the action of endogenous or exogenous kininogenases on plasma kininogen, and is a potent algesic, hypotensive, and proinflammatory endogenous substance. It is rapidly hydrolyzed by the action of angiotensin-converting enzyme (ACE). BK and BK-related peptides (BRPs) are also widely distributed in venomous animals, including frogs and insects, including wasps. In a survey of the solitary wasp *Cyphononyx fulvognathus* venom three novel BRPs, besides the well-known Thr^6-BK, were identified. Herein we report the chemical, biochemical and pharmacological characterization of Thr^6-BK and of these novel BRPs, named *Cf*-32, *Cf*-46, and *Cf*-146. **Objective:** Aiming a better understanding on the wasp envenomation process, the extract of solitary wasp *Cyphononyx fulvognathus* venom was surveyed. The identified compounds were both biochemically and pharmacologically characterized. **Material and Methods:** The venom extracts of *C. fulvognathus* were subjected to reverse-phase HPLC, and the purity and complexity of each fraction was examined by MALDI-TOF MS. The primary sequence of observed peptides was determined by Edman degradation and ladder sequencing. Enzyme activity assay and determination of kinetic parameters of ACE for these wasp peptides were performed using the synthetic analogs obtained by synthesis on an automated PSSM-8 peptide synthesizer. These same analogs were employed for the BK-potentiation assays on isolated guinea pig ileum and pain threshold evaluations (CEUAIB n° 532/2008). **Results and Discussion:** Interestingly, we verified that, those peptides presenting the highest structural similarity to BK, which were the case of Thr^6-BK and *Cf*-46, were shown to be able to contract smooth muscle preparation, while the other two peptides studied herein, namely *Cf*-32 and *Cf*-146, could not. On the other hand, all these peptides were able to inhibit ACE as well as to induce the hyperalgesic effect in living rats after intraplantar injection. The use of specific BK-receptors antagonists also allowed the identification of BK-receptors as the target of these wasp peptides. We believe that this knowledge will contribute to a better understanding on the wasp envenomation process, which might be a good support to propose the inclusion of novel elements to be considered for the treatment of current wasp accident symptoms. **Financial Support:** Fapesp and CNPq.

01.021

Enhanced expression of alpha-synuclein and Activation of NF-kB induced by reactive astrocytes in SH-SY5Y cells. Yshii LM[1], Sá Lima L[1], Demasi MAA[2], Sogayar MC[2], Kawamoto EM[1], Munhoz CD[1], Scavone C[1] [1]ICB-USP - Pharmacology, [2]IQ-USP-Biochemistry

Introduction: Alpha-synuclein (alpha-syn) is a small soluble protein expressed primarily at presynaptic terminals in the central nervous system (CNS). Interest in alpha-syn has increased after the discovery of a relationship between its dysfunction and several neurodegenerative diseases, including Parkinson´s disease (PD)[a]. Microglial neuroinflammatory responses affect the onset and progression of PD. However, results also suggest that the fundamental property of the wild-type form of this protein may be neuroprotection, as it can inhibit apoptosis in response to various pro-apoptotic stimuli[b]. Nuclear-kB factor (NF-kB) is a transcription factor involved in cellular defense response, apoptosis and regulation of pro and antiinflammatory genes expression[c]. The aim of the present study is to evaluate the influence of reactive astrocytes in the alpha-synuclein expression and NF-kB activation in SH-SY5Y cells infected with lentiviral (LV) vectors expressing wild-type (WT) alpha-syn. **Methods:** This study was submitted and approved by National Committee of Ethics in Animal Researches – CONEP (868/CEP December 10, 2008. ICB - USP). Astrocytes cultures were prepared from 1-day old Wistar rats (ICB-USP). Brains were dissected, cortices were isolate and cells were dispersed by mechanical and enzymatic dissociation with 0.25% trypsin solution (Invitrogen). Cells were placed onto 75 cm^2 flasks and maintained in DMEM supplemented with 10% Fetal Calf Serum (FCS), penicillin (100 U/ml)/ streptomycin (100 ug/ml) and 2 mM glutamine (Invitrogen). After 2 weeks, confluent cultures were shaken overnight at 37°C to remove microglia and were replated for experimental use. We challenged SH-SY5Y cells infected with Lentiviral system with conditioned medium (CM) from reactive astrocytes previously treated with LPS (1 mg/ml). The expression of alpha-syn protein was evaluated by Western blot assay. Cell nuclear extract was used to run eletrophoretic mobility shift assay (EMSA) to measure NF-kB binding activity. Data were analyzed by one-way ANOVA, followed by Newman-Keuls post-test. $p \leq 0,05$ was considered statistically significant. **Results:** Exposures of SH-SY5Y cells infected with lentiviral (LV) vectors expressing wild-type (WT) protein to CM from treated astrocytes (CM with TNFalpha 122 ± 6.72 pg/mL, n = 3) produced a pronounced increase in alpha-synuclein expression when compared to control treated cells. 0.014 ± 0.01 vs 0.05 ± 0.01, $p<0,05$; n = 5). In addition, CM induced an increase in NF-kB activation in nuclear extracts from SH-SY5Y cells infected with lentiviral vectors expressing wild-type (WT) protein (201.4 ± 0.2%, n = 4) when compared to control group (100 ± 0.34%). **Discussion:** Our results suggest a putative interaction between alpha-syn expression and NF-kB signaling in response to an inflammatory stimulus. Therefore, although alpha-syn is not a toxic compound, it can be converted to a toxic compound in the presence of a chronic inflammatory condition which might be linked to NF-kB signaling cascade in the striatum. **References**: a. Lotharius & Brundin, *Nat Rev Neurosci*, 3: 932-942, 2002; b. Alves da Costa et. al., *J Biol Chem*, 277: 50980-50984, 2002; c. Camandola & Mattson, *Expert Opin Ther Targets*, 11(2):123-32, 2007. **Financial Support:** FAPESP

01.022

Influence of transcription factor NF-kB on human uremic serum induced neurotoxicity. Degaspari, D.[1], Kawamoto EM[1], Abe, MY[2], Sá Lima L[1], Branco-Martins JPL[2], Ribeiro Junior E[2], Martins CBT[2], Scavone C[1] [1]ICB-USP - Pharmacology, [2]CINE

Introduction: Chronic kidney disease (CKD), a condition which describes extensive and irreversible alteration of kidney function has been related to cognitive function changes [1]. Due to vascular effects found in these patients, the relationship between CKD and cognitive dysfunction is related to brain tissue chronic hipoperfusion[2]. Nuclear-kB factor (NF-kB) is a transcription factor which is involved in cellular defense response, apoptosis and regulation of pro and anti-inflammatory genes expression. It could be modulated by different kinds of stimuli-like inflammation, ischemia, oxidative stress in neurons and glia[3]. Therefore the aim of this study was to evaluate human uremic serum (pre-hemodialysis) effects on NF-kB modulation in the rat cerebellar granule neuronal culture. **Methods:** This study was submitted and approved by the National Committee of Ethics in Human Being Researches –CONEP (869/CEP December 10, 2008. ICB - USP). Primary rat cerebellar granule neuronal culture was incubated with different concentrations of human uremic serum (1, 5 and 9%) for 1, 3 and 7 days (periods of incubation). Serum from age-matched individuals with no CKD or cognitive deficit was used as control. Cell viability was evaluated by 3-[4,5-dimethyltiazol-2-il]-2,5-diphenyl tetrazolium (MTT) bromide reduction assay and cytotoxicity was measured by lactate dehydrogenase (LDH) release assay. Cell nuclear extract was used to run eletrophoretic mobility shift assay in order to measure NF-kB binding activity. Data were analyzed by one-way ANOVA, followed by Newman-Keuls post-test. $p \leq 0,05$ was considered statistically significant. **Results:** Neuronal toxicity induced by uremic serum was not related to concentrations or time-course assayed. However, the toxicity induced by uremic serum at 1% of concentration was similar in all time points studied and NF-kB activation was observed after 7 days of incubation. This result suggests that CKD patients in chronic hemodialysis show more susceptibility to develop neurologic diseases since their serum contains compounds which even at low concentrations can keep the signaling pathway associated to activated inflammatory response. **Discussion:** Our results suggest the NF-kB signaling pathway is activated even in low concentration of human uremic serum in rat cerebellar granule neuronal culture which is in accordance to the literature indicating NF-kB binding activity increases in situations of neuronal stress that could be elicited by uremic serum. **References**: *1*. Madero, M. et al., *Dis. Sem. Dial*, 21, 29-37, 2008; *2.* Murray, A. M., *Adv. Chron. Kidney Dis*., 15 (2), 123-132, 2008; *3*. Munhoz et al., *Eur. J. Pharmacol*, 2: 3-4, 2005. **Financial Support:** FAPESP, CNPq and CINE.

01.023

Effect of bufadienolides in new intracellular signaling pathways mediated by the Na^+/K^+-ATPase. Amaral LS[1], Figueira R[1], Bezerra MA[1], Loureiro-Tomaz E[1], Cunha-Filho GS[2], Santos ML[2], Noel F[1], Quintas LEM[1] [1]ICB-UFRJ - Farmacologia, [2]IQ-UnB - Isolamento e Transformação de Moléculas Orgânicas

Introduction: The Na^+/K^+-ATPase is a transmembrane protein whose function occurs in two possibly distinct ways: as a carrier of ions Na^+ and K^+ generating electrochemical gradients essential for cell viability and as a signal transducer mediated by protein-protein interactions. This transduction function was recently discovered and is a challenge for the development of Na^+/K^+-ATPase ligands. Ouabain, a cardenolide considered as a steroid hormone in mammals, was shown to inhibit ion transport and also to stimulate the signaling cascade. In this context, we evaluated the ability of different bufadienolides, a class of cardiotonic steroids that remains largely unexplored despite their structural abundance and great natural profusion, to stimulate these new transduction pathways (i.e., Na^+/K^+-ATPase-Src-EGFR-Ras-ERK) and compare with their inhibitory activity on Na^+/K^+-ATPase, trying to establish the structural requirements of potential selective modulators of each of these mechanisms. **Methods:** Primary culture of rat endothelial cells, resistant to ouabain action, and LLC-PK1 cell line (proximal tubule of pig kidney), sensitive to ouabain, were treated 15 min with different bufadienolides – bufalin (BFL), telocinobufagin (TCB), marinobufagin (MBG), isolated from the venom of Brazilian toad *Rhinella schneideri* – lysed with RIPA buffer, centrifuged at 13,000*g* for 15 min and the supernatants (membrane fraction) were used in Western blot for evaluation of MAP kinase ERK1/2 activation (phosphorylation). To assess the ability of Na^+/K^+-ATPase inhibition, inhibition curves were performed with increasing bufadienolide concentrations in membrane preparations of rat kidney (ouabain resistant) and of rat cerebral hemispheres/LLC-PK1 cells (ouabain sensitive) by the colorimetric method of Fiske and Subbarow. For comparison purpose, ouabain was used as standard. **Results and Discussion:** TCB and BFL inhibited Na^+/K^+-ATPase activity in a concentration-dependent fashion at the same range of inhibition achieved by ouabain (rat kidney, IC_{50} = 46 ± 6 μM TCB and 64 ± 10 μM BFL; rat cerebral hemispheres, IC_{50} = 0.037 ± 0.006 μM TCB, 0.056 ± 0.010 μM BFL, 0.85 ± 0.11 μM MBG). MBG, however, was unable to significantly inhibit the rat kidney enzyme even at the concentration of 100 μM. Preliminary experiments for evaluating phospho-ERK1/2 showed that the ERK1/2 activation was also achieved in a concentration-dependent manner but such stimulation seemed to occur at concentrations where no significant inhibition of enzyme activity was observed. These results suggest that bufadienolides present functional selectivity, being, able to activate the transduction pathway at lower concentrations that the ones needed for Na^+/K^+-ATPase inhibition. The study of original natural and semisynthetic bufadienolides and the evaluation of inhibitory function in human Na^+/K^+-ATPase isoforms heterologously expressed in yeasts are in course. **Financial support:** FAPERJ; CNPq, UnB, CAPES

01.024

Characterization of the antimicrobial activity of crotamine. Yamane ES[1], da Silva Junior, PI[2], Rádis-Baptista G[3], Oliveira EB[4], Yamane T[5], Lapa AJ[1], Hayashi MAF[1] [1]UNIFESP - Produtos Naturais, [2]CAT-CEPID-Instituto Butantan, [3]UFC - Ciências do Mar, [4]FMRP-USP – Bioquímica e Imunologia, [5]CBA - Bioquímica e Biologia Molecular

Introduction: Nowadays, different functional classes of biologically active peptides and toxins isolated from many organisms are known. These compounds can be directly used in medicine or may serve as models for the generation of molecules with medical interest. Crotamine is the most abundant toxin found in the venom of the South American rattlesnake *Crotalus durissus terrificus* and it has been shown to be one of these active peptides with potential pharmaceutical application. This toxin is a strongly basic 42-amino acid residues polypeptide with a molecular weight of about 4.9 kDa. Injection of crotamine in mice induces skeletal muscle spasms, leading to spastic paralysis of the hind limbs, which led to its inclusion into the small basic myotoxin family. The presence of three disulfide bridges in crotamine structure gives a high conformational stability to this compound. Interestingly, this same disulfide bridges pattern is also found in antimicrobials peptides from mammals, which usually also present a positively charged surface similarly to crotamine. However, up to now, crotamine was never consistently evaluated as an antimicrobial compound. **Objective:** This study aims to characterize the antimicrobial activity of crotamine against fungus and bacteria (Gram-negative and positive). **Material and Methods:** Aiming to study the antimicrobial activity of crotamine purified from the rattlesnake venom, 10 microorganisms were tested. For that, a colorimetric broth microdilution method was employed for MIC (minimum inhibitory concentration) determination. Microdilution testing was performed according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations (NCCLS document M27-P). By using the growth control for comparison, reference microdilution MIC endpoints for crotamine were scored (+) as the lowest concentration at which an absence of growth was observed, and those in which a prominent decrease in turbidity was observed were scored as (++). **Results and Discussion:** The antimicrobial assay demonstrated that crotamine is mainly able to inhibit the growth of fungus, either from reference strains from American Type Culture Collection (ATCC) as well as from clinical isolates. The growth of *Candida krusei, Trichosporon klebahnii, Candida guilliermondii, Candida glabrata, Candida albicans, Candida parapsilosis,* and *Candida tropicalis* were clearly observed to be inhibited by crotamine in the employed conditions. **Conclusion:** The antimicrobial activity of crotamine was characterized and the obtained data suggest this natural compound as a potential candidate for the development of a novel class of antimicrobial compound to treat clinical infections. **CEP:** 1474/07. **Financial Support:** Fapesp and CNPq.

01.025

Interaction of (-)-3-O-acetyl-spectaline hydrochloride (LASSBio-767) with the acetylcholinesterase peripheral anionic site. Gambôa NF[1], Vieira KST[1], Areas TFMA[1], Fraga CAM[2], Barreiro EJ[2], Bolzani V[3], Castro NG[1] [1]ICB-UFRJ - Farmacologia Molecular, [2]LASSBio-UFRJ Farmácia, [3]NuBBe-UNESP-Araraquara - Química. Orgânica

Introduction: Alzheimer's disease (AD) is the most frequent dementia in the elderly. Being correlated mainly with cholinergic system dysfunction, AD has the acetylcholinesterase (AChE) inhibitors as the mainstay of treatment, because they promote the increase in acetylcholine levels in the synaptic cleft. It has been proposed that some AChE inhibitors that interact with the enzyme's peripheral anionic site (PAS) may have an additional benefit compared to those that interact with the catalytic site (Johnson, *Curr. Pharm. Des.* 12:217, 2006). LASSBio-767, or (-)-3-*O*-acetyl-spectaline hydrochloride, was recently described as an efficient non-competitive cholinesterase inhibitor with central activity (Castro et al., *Eur. J. Pharmacol.* 580:339, 2008). Our aim was to investigate effects beyond AChE inhibition to further characterize the prototype LASSBio-767 as a candidate drug for AD. *Methods:* In order to investigate the compound's binding to the purified enzyme from *Electrophorus electricus*, we performed a spectrofluorimetric competition assay with the PAS ligand propidium. A binding curve of propidium (fixed at 1 microM) was initially obtained by successive dilutions of the enzyme, in the presence and in the absence of LASSBio-767 10 microM. Enzyme kinetics experiments were performed using Ellman's method, adapted to a microplate reader, to determine cholinesterase IC50 for propidium and LASSBio-767. Modulation of neuritogenesis was assayed in differentiating PC12 cells incubated with nerve growth factor (NGF) and followed during three days, in the absence and in the presence of LASSBio-767 (1, 10 and 100 microM). Cultures were photographed and the possible neuritogenic effect was evaluated by counting and measurement of the neurite lengths. **Results and Discussion:** Propidium fluorescence increased by about 12 times in the presence of AChE 10 microM in vehicle (phosphate buffer 1 mM, pH 7.4). In the presence of LASSBio-767 10 microM, the fluorescence increase was of only 4.6 times, indicating a lower formation of AChE-propidium complexes. The propidium-AChE binding curve was displaced to the right in the presence of LASSBio-767, with a reduction of 5 times in apparent affinity. Accordingly, the enzyme inhibition curve of LASSBio-767 was also displaced to the right in the presence of propidium. These experiments demonstrate that LASSBio-767 competes with propidium at the AChE PAS. The PC12 cells showed a concentration-dependent increase in neuritogenesis in the presence of LASSBio-767. This effect may depend on binding to the PAS, as suggested for other AChE inhibitors, and may be relevant in the context of neurodegeneration. Thus, we found that LASSBio-767 interacts with the PAS of AChE, a property that may confer additional therapeutic benefit in AD drugs. Support: Finep (Ações Transversais) and Apsen Farmacêutica.

01.026

The role of osteopontin in muscle regeneration process after injury by injection of *Bothrops lanceolatus* venom: an immunohistochemical study. Barbosa-Souza V[1], Contin DK[2], Bonventi W[3], Lôbo de Araújo A[2], Irazusta SP[3], Cruz-Höfling MA[4]
[1]UNICAMP – Farmacologia / Histologia e Embriologia, [2]UNICAMP - Farmacologia, [3]CEETEPS [4]IB-UNICAMP - Biologia Celular e Estrutural

Introduction: Envenomation by snakes of the genus *Bothrops* are characterized by prominent local tissue damage, such as edema, hemorrhage and myonecrosis. *Bothrops lanceolatus*, the species responsible for snakebite in Martinica and Caribe, is known to induce thrombotic events in human victims. Studies have shown that *B. lanceolatus* venom is devoid of coagulant and defibrinogenolytic effects, but shows PLA_2 and metalloproteinase activities with only mild local myonecrosis in mice. Nevertheless myofibers alterations induce an inflammatory response. Inflammation is known to contribute for removal of the necrotic material and secretion of cytokines and growth factors which by their turn cause satellite cell activation. Here, we investigate the expression of osteopontin (OPN), a cytokine–which plays an important role in inflammation process and tissue repair, after different time-points of the *B. lanceolatus* i.m injection in muscle. **Materials and Methods:** The expression of osteopontin (Sigmaâ) was investigated by immunohistochemistry (IHC) at 1, 3, 6, 18 hr, 1, 2, 3, 7, 14, 21 days after i.m.-injection in mice gastrocnemius of the *B. lanceolatus* snake venom (100 µg/100 µl), or PBS (100 µl). The data of the osteopontin expression were analyzed by the software GIMP 2.6.4 (Gymp program, CNET Networks, Inc. Australia), that considered the difference of the percentage of the area labelled by OPN between treated and control groups, and correlate with the injury/regeneration process in skeletal muscle along the period investigated. The statistical analysis was accomplished using one-way ANOVA followed by Bonferroni test. $P<0.05$ was considered significant. The research was approved by the Committee for the care and use of Animals (CEEA-IB-UNICAMP), protocol nº 945-1.**Results and Discussion:** The osteopontin expression was crescent from 6h after injection in the treated animals, with the highest values between 3-14d time interval (31±3,1; 27,0±1,2 and 24,2±3,2%, respectively), suggesting that this glycoprotein participates in the regeneration process of affected myofibers. There was not significant difference in the osteopontin expression between treated and control animals at the 1 and 3h interval. Muscle fibers regeneration after injury requires satellite cells activation, their proliferation into myoblasts and fusion into elongated multinucleate myofibers. Literature reports that myoblasts are na important source of osteopontin in damaged muscle and that osteopontin expression significantly increases in thrombotic events and when released from myoblasts may assist in controlling both the myogenic and inflammatory processes during the early phases of muscle regeneration. As far as we know this is the first study dealing with muscle damage by snake venom which investigates the expression of osteopontin in muscle repair. Further studies are underway to substantiate these preliminary findings. Financial support: CAPES, CNPq, FAEPEX/UNICAMP, FAPESP.

01.027

Effect of glucagon on number and reactivity of mast cells. Insuela DBR, Torres RC, Cordeiro RSB, Martins MA, Silva PMR, Carvalho VF IOC-FIOCRUZ - Inflamação

Introduction: The human body wants blood glucose to keep in homeostasis. Insulin and glucagon are the major hormones that regulating the levels of blood glucose. The imbalance between the systemic levels of insulin and glucagon hormones seems to be implicated in the persistent hyperglycaemia (Li *et al.*, Clin. Sci. 114: 591, 2008). We previously described that hypoinsulinemia induce reduction in mast cell population and reactivity (Diaz *et al.*, Int. Arch. Allergy Immunol. 111: 36, 1996). For this, the aim of this work was evaluated the effect of glucagon on number and reactivity of mast cells. **Methods:** The animals were obtained from the Oswaldo Cruz Foundation breeding colony and used in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (CEUA-FIOCRUZ, protocol 0085–02). Swiss-Webster mice were treated with the glucagon (0.01 or 0.1 mg/kg, i.p.), once a day during 7 days. After treatment, peritoneal and mesenteric mast cell numbers were evaluated by means of toluidine blue and Giemsa dyes, respectively. Moreover, the plasma of animals was recovered to insulin quantification by radioimmunoassay. For analysis of mast cell reactivity hypodermic tissue was removed from actively sensitized rats and challenged with OVA (300 ug/ml) in vitro during 1h, after the supernatant was recovered to histamine quantification by fluorimetric assay. The treatment with glucagon (0.1 or 1 uM) was realized in vitro 1h before challenge. **Results:** We showed that animals treated with glucagon showed a marked reduction in mast cell population present in peritoneal cavity (from 32.18 ± 2.41 to 9.9 ± 1.2 mast cells (10^3)/cavity; mean ± SEM) and mesenteric tissue (from 494.36 ± 67.93 to 232 ± 77.17 mast cells/μm^2; mean ± SEM), a phenomenon that paralleled with the reduction in the plasma insulin levels (from 97.2 ± 23.1 to 32.2 ± 13 µU/mL; mean ± SEM). Also, fragments of the hypodermic tissue from sensitized rats treated with glucagon showed a reduction in histamine release after stimulation with antigen in vitro (from 200.7 ± 6.24 to 82.3 ± 3.56 histamine release (ng)/tissue (mg); mean ± SEM). **Discussion:** Our findings show that glucagon down-regulated mast cell number, in a clear association with reduction of plasma insulin levels, as well decreased the mast cell reactivity in vitro. Therefore, we propose that glucagon could be act as an anti-inflammatory hormone. **Financial Support:** CNPq, FAPERJ, FIOCRUZ.

01.028

Melatonin inhibits preconditioning of cultured endothelial cells induced by lipopolysaccharide (LPS). Tamura EK, Marçola M, Fernandes PACM, Monteiro AWA, Cruz-Machado SS, Markus RP IB-USP - Fisiologia

Introduction: The hormone melatonin is released at night by the pineal gland and when synthesized by immune competent cells can reach high concentrations at the site of production (Pontes, *J Pineal Res*, 41:136, 2006). We have previously shown that concentrations compatible to nocturnal melatonin surge inhibits the activation of constitutive nitric oxide synthase (Tamura, *J Pineal Res*, 41:267, 2006) as well as impairs neutrophil migration (Lotufo, *Eur J Pharmacol*, 430:351, 2001) but need a 1000-10000 fold higher concentrations to block the inducible enzyme (iNOS) (TAMURA, J Pineal Res, 46:268, 2009). These cells were shown to be preconditioned by adrenalectomy, when the adherence to neutrophils increases, due to the reduction of circulating glucocorticoids (Cavalcanti, *Brit J Pharmacol*, 152:1291, 2007). Here we investigated whether the treatment of the donor animal with LPS or LPS plus melatonin could prime endothelial cells in such a way that the expression of iNOS, adhesion molecules and neutrophil adherence capability of cultured cells would depend on the origin of the cells. **Methods**: Male rats (2 month-old) maintained in 12/12 h light/dark cycle, injected after four hours of lights off with LPS (0.5 mg/Kg) or LPS plus melatonin (3 mg/Kg), were killed after two hours. Two groups were killed after 6 hours of lights off (naïve, or saline injected after four hours of lights off). Endothelial cells culture (TAMURA, 2006) experiments were performed in cells that reached confluence (±14 days). All animal procedures were performed according to approved institutional protocols (086/2008). The expression of the adherence molecules (ICAM and PECAM) and iNOS were done by immunofluorescence. The neutrophil-endothelium adherence assay was done in according to protocol described by Lotufo (*Eur J Pharmacol*, 534:258, 2006). All the results are expressed in % relative to naïve group (100%). **Results**: Endothelial cells from LPS injected rats had a higher ability to adhere neutrophils *in vitro* (276.5±32.2%) when compared to naïve or saline group. Cells from rats treated with melatonin + LPS adheres leukocytes in a manner similar to control or naïve animals. The expression of iNOS (639.5±212.2%) and adhesion molecules (PECAM: 357.0±179.6%; ICAM: 513.1±150.2%) was significantly higher in the LPS group. This increase in expression of molecules that mediate inflammatory response was inhibited by melatonin (iNOS: 121.9±15.8%; PECAM: 85.42 ± 42.5%; ICAM: 62.7±19.69%). **Discussion**: Our results show that cultured endothelial cells preserve the memory of the donor animal state, in such a way that cells of animals treated with LPS express a higher amount of adherence molecules and adheres neutrophils significantly more than cells from control and naïve animals after ±16 days in culture. Melatonin treatment, which was shown to reduce neutrophil adherence *in vivo* (Lotufo, 2001), also primes the cells for a future *in vitro* response. Taking into account that melatonin is the hormone of darkness, our result opens the possibility that cells for storage in cell banks could have different properties depending on the hour of collection and on the state of the donor. **Acknowledgments**: The technical support of Debora A. de Moura is gratefully acknowledged. **Financial Support**: FAPESP (07/07178-06), CAPES, CNPq and PRPesq USP.

01.029

LASSBio-579, an antipsychotic lead compound, increases synaptic glutamate release in rat cultured hippocampal neurons. Neves G[1], Rates SMK[1], Castro NG[2] [1]UFRGS - Ciências Farmacêuticas, [2]ICB-UFRJ - Farmacologia Molecular

Introduction: LASSBio-579 was recently characterized as an antipsychotic lead compound active in animal models of positive symptoms of schizophrenia with a mild propensity to induce motor side effects. Investigation of LASSBio-579 mechanism of action revealed a multireceptor profile, including binding to D_2-*like* (Ki = 0.11 μM), 5-HT_{1A} (Ki = 0.09 μM) and 5-HT_{2A} (Ki = 2.2 μM) receptors. The ability of this compound to modulate dopaminergic and serotonergic neurotransmission *in vivo* was also demonstrated. Although the binding profile could explain in part LASSBio-579's *in vivo* effects, modulation of other molecular targets or neurotransmitter systems cannot be ruled out. The aim of this work was to evaluate the effect of LASSBio-579 upon the glutamatergic neurotransmission using electrophysiological assays. **Methods:** Miniature excitatory post-synaptic currents (mEPSCs) were recorded in rat cultured hippocampal neurons (E18-20) using the whole-cell patch-clamp technique. Recordings were made under voltage clamp (-70 mV) and lasted 150 s. Test solutions were perfused using an ultrafast parallel tubes system. Bicuculline 20 μM and tetrodotoxin 150 nM were used to block inhibitory currents and action potentials, respectively. Effects of LASSBio-579 10 μM on mEPSCs were evaluated with or without co-perfusion with WAY 100,635 1 μM or ketanserin 1 μM. All experimental procedures were approved by the Ethics Committee on Animals Research of CCS/UFRJ, protocol DFBICB 0290. **Results and Discussion:** Perfusion of LASSBio-579 10 μM induced a decrease on inter-mEPSCs interval and did not affect the events amplitude. A selective effect upon frequency of mEPSCs points to a pre-synaptic mechanism. Thus, LASSBio-579 increased glutamate release in cultured hippocampal neurons. In order to investigate the involvement of serotonin receptors in this effect, co-perfusion of LASSBio-579 with 5-HT_{1A} and 5-HT_{2A} antagonists was also performed. The results obtained with WAY 100,635 1 μM are inconclusive and require more experiments to be clarified. However, co-perfusion of ketanserin 1 μM significantly reversed LASSBio-579's induced increase in mEPSCs frequency, indicating that this compound is acting as a 5-HT_{2A} agonist in this experimental condition. An increase in synaptic glutamate release in the hippocampus has been related with cognitive improvement in neurodegenerative disorders, such as Alzheimer's disease. Thus, LASSBio-579's effect on glutamatergic neurotransmission demonstrated in this work might contribute to a beneficial effect on schizophrenia cognitive symptoms through a mechanism different from those of second generation antipsychotics. **Financial support:** PROCAD/CAPES; INCT de Fármacos e Medicamentos, INOFARMED, CNPq.

01.030

ANG(1-7) activates the AKT phosphorylation pathway by directly binding to the MAS receptor. de Souza LL, Santos GA, Costa-Neto CM FMRP-USP - Bioquímica e Imunologia

Introduction: The renin-angiotensin system (RAS) plays a critical role in blood pressure and body fluid and electrolyte homeostasis. Angiotensin (Ang) II and Ang(1-7) are active peptides of RAS that play a role in regulation of cardiovascular and renal function, where some of the Ang(1-7) effects are opposite to those of Ang II. In this sense, AngII is involved in growth stimulatory pathways while Ang(1-7) has been shown anti-proliferative effect in human lung cancer cells. Since 2003 it is known that Ang(1-7) exerts its effects by binding to the MAS receptor. Although many studies have reported the *in vivo* functions for this heptapeptide, the precise signaling pathways involved in those effects are still to be unveiled. In this study, we investigated the effects of Ang(1-7) in the activation of a specific signaling pathway involved in cell proliferation in a system where only MAS receptor is expressed. **Methods**: HEK293T cells were cultured for 2-3 days as a monolayer in DMEM with 10% bovine serum albumin, glutamine and gentamycin. Cells were transiently transfected with MAS DNA by the calcium chloride method. MAS-transfected cells were treated with 1μM of Ang(1-7) for different times. AKT phosphorylation was determined by western blotting. MTT assay was performed to evaluate cell proliferation. MAS-transfected cells were seeded in 48-well plates ($2x10^4$ cells/well) and incubated for 24h with 1μM of Ang(1-7) at 37ºC. MTT reagent was added to the plate and the relative rate of cell grown was measured by absorbance. **Results and Discussion**: HEK293T cells do not express endogenous mRNA for AT1 and AT2 receptors, nor for MAS receptor. Therefore, this cell line allowed us to analyze the effects of Ang(1-7) in a “pure system” where only MAS receptor was present. The efficiency of transfection was confirmed by real-time PCR, where the MAS receptor transcripts were detected only in transfected cells. A time course in MAS-transfected cells stimulated with Ang(1-7) showed an increase of about 30% in AKT phosphorylation after 30 minutes of treatment as compared to the control. Since AKT signaling pathway is involved in cell proliferation, a proliferation assay was performed to evaluate cell growth. We found no significant difference in cell growth after 24h of incubation with increasing doses of Ang(1-7) (0.1, 1 e 10μM). Our results evidence that AKT signaling pathway is activated by Ang(1-7) directly and solely acting on MAS receptor. We also conclude that the MTT assay should be redesigned to a longer time-course to further investigate cell proliferation rates. **Financial support**: FAPESP, CAPES and FAEPA.

01.031

Reactivity of cultured endothelial cells are influenced by the endogenous rhythmicity of animal donor. Marçola M, Tamura EK, Fernandes PACM, Monteiro AWA, Markus RP
IB-USP - Fisiologia

Introduction: The endothelial cells, responsible for vascular homeostasis and for several aspects of the mounting of inflammatory responses, are privileged target for circulating substances. Endothelial layer is the gate for entrance of immune cells, which initiate innate immune response. The putative use of these cells in cell therapy or therapeutic devices is being impaired by the diversity of response among the sources, either regarding the tissues or the conditions of collecting them. We have previously shown that melatonin, in concentrations compatible with nocturnal surge, reduces leukocytes rolling and adherence (Lotufo, *Eur J Pharmacol*, 430:351, 2001). In addition, melatonin inhibits the activation of the nuclear factor kappa B (NFkB) in cultured endothelial cells challenged by LPS, resulting in a reduction of the expression of inducible nitric oxide synthase (iNOS). In order to allow the mounting of inflammatory responses endothelial cells express adhesion molecules such as PECAM and ICAM and the enzyme iNOS. The ability of endothelial cells in culture to adhere neutrophils is changed by adrenalectomy of the animal donor (Cavalcanti, *Brit J Pharmacol*, 152:1291, 2007). Here we evaluated whether the endothelial cells obtained from animals killed during daytime or nighttime could, in culture, show a variation in the PECAM, ICAM and iNOS expression and in neutrophils adherence assay. **Methods:** Male rats were maintained in 12/12h light/dark cycle and separated in two groups, one killed 6 hours after lights on (day) and other 6 hours after lights off (night). All animal procedures were performed according to approved institutional protocols (086/2008). The endothelial cells obtained from the cremaster muscle were used after confluence (±14 days *in vitro)*. Neutrophil adherence was quantified by mieloperoxydase enzyme assay, as described previously (Lotufo, *Eur J Pharmacol*, 534:258, 2006). The expression of adhesion molecules and iNOS was determined by immunofluorescence. All the results are expressed in % relative to day group. **Results:** Neutrophils adherence in endothelial cells from animals sacrificed during the dark phase was lower (39.99±7.53%) than in cells obtained from animals killed during the light phase. Similar profiles were observed on the expression of adhesion molecules (PECAM:32.09±18.35%; ICAM:31.04±14.69%) and iNOS (23.55±10.57%). Therefore, endothelial cells obtained from animals sacrificed at night showed a decrease in the expression of these proteins than cells obtained on day group. **Discussion:** Our results clearly showed that after 14 days in culture endothelial cells kept the same ability to adhere neutrophils as observed with intravital microscopy. The lowest ability to adhere neutrophils observed in endothelial cultures obtained from animals killed at night was due to a reduction in the expression of the adhesion molecules PECAM and ICAM. These cells also expressed a lower amount of iNOS, strongly suggesting that daytime information was retained even after 14 days in culture. This is the first evidence that the hour of the day is an important consideration in recording information for storing cells for future therapeutic uses. **Acknowledgments**: The technical support of Debora Aparecida de Moura is gratefully acknowledged. **Financial Support**: FAPESP (07/07178-06), CAPES and CNPq.

01.032

Minocycline reduces pge_2 production and mPGES-1, but not COX-2, expression in LPS-activated primary rat microglia. Bastos LS, Oliveira ACP, Fiebich BL Albert-Ludwigs - Neurochemisches

Introduction: Minocycline induces beneficial effects in animal models of inflammation, nociception and neurodegeneration. These effects are associated with inhibition of microglial activation, cells whose roles played in pain modulation and neurodegeneration have been widely studied. The present study aimed to investigate the effect induced by minocycline on prostaglandin (PG)-E_2 production in primary rat microglial cells activated by lipopolysaccharide (LPS), and mechanisms related to this effect. Therefore, the effects on the expression of cyclooxygenase (COX)-2 and microsomal prostaglandin synthase (mPGES)-1 expression were tested. These enzymes are up-regulated in inflammatory conditions, which in turn augments PGE_2 production. **Methods**: Primary microglial cell cultures were established from cerebral cortices of one-day neonatal Wistar rats. LPS (10 ng/ml) was added into the culture media 30 min after minocycline hydrochloride (3, 10 or 30 μM) or its vehicle (phosphate-buffered saline). After 24 h incubation, supernatants were harvested and PGE_2 was determined by enzyme immunoassay. COX-2 and mPGES-1 protein levels were then measured by Western blot analysis. The cell viability was assessed by an adenosine triphosphate (ATP)-based assay. Results from 3 independent experiments were analyzed by one-way analysis of variance followed by Newmann-Keuls test. This study was approved by the local ethics board of the University Hospital Freiburg (protocol 110/06). **Results and Discussion**: Minocycline reduced the PGE_2 production in a concentration-dependent manner. The highest concentration used, 30 μM, reduced 53% when compared with the LPS-treated control group ($p < 0.05$). Moreover, mPGES-1 expression was markedly reduced (3, 10 or 30 μM inhibited 37, 41 and 49%, respectively, $p < 0.01$), whereas the expression of COX-2 was only slightly affected (20%), but not in a statistically significant manner. The cell viability was not affected by minocycline at concentrations used in the present study. On the other hand, dimethylsulfoxide (5% v/v), used as a positive control, reduced markedly the cell viability (90%, $p < 0.05$). Altogether, these results indicate that mPGES-1, rather than COX-2, appears to be a preferential minocycline target to inhibit PGE_2 production. As far as we know, this is the first demonstration that a clinically available drug inhibits mPGES-1, but not COX-2, expression. Regarding the cardiovascular risk associated with the use of nonsteroidal anti-inflammatory medicines that selectively inhibit COX-2 activity, mPGES-1, a downstream enzyme in the arachidonic acid pathway, might be a promising therapeutic target rather than COX-2. In conclusion, this study shows mechanisms that support previous findings of anti-inflammatory, antinociceptive and neuroprotective activities exhibited by minocycline. **Financial support**: VivaCell Biotechnology GmbH (Germany) and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG, Minas Gerais, Brazil).

01.033

Pineal nuclear factor kappa B (NFkB) pathway is triggered by nicotinic acetylcholine receptor activation. Cecon E, Fernandes PACM, Ferreira ZS, Markus RP IB-USP - Fisiologia

Introduction: The relevance of maintaining the synchronization of the internal milieu is now recognized. The hormone of the pineal gland, melatonin, is the endogenous marker of the dark phase of the day and it was known to be suppressed only by light. We have recently demonstrated that pro-inflammatory cytokines are able to suppress melatonin production even at night, due to the activation of the NFkB pathway (Markus, *Neuroimmunomodulation* 14:126, 2007). NFkB pathway, which is constitutively activated in rat pineal glands, shows a daily rhythm, with high nuclear translocation during the light phase and an abrupt reduction of nuclear content as soon as darkness occurs (Cecon, FASEB Summer Res. Conference, 75, 2008). Taking into account that propranolol treatment was not effective on preventing NFkB reduction at night, and that carbachol mimics the effect of light on inhibiting the darkness-induced melatonin synthesis (Zatz, Brain Res. 213:438, 1981), here we addressed the effect of acetylcholine on NFkB pathway in the pineal gland. **Methods:** Pineal glands were obtained from male Wistar rats (Animal Committee Care: 047/2007), 2 month-old, kept under 12h:12h light/dark cycle, sacrificed at the end of the light phase or at the beginning of the dark phase. The glands were immediately placed in 24-wells plate with BGJb medium and incubated with norepinephrine (NE, 100 nM, 30 min to 1h) or acetylcholine (ACh, 1μM to 1mM, 30 min). NFkB-DNA binding activity were assessed by eletromobility shift assay (EMSA) and quantified by optical densitometry. **Results:** The non-stimulated pineal glands obtained at the end of the light phase of the day presented high levels of nuclear NFkB, which drops immediately after lights off. NE had no effect on nuclear NFkB content and ACh increased in a dose-dependent manner the amount of NFkB found in the nucleus. The comparison of fitting between single and double site log-dose-response curve showed a best fitting to two sites ($p=0.0374$). **Discussion:** Here we show that NE is not responsible for the abrupt reduction on nuclear NFkB content in the early night, while ACh is able to keep the high levels of NFkB-DNA binding activity observed during the light phase. Even more, the high concentrations of ACh used to attain maximal response highly indicates that the effect is mediated by nicotinic cholinergic receptors, which are known to mimic light (Zatz, Brain Res. 213:438, 1981). Therefore, our data open a new perspective for evaluating the interaction between cholinergic and adrenergic neurotransmission in the pineal gland, as well as understanding how light mediates nocturnal melatonin suppression independent of the sympathetic pathway. **Acknowledgements:** The technical support of Alex W. A. Monteiro and Débora Aparecida de Moura are gratefully acknowledge. **Financial Support:** FAPESP, CNPq, CAPES.

01.034

Anti-inflammatory and antimicobacterial activity of *N-(aryl)-2-thiophen-2-ylacetamide* against *Mycobacterium tuberculosis* (MTB) and *Mycobacterium bovis* (BCG). Vergara FMF[1], Candea ALP[1], Ferraris FK[1], de Souza, MVN[2], Henriques MGMO[1] [1]FarManguinhos-FIOCRUZ - Farmacologia Aplicada, [2]FarManguinhos-FIOCRUZ - Síntese Orgânica

Introduction: Tuberculosis killed approximately 1.6 million in 2005 alone, according to the latest estimates from the World Health Organization (WHO). There is an urgent need for new drugs to fight against this disease. 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, which is present in many natural and synthetic products with a wide range of pharmacological activities. Our group presented a new class of compounds and demonstrated its activity against *Mycobacterium sp.* (M.C.S. Lourenço *et al*, *Bioorg. Med. Chem. Lett*. (17) 6895, 2007). Here we described the *in vitro* and *in vivo* effects of *N-(aryl)-2-thiophen-2-ylacetamide* against micobacteria infection. **Methods:** The direct growth inhibition of *M. bovis*. was evaluated by Alamar blue® method. The bacteria suspension ($3x10^6$/well) was incubated with different concentrations (0,1 1,0; 10; 25; 100µg/mL) of the compound, for 24hs. Citotoxicity of the compound was evaluated by MTT method (Mosman, *J Immunol Methods* (65) 55, 1985) after 48h treatment of infected cells. Mesotelial cells or macrophages were infected with *M. bovis* (1/1) and treated for 48hs. The bacterial growth was counted 30 days after been cultivated in Lowenstein Jensen (LJ) medium. Cytokines were evaluated by ELISA assay and nitric oxide (NO) by Greiss method, using supernatant collected from infected and treated (24hs) cells. Translocation of NF-κB factor was analyzed by confocal microscope. Neutrophil adhesion was investigated using plated mesothelial cells (10^4/well), infected and treated them with the compound and counted at Olympus IX70 microscope and analised at ImageJ. The expression of receptors was analyzed by flow cytometer. The anti-inflammatory and the antimicobacterial effect *in vivo* were evaluated in BCG-induced pleurisy model in C57BL/6 mouse (CEUA, Fiocruz; licence n. L-0004/08), the compound was administrated intrapleural at (0,5-50mg/Kg). **Results and Discussion**: *N-(aryl)-2-thiophen-2-ylacetamide* wasn't citotoxicity in both cells in all concentrations used. *In vitro*, this compound was able to inhibit directly the growth of MTB and BCG and the intracellular BCG growth. The treatment with the compound was able to reduce TNF-α, IL-6, Il-1β and nitric oxide in infected macrophages and decreased the translocation of NF-κB factor. The expression of mannose receptor was increased, indicating that this compound was able to modulate this phagositosis via. The expression of CD14 molecule was decreased in treated cells, indicating that this compound possibly interferes with the interaction between the receptor and the lipoarabinomanan and lipomanan. The compound affects neutrophil adhesion on mesothelial cells, possibly interfering in the cellular communication. Moreover the compound was able to decrease the liberation of IL-12 by mesothelial cells. *In vivo* treatment induced a decrease of total leukocyte, mononuclear, eosinophils and, especially neutrophils influx to the pleural cavity at the higher dose used (50mg/Kg). Also *in vivo,* we demonstrated that $CD3^+$lymphocytes presented in pleural cavity increased the expression of IFN-γ and decreased the expression of IL-4 after treatment with the compound. Our data indicate that this new compound, *N-(aryl)-2-thiophen-2-ylacetamide,* presents *in vitro* and *in vivo,* an important antimicobacterial activity and immunomodulatory effect. Financial Support:CNPq, FarManguinhos, PDTIS

01.035

Angiotensin II modulates different functions in smooth muscle cell via a2b1/b2 integrin: role of ILK. Dias AM[1], Moraes JA de[1], Marcinkiewicz C[2], Assreuy J[3], Barja Fidalgo TC[4]
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Introduction and goals: Cardiovascular diseases represent the major cause of mortality and morbidity in western countries. Among these conditions, atherosclerosis is the most prominent one. A hallmark of atherosclerosis is the atheromatous plaque formation, where occurs vascular smooth muscle cell (SMC) accumulation. The pathological effect of SMC in response to different stimuli that are able to induce SMC migration and proliferation, leads to fibrous cap (atherosclerosis) or neointima (restenosis) formation. An important inflammatory mediator released during atherogenesis is Angiotensin II (AngII) that can represent relevant importance in vascular injury because induces reactive oxygen species (ROS) production, migration and proliferation of SMC. AngII acts via Angiotensin Receptor I (AT1), therefore integrin-mediated signaling pathways also are activated. Activation of AT-1, a G-protein coupled receptor, induces PKC-alpha activation, which in turn can modulate different signaling pathways. To elucidate the molecular mechanisms involved in restenosis/atherosclerosis, we have studied the integrin signaling pathways that modulate the effect of AngII on SMC functions. **Methods:** SMC linage from rat thoracic aorta (A7r5) was used throughout. The disintegrin obtustatin, was used as a selective ligant of a1/ a 2 b1 integrin. Chemotaxis assay was performed in Boyden chambers. SMC was pre-treated for 30min with different antagonists and then incubated for 4 hours to migrate towards AngII. The cell images of ILK-actin cytoskeleton colocalization were obtained by fluorescence microscopy. Cell proliferation was aaccessed evaluated by thymidine (H3) incorporation assay. Thymidine (1 µCi/mL) was added after last 24h of incubation of AngII. Protein expression was analysed by immunoblotting. ROS production was quantified by CM-DCFDA probe analysis. Cell cycle investigation was observed by PI analysis in a Cytometer. **Results:** Firstly, we observed that the chemotactic effect of AngII (100nM) on SMC was abolished when the cells were pretreated with the a1/ a 2 b1 integrin ligand, Obtustatin (Obt 100nM). Other disintegrins were also tested (eristostatin and kistrin) therefore Obt showed the best inhibitory effect. Obt abrogated AngII effect on FAK phosphorylation in SMC. We also observed that integrin-sinaling pathway was induced after SMC activation by AngII, because RO 31-8220, PKC-alpha inhibitor also blocked FAK phosphorylation induced by AngII. When integrin-signaling pathways are activated, ILK can colocalize with actin cytoskeleton, promoting its rearregement. When SMC was pretreated with Obt we did not observed actin cytoskeleton-ILK colocalization, as observed in SMC only treated with AngII. We also observed that RO 31-8220 inhibited actin cytoskeleton-ILK colocalization induced by AngII, confirming that integrin-signaling pathway is downstream AT1-PKC-alpha activation. Interestingly, Obt accelerated the ROS production return to basal levels after AngII treatment. We also observed that AngII induces, via a1/a2b1 integrin, AKT phosphorylation, and p21 expression reducement, probably via ILK. Corroborating these data we demonstrated that, pretreatment with Obt, induced G1 phase arrest and diminishment AngII effect on SMC proliferation. In this work we suggest that a1/ a 2 b1 integrin may be an important target molecule to the development of more effective therapeutic interventions in restenosis/atherosclerosis. **Supported by**: FAPERJ, CAPES, CNPq

01.036

Effect of purmorphamine on proliferation and osteoblastic differentiation of rat mesenchymal stem cells. Oliveira FS[1], Bellesini LS[1], Crippa GE[2], De Oliveira PT[2], Beloti MM[2], Rosa AL[2] [1]USP – Farmacologia, [2]FORP-USP

Introduction: Purmorphamine is a cell permeable purine compound that is reported to activate the Hedgehog signaling pathway, involved in multiple developmental processes during formation of various organs and tissues including skeletal tissues. Recently, RUNX2, one of the master genes involved in late-stage osteogenesis, was induced following purmorphamine treatment. Although purmorphamine is known to induce osteoblast differentiation in C3H10T1/2 multipotent mesenchymal progenitor cells and in MC3T3-E1 lineage-committed preosteoblasts, the molecular details of its function have remained vague. This study aimed at evaluating the effect of purmorphamine on proliferation and osteoblastic differentiation of mesenchymal stem cells derived from rat bone marrow. **Methods**: Experiments were carried out under approval of Committee of Ethics in Research (Number 07.1.495.53.9). Cells were obtained by flushing out the bone marrow from femurs and cultured in osteogenic medium until subconfluence. First-passage cells were cultured in 24-well culture plates (2×10^4 cells/well) in presence of purmorphamine 2 μM or vehicle. At 3, 7 and 10 days, cell proliferation was evaluated by MTT assay. At 7, 10 and 14 days alkaline phosphatase (ALP) activity was evaluated using a commercial kit. Gene expression of the osteoblastic phenotype markers RUNX2, osterix (OST), bone sialoprotein (BSP) and ALP were evaluated by quantitative real-time PCR after 7 days of drug exposure. All experiments were done in triplicate and submitted to Mann-Whitney test. **Results**: Purmorphamine did not affect cell proliferation at day 3 ($p=0.2506$) but at 10 ($p=0.0163$) and 14 days ($p=0.0163$) cell proliferation was increased by drug exposure (1.08 fold and 1.24-fold, respectively). ALP activity ($p=0.0090$) was increased 1.43 fold by purmorphamine at 14 days. Gene expression of RUNX2 ($p=0.0463$), BSP ($p=0.0463$), OST ($p=0.0463$) and ALP ($p=0.0495$) were upregulated after 7 days of drug exposure (1.41-fold, 1.28-fold, 1.20-fold and 1.11-fold, respectively). **Discussion**: These results indicate that purmorphamine presents a positive impact on osteoblasts derived from mesenchymal stem cells as it increased both proliferation and osteoblastic differentiation. The latter by upregulating the transcription of some of the master regulator of osteogenesis, such as RUNX2 and OST and the osteoblastic marker, ALP. **Acknowledgements**: FAPESP for financial support.

01.037

Effect of the ppar-gamma agonist rosiglitazone on mast cell population in alloxan-diabetic mice. Torres RC, Silva AR, Cordeiro RSB, Martins MA, Silva PMR, Carvalho VF IOC-FIOCRUZ - Inflamação

Introduction: *Diabetes mellitus* is a group of metabolic disorders characterized by chronic hyperglycemia, which is considered as the primary cause of several diabetic complications. Increase of infection susceptibility and low prevalence of allergic diseases associated with a decrease in the inflammatory response are examples of those complications (de Luis *et al.,* Diab. *Res. and Clin. Prac*. 52: 1, 2001; Huang, The Lancet 354: 515, 1999). We previously described that alloxan-diabetic rats were refractory to antigen challenge by a mechanism dependent on the down-regulation of mast cell population (Diaz *et al.*, *Int. Arch. Allergy Immunol*. 111: 36, 1996). It has been reported that PPAR-g is expressed in mast cell and regulates mast cell proliferation and various functions as synthesis and release of inflammatory mediators (Maeyama *et al.*, J. Pharmacol. Sci. 97: 190, 2005). This study was carried out to investigate the potential involvement of rosiglitazone in the mast cell alterations noted in diabetic mice. **Methods:** The animals were obtained from the Oswaldo Cruz Foundation breeding colony and used in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (CEUA-FIOCRUZ, protocol 0085–02). Male Swiss-Webster mice were rendered diabetic by an intravenous injection of alloxan (65 mg/kg) and the analyses were performed 7 or 21 days later. Mice were treated with the PPAR-g agonist, rosiglitazone (0.5 mg/kg, i.p.), once a day starting on day 3 after alloxan injection. Peritoneal and mesenteric mast cell numbers were evaluated by means of toluidine blue and Giemsa dyes, respectively. Moreover, the plasma of animals was recovered to insulin quantification by radioimmunoassay. **Results:** We showed that treatment with rosiglitazone inhibited the reduction in the number of mast cells present in the peritoneal cavity and mesenteric tissue of diabetic mice so in 7- as in 21-days of diabetes. Besides, we noted that 21-days diabetic mice treated with rosiglitazone presented a greater plasma insulin levels than untreated diabetic mice. **Discussion:** Our findings show that rosiglitazone attenuated the alloxan-induced mast cell depletion, in a clear association with increase of plasma insulin levels, indicating that PPAR-g seems to play a critical role in these phenomena. **Financial Support:** CNPq and FAPERJ.

01.038

Possible involvement of the kinin B_1 receptor in skeletal muscle atrophy. Parreiras-e-Silva LT[1], Gomes MD[1], Pesquero JB[2], Pires-Oliveira M[3], Godinho RO[3], Costa-Neto CM[1] [1]FMRP-USP - Bioquímica e Imunologia, [2]UNIFESP - Biofísica, [3]UNIFESP - Farmacologia

Introduction: Skeletal muscle fibers loose mass due to catabolic signals, like pro-inflammatory cytokines, which effects are mainly mediated by the ubiquitin-proteasome system (UPS), but also by other proteolytic enzymes. Muscle proteolysis is increased during several pathologies like cancer, diabetes and sepsis. OBJECTIVE: Since kinin receptors (B_1 and B_2) are involved in inflammatory responses, we decided to analyze participation of the kallikrein-kinin system in two different models of muscle atrophy. Methods: In one model, Wistar rats, Balb-C mice and C57BL/6 wild-type and kinin B_1 receptor *knockout* mice were gonadectomized for 2, 7, 15 and 30 days to induce levator ani (LA) muscle atrophy. In another atrophy model, Swiss mice were deprived of food for 2 days and gastrocnemius muscles were collected. Total RNA from each muscle was extracted, cDNA was produced and mRNA expression of target genes was analyzed by PCR. All experiments were conducted in accordance with the local Animal Care and Use Committee (FMRP-USP 046/2006). Results: We showed that mRNA expression levels of atrogin-1 and MuRF-1, key enzymes of UPS, were increased in the muscles collected from animals submitted to both models. Kinin B_1 receptor mRNA was also increased in the same muscles. Moreover, LA muscles from B_1 receptor *knocktout* mice did not induce MuRF-1 mRNA expression. Discussion: These data suggest the participation of kinin B1 receptor in muscle atrophy, possibly due to an activation of NF-kB, production of inflammatory cytokines and ultimately activation of UPS. Financial support: FAPESP, CAPES, CNPq, FAEPA.

01.039

Extracellular calcium and cyclic AMP signaling pathways cross-talk in the regulation of skeletal muscle contraction. Rodrigues FSM, Pires-Oliveira M, Andrade-Lopes AL, Chiavegatti T, Godinho RO UNIFESP - Farmacologia

Introduction: Skeletal muscle contraction is triggered by sequential activation of postsynaptic nicotinic acetylcholine receptors, depolarization of the sarcoplasm and release of Ca^{+2} from sarcoplasmic reticulum. Although extracellular calcium is not required to maintain skeletal muscle contraction, it has been shown that calcium channel blockers have an inotropic effect on frog skeletal muscle (Kawata, Jpn J Physiol, 40:337, 1990). However, the mechanism by what this process occurs is not known yet. Since skeletal muscle fiber expresses Ca^{2+}-sensitive adenylate cyclase (AC) and the second messenger cAMP is known to increase skeletal muscle contraction force, the goal of this work was to investigate the role of Ca^{2+} influx on mammalian skeletal muscle contraction and the putative crosstalk between extracellular Ca^{2+} and cAMP signaling pathways. **Material and Methods**: The effect of extracellular Ca^{+2} on isometric contraction was studied in mouse diaphragm muscle under direct electrical stimulus (supramaximal voltage, 2 ms, 0.1 Hz) incubated with L type calcium channel blockers (30 µM and 50 µM verapamil, 5 µM nifedipine) or vehicle in Tyrode's solution, containing 2 mM Ca^{2+}. The effect of the channel blockers on diaphragm contraction was compared to those obtained by calcium chelation with 5 mM EGTA or 1 mM EGTA in Ca^{+2}-free Tyrode. To investigate possible mechanisms that could explain Ca^{+2} influx effects, muscles were incubated with phosphodiesterase inhibitor IBMX (50 µM) or AC inhibitor SQ 22536 (300 µM). In addition, cAMP content was measured after 25 min incubation with 50 µM verapamil by radiometric assay. All procedures were approved by the institution's ethics committee (0011/08). **Results:** Ca^{2+}-channel blockers verapamil (30 µM or 50 µM) and nifedipine (5 µM) increased basal diaphragm contraction by 18%, 40% and 11%, respectively. IBMX also potentiated by 12% muscle contraction. While verapamil and IBMX effects were sustained for at least 40 min, potentiation induced by nifedipine was transient, peaking 5 min after the onset stimulation. Nifedipine short-term effect was mimicked by extracellular Ca^{2+} depletion with chelating agent EGTA, which increased the basal force of contraction by 20%. Pre-incubation of diaphragm with 10 µM verapamil potentiated by 63% the inotropic effect of IBMX. On the other hand, inhibition of AC with SQ 22536 reduced muscle contraction by 9% and abolished the inotropic effect of nifedipine, verapamil and EGTA, indicating the involvement of AC/ cAMP signaling on inotropic effect of extracellular Ca^{2+}. In fact, a 176% increase in intracellular cAMP content was observed in verapamil treated diaphragm, in comparison with non-treated muscles. **Discussion**: Our data showed that electromechanical activity alone leads to skeletal muscle cAMP production and extracellular Ca^{2+} influx. The blockade of Ca^{2+} influx increases muscle contraction via cAMP dependent pathway. These results demonstrate that, although not essential to maintain muscle contraction, extracellular Ca^{2+} provides a fine control of skeletal muscle contraction, attenuating twitch amplitude, probably by inhibiting Ca^{2+}-sensitive AC (isoforms 5 and 6) enzymes. **Support:** FAPESP, CNPQ and CAPES.

01.040

Efflux of cyclic AMP VIA probenecid-sensitive ABC transporters is responsible for increased plasma cyclic AMP following β2-adrenoceptor activation. Aparecida-Santos E, Andrade-Lopes AL, Chiavegatti T, Godinho RO UNIFESP - Farmacologia

Introduction: Asthma is a chronic inflammatory obstructive disease characterized by airways hyperresponsiveness. One of the most used strategies to overcome asthma acute crisis is the administration of β2-adrenoceptor agonists, which causes bronchodilation by increasing intracellular cAMP. We have previously showed that intracellular production of cAMP induced by adenylyl cyclase activation is followed by the efflux of the cyclic nucleotide through probenecid-sensitive ABC transporters (Godinho & Costa, 2008; Br J Pharmacol 138:995). At the extracellular space, cAMP is metabolized to adenosine (Chiavegatti, 2008; *Br J Pharmacol* 153:1087), a bronchoconstrictor agent in asthmatics. In addition, expression of inhibitory G protein-coupled A_1 adenosine receptors is increased in bronchial tissue from asthmatics, whereas the stimulatory G protein-coupled A_{2a} receptor is downregulated. Considering the role of the cAMP extracellular metabolite adenosine in asthma and the importance of β2-adrenoceptor agonists in the management of asthma, the aim of this work was to study the effect of fenoterol administration on the plasma cAMP levels and the putative autocrine or paracrine effects of this cyclic nucleotide. **Methods:** Adult rats were treated *p.o.* with fenoterol (FEN; 3 or 10 mg/kg), 3 mg/kg FEN plus probenecid (PROB; 600mg/kg doses) or vehicle. After 2 h, animals were sacrificed, blood from abdominal aorta was collected and plasma was obtained by centrifugation. Paracrine actions of cAMP were studied in cultured L6 cells fully differentiated in to skeletal muscle fibers. Cells were pre-incubated with Krebs containing 0.1 mM IBMX and treated with exogenous cAMP (1 or 10 μM), in the absence or presence of the non-selective adenosine receptor antagonist CGS 15943 (10 nM). After 30 min, cells were rinsed with cold Krebs and harvested with Krebs containing 1 mM IBMX. Samples were boiled and cAMP content was quantified by competition against ^{3}H-cAMP for a binding protein. All procedures were approved by the institution´s ethics committee (0876/08). **Results:** Acute treatment with 3 mg/kg (n=8) or 10 mg/kg (n=3) FEN increased plasma cAMP by 84% in comparison to control (62.3 ± 7.6 pmol/mL; n=7). Previous treatment of rats with PROB (n=10) abolished the effect of FEN. Incubation of L6 cells with exogenous cAMP (1 or 10 μM), which is unable to cross cell membrane, increased by 56% and 215%, respectively, the intracellular levels of the cyclic nucleotide. On the other hand, non-selective adenosine receptor CGS 14953 reduced by 25% the effect of 10 μM exogenous cAMP. **Discussion:** Our results show that FEN increases cAMP plasma concentration in rats. This effect was blocked by PROB, demonstrating the cAMP efflux through organic anion transporters *in vivo*, as previously showed *in vitro*. Once outside the cell, cAMP may have paracrine or autocrine actions mediated by activation of adenosine receptors, as shown here in L6 cells. In this model, extracellular cAMP is metabolized to adenosine, which in turn increases intracellular cAMP through Gs-coupled adenosine receptors. Since adenosine receptors expressed at bronchial tissue in asthmatics are negatively coupled to cAMP production, it is possible that adenosine generation via extracellular cAMP degradation could reduce the efficacy of β2-adrenoceptor in these patients. **Support:** FAPESP, CNPq.

01.041

NADPH oxidase-derived ros control the antiapoptotic effect of leukotriene B_4 in neutrophils through NF-kB activation. Barcellos-de-Souza P[1], Lima-Resende A[1], Barja Fidalgo TC[1], Arruda MA[2] [1]UERJ - Farmacologia, [2]UERJ / Farmanguinhos-Fiocruz - Farmacologia

Introduction: Leukotriene B4 (LTB_4), an arachidonic acid-derived lipid mediator, is a known proinflammatory agent released in many inflammatory situations and it is able to activate biological responses in human neutrophils (PMN) including reactive oxygen species (ROS) generation by the NADPH oxidase complex. LTB_4 delays neutrophils spontaneous apoptosis through the activation of classical pro-survival signaling, which in turn may corroborate to the onset of a chronic inflammatory condition. Recently, ROS have emerged as second-messengers, coordinating intracellular signaling cascades, and thus modulating several biological phenomena, including apoptosis. In this study, we aim to elucidate the putative role of NADPH oxidase-derived ROS in LTB_4 antiapoptotic effect. **Methods**: PMN were isolated from whole blood of healthy volunteers by Ficoll-Paque™ density gradient. ROS production was evaluated by cytochrome *c* reduction (one hour after stimulation) as well as lucigenin and luminol enhanced-chemiluminescence (time kinetics of 0-10 minutes). Constitutive apoptosis after 20 hour was determined by cell morphology and annexin V-phosphatidylserine binding. Mitochondrial membrane potential was assessed by flow cytometry of JC-1-stained cells. Protein expression was evaluated by western blot analysis of total or nuclear extracts (1-60 minutes). **Results and discussion**: Our data show that NADPH oxidase-derived ROS are critical to LTB_4 (100 -300 nM) pro-survival effect on neutrophils. This event relies on redox modulation of NF-kB translocation and IkB-a phosphorylation/degradation. We have also observed that LTB_4-induced Bad degradation and mitochondrial stability requires NADPH oxidase activity. Our results strongly indicate that LTB_4-induced antiapoptotic effect in neutrophils occurs via ROS-dependent signaling routes and we do believe that a better knowledge of molecular mechanisms underlying neutrophils spontaneous apoptosis may contribute to design better strategies to control chronic inflammation. **Financial Support:** CNPq, FAPERJ, SR-2/UERJ, ABC/UNESCO/L'Oreal.

01.042

Hepatic protection of the antocianidin in rabbits hypercholesterolemia. Silva DJC[1], Bispo RFM[2], Caldas RAA[3], Ramos FWS[4], Souza ET[1], Queiroz AC[1], Rodrigues CFS[2]
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Introduction: Antioxidants are compounds that can delay or inhibit oxidation of lipids or other molecules, preventing the initiation or propagation of oxidation reactions in jail. The Oxygen, essential for life, can result in irreversible damage or even reverse, when all living beings are exposed to it at concentrations higher than found in the atmosphere and may even lead to cell death. In recent years, interest in these pigments are intensified since research has shown that the anthocyanins and their aglycones are bioactive compounds and that, among various other physiological effects, have antioxidant capacity and anti-inflammatory properties, promotes vasodilatation, act the prevention of hyperglycemia, stimulate insulin secretion, improve the adaptation of night vision and prevent eyestrain. Flavonoids also modify several physiological functions by interaction with cytochrome P450 enzymes and transporters and the membrane proteins as carriers of glucose. Act against free radicals, allergies, inflammations, ulcers, viruses, tumors and hepatotoxin. **Objectives:** To evaluate the protector effect in liver of lyophilized powder of antocianidin (acquired in the Formula Manipulation of Pharmacy) in hypercholesterolemic rabbits, quantify the blood plasma GOT and GPT and the more classification of liver injury. **Methods**: We used 21 rabbits of the New Zealand breed, adults between 8 and 10 months old, weighing between 3 kg and 3.4 kg divided into 3 groups: control group with 200 g and water diet ad libitum (GR), the group with 1.5 g of cholesterol from egg yolk p.o + 10mL (GC) group treated with 60mg/kg of antocianidin p.o (GA). All groups were fed during the period of 100 days. The collection of blood for the measurements of AST and ALT occurred in animals at 0, 50 and 100 days. At the end of the trial period, the animals were sacrificed. Segments of liver were collected for histological analysis and were stained with the Gomori trichrome. The Ethical Committee of Federal University of Alagoas (N º 014869/2006-86) approved all experimental protocols described in this study. **Results:** As expected the group racion observed no change diet liver, the group of GC formation macrovesicular hepatic steatosis (71.5%) and microvesicular (at 28.5%), the GV group was the formation of macrovesicular steatosis (28, 5%) and macrovesicular (71.5%). This classification was made according to microscopic examination, which were the size of hepatocytes. **Discussion:** As the above results we can say that antocianidinas are effective in reducing the cholesterol and thus an effect hepatoprotetor. Thus, the economic interests of flavonoids is of arising from their different properties. Biological tests using combinations show that the isolated flavonoids exhibit a large effect on biological systems showing effects antimicrobial, antiviral, antiulcerogenic, cytotoxic, antineoplastic, antioxidant, antihepatotoxic, antihypertensive, hypolipidemic, anti-inflammatory, antiplatelet. **Conclusion:** The antocianidin had a significant reduction of hepatic steatosis during the 100 days of experiment. **Acknowledgements:** CNPq, FAPEAL, IM-INOFAR, FITs.

01.043

Activation of PGD_2 receptors, DP1 and CRTH2, elicits complementary signaling that evokes LTC4 secretion from eosinophils. Mesquita-Santos FP[1], Ferreira-Souza L[1], Bakker-Abreu I[1], Luna TGS[1], Bozza PT[2], Diaz BL[1], Bandeira-Melo C[1] [1]IBCCF-UFRJ, [2]IOC-FIOCRUZ

Introduction: During allergic response several inflammatory mediators are produced. Among these products, prostaglandin D_2 (PGD_2) have been described as a major metabolite of arachidonic acid released by activated mast cells, that is able to induce eosinophil migration. Recently, we described that PGD_2 is able to trigger eosinophil activation, inducing lipid bodies (LB) biogenesis and leukotriene C_4 (LTC_4) synthesis (Mesquita-Santos,F.P., *J.Immunol*.176(3):1326-30,2006). PGD_2 is known to exerts its effects through 2 receptors constitutively expressed on eosinophils, DP1 and CRTH2. Specifically concerning PGD_2 ability to trigger eosinophil chemotaxis, simultaneous activation of DP1 and CRTH2 displays opposing effects. Here, we investigated how PGD_2 receptors interact to elicit LB biogenesis and LTC_4 synthesis. **Methods:** Human eosinophils were isolated from peripheral blood of health volunteers with a negative selection kit (StemCell Technologies; human studies approved by 052/09 CEP UFRJ/HUCFF). Cells were stimulated for 1h (37°C) with PGD_2 (25nM) and pre-treated with PGD_2 receptors antagonists – BWA868c, BAY-u3405 or CAY10471 (20uM) – 30 min before stimulation. PKA inhibitors pre-treatment was 15 min before stimulation. Eosinophil lipid body biogenesis was analysed by osmium staining, and LTC_4 levels by EIA. *Swiss* mice (18-20g) sensitised with $Al(OH)_3$ and OVA at day 0 and 7, were stimulated with intrapleural injection of PGD_2 (35 pmol/cavity) at day 14, 30 min after pre-treatment with DP receptors antagonists. Eosinophils influx, LB biogenesis and LTC_4 synthesis were evaluated within 24h (L002-08 CEUA-FIOCRUZ). **Results:** By employing a mouse model of PGD_2-induced eosinophilic inflammation, we observed that although BWA868c treatment (DP1 antagonist) did not affect eosinophil influx, it did decreased eosinophil LB numbers. In contrast, BAY-u3405 (CRTH2 antagonist) inhibited PGD_2-elicited pleural eosinophil influx but failed to affect *in vivo* LB biogenesis within remaining infiltrating eosinophils. Both treatments were able to inhibit LTC_4 release by eosinophils into pleural fluid (from 80±10 to 30±8 and 20±10 pg/ml when treated with BWA868c and BAY-u3405, *n=6*). *In vitro* assays with purified human blood eosinophils, BWA868c blocked PGD_2-induced LB formation (from 19,8±0,6 to 10,0±0,7, *n=7*), while BAY-u3405 had no effect. Both antagonists inhibited PGD_2-induced LTC_4 synthesis by eosinophils, reinforcing the hypothesis of complementary roles for PGD_2 receptors. Moreover, eosinophils stimulated *in vitro* with selective DP1 (BW245c) and $CRTH_2$ (DK-PGD_2) agonists, revealed that BW245c, but not DK-PGD_2, triggered LB biogenesis. Neither agonist evoked LTC_4 synthesis. However, by simultaneous stimulation with both agonists induced LB formation and LTC_4 production. Pre-treatment with H-89 and PKI (PKA inhibitors) blocked LB genesis and LTC_4 synthesis induced by PGD_2, suggesting involvement of this signaling pathway (*n=2*). **Conclusion:** Our findings suggest that simultaneous activation of both PGD_2 receptors works in a complementary manner to evoke PGD_2-driven LTC_4 synthesis that takes place within eosinophil lipid bodies. **Financial Support**: CNPq/FAPERJ

01.044

Cyclic AMP signaling pathway of fast and slow twitch skeletal muscles: effects of denervation. Bergantin LB, Andrade-Lopes AL, Chiavegatti T, Godinho RO UNIFESP - Farmacologia

Introduction: Cyclic AMP (cAMP) signaling pathway is involved in neurotrophic control of many physiological processes in skeletal muscle, such as maintenance of muscle mass and expression of nicotinic acetylcholine receptors and acetylcholinesterase, which are drastically affected by chronic denervation. However, little is known about the impact of motoneuron influence on postsynaptic cAMP signaling cascade of fast and slow twitch skeletal muscles. Taking into account that adenylyl cyclase (AC) and G protein expression are identified as key steps of cAMP biosynthesis; we evaluated the effect of denervation on AC activity and on G protein expression of extensor digitorium longus (EDL, fast twitch) and soleus (SOL, slow twitch) muscles. **Material and Methods:** The effect of 7- to 60- day tibial nerve section of 3-month old Wistar rats was evaluated on EDL and SOL muscle weight, G protein expression and AC activity (n=3-4). AC activity was performed using forskolin as activator and ATP as substrate. The amount of cAMP produced was determined by radiometric assay. Functional binding assay of [^{35}S]GTPγS (non-hydrolysable GTP analogue) to membranes from control or denervated SOL and EDL was performed to quantify G proteins (n=4). **Results:** Denervation of EDL induced a progressive atrophy and loss of protein content that occurred in parallel with reduction of total AC activity. The concomitant reduction of protein content justified the maintenance of constant AC activity/protein after 14- and 60-day denervation (control: 2.6 ± 0.15 pmol cAMP/h/µg protein). Conversely, a delay between loss of SOL protein and reduction of AC activity, respectively detected after 7 to 28-day denervation, caused an increment of AC activity/protein (control: 2.7 ± 0.28 pmol cAMP/h/µg protein) which returned to control values after 60 days. Interestingly, 14 to 28-days denervation increased AC substrate apparent affinity (Km) at SOL (control: 0.33 ± 0.03 mM), but not at EDL (control: 0.34 ± 0.06 mM). Total G protein content remained unchanged at 7- and 14-days denervated EDL (control = 22,8 ± 1,3 fmol/mg, n=4) and SOL (control= 10,7 ± 0,6 fmol/mg, n=4). **Conclusions:** Our results showed differential adaptive changes in AC activity of fast- and slow-twitch muscles induced by chronic denervation. While EDL AC is reduced proportionally to protein content loss, at SOL the enzyme turnover appeared to be less susceptible to denervation. Denervation did not modify G protein/mg protein in EDL neither in SOL, indicating that at fast- and slow- twitch muscle fiber types, this step of signaling cascade is equally affected by innervation. Besides, changes in SOL AC Km suggest that motoneuron regulates the expression balance among the isoforms of the enzyme expressed in the skeletal muscle. These results provide strong evidences that neural factors differentially influence the functional properties of AC in slow- and fast- twitch muscle, explaining important changes in cAMP production observed after removal of SOL neural input. These findings may also elucidate the mechanisms by which innervation controls pivotal cAMP-dependent processes in skeletal muscle, including maintenance of muscle mass and expression of the synaptic proteins. **Support:** Fapesp & CNPq. **Ethical committee:** 0057/08

01.045

Experimental liver regeneration: role of verapamil and amniotic membrane as studied by PCNA immunohistochemical and biochemical analysis. Vilela-Goulart MG[1], Gomes MF, Bastos-Ramos WP, Oliveira MAC, Imae MA CEBAPE-FOSJC, UNESP-São José dos Campos

Introduction: The influence of the homogenous amniotic membrane (hAM) and the calcium blocker drug verapamil (V) on the morphological and functional regeneration of surgically damaged liver, in rats, was analyzed by the Proliferating Cellular Nuclear Antigen (PCNA) immunohistochemical reactivity and the enzymatic activity of aspartate and alanine aminotransferase (AST, ALP) and alkaline phosphatase (ALP). **Methods**: Ninety six male adult *Wistar* rats (as authorized by local Ethics Committee, 0232005) were used. 1)-LH group: control-rats with surgical liver damage; 2)-rats with liver damage plus hAM; 3)-V group: rats with liver damage plus verapamil; 4)-VhAM group: rats with liver damage, under verapamil treatment and hAM. The hAM was obtained from 24 pregnant females. Surgeries were performed under ketamine-xylazine anesthesia and hAM dressed the liver injured area. At the 10^{th}, 20^{th}, 30^{th} and 40^{th} days after the surgery, the animals were sacrificed and the liver excised to morphological and biochemical analysis (liver tissue and plasma). **Results and Discussion**: Metaplasia was observed in the hAM dressed injured liver, resulting in an amniohepatic tissue, which demonstrated the biocompatibility of the membrane. It was also very effective in stopping the wound bleeding. When associated with verapamil, the membrane significantly enhanced the PCNA immunohistochemical reactivity of the hepatocytes population, indicative of a increased cellular proliferation. The plasmatic enzymatic activity of AST and ALT were significantly lower ($p<0.001$) in the V and VhAM groups, as compared to control LH while the ALP enzymatic activity was significantly higher ($p<0.001$). The results of AST and ALT indicate a liver tissue protective action of the verapamil, enhancing the regenerative process. The higher ALP would result of a higher tissue reparation favored by verapamil. The present research demonstrates that the association of the homogenous amniotic membrane and verapamil stimulates the regeneration of the surgically damaged liver, in rats. The membrane was very effective as a biological curative and hemostatic. **Supported by FAPESP:** 2004/08656-3

01.046

Efeito de bloqueadores de co-transportadores NKCC1 e KCC2 nos relaxamentos induzidos pelo GABA e pelo óxido nítrico no duodeno proximal de rato. Silva JDP[1], Freitas DG[1], Cavalcanti PMS[2] [1]NPPM-UFPI, [2]CCS-UFPI - Bioquímica e Farmacologia

Introdução: No duodeno isolado de rato o Ácido Gama-Amino Butírico (GABA) ao ativar receptores $GABA_A$ é capaz de induzir relaxamentos neuronais semelhantes àqueles provocados pela estimulação de nervos não-adrenérgicos não-colinérgicos (NANC) (Maggi e cols, J Auton Pharmacol *4*: 77, 1984). No sistema nervoso central (SNC) de neonatos, a ativação de receptores $GABA_A$ promove despolarização neuronal que é dependente do gradiente eletroquímico de Cloreto (Cl^-), que é determinado pela ação de co-transportadores NKCC1, que medeiam a captação de Cl^-, e por co-transportadores KCC2, responsáveis pela extrusão de Cl^- da célula (Dzhala e cols, Nat Med *11*: 1205, 2005). Com o objetivo de determinar como o GABA, maior neurotransmissor inibitório do SNC de mamíferos (Bloom e Iversen, Nature *229*: 628, 1971), é capaz de ativar nervos NANC e relaxar o duodeno de rato, decidimos investigar o efeito de bloqueadores de cotransportadores NKCC1 e KCC2 nos relaxamentos neuronais induzidos pela Estimulação Elétrica de campo (EEc), GABA e Nicotina, bem como nos relaxamentos musculares produzidos pelo Óxido Nítrico (NO) e pela Noradrenalina (NA). **Material e métodos:** Segmentos da porção proximal do duodeno de rato (2,0-2,5 cm) foram suspensos em cuba para órgão isolado (10 ml; 1g de tensão; 32ºC; continuamente borbulhado com ar), contendo solução nutritiva de Tyrode livre de cálcio (com Atropina, Guanetidina e Indometacina, todos a 1 µM), para registro das contrações isotônicas. Após 2 horas o tônus foi induzido adicionando $CaCl_2$ (1mM). Respostas com EEc (1-8 Hz, 2 ms, voltagem supramáxima/5s), GABA (100 µM), Nicotina (100 µM), NO (10 µM) e NA (0,3 µM) foram obtidas na ausência e na presença de Furosemida (0,3-30 µM) e Bumetanida (1 µM), bloqueadores seletivos de NKCC1, e DIOA (1-3 µM), bloqueador seletivo de KCC2. O NO foi preparado a partir de solução acidificada de Nitrito de Sódio ($NaNO_2$) (Cocks e cols, Naunyn-Schim Arch Pharmac *341*: 364, 1990). **Resultados:** A EEc, a adição de GABA (100 µM), Nicotina (100 µM), NO (10 µM) e NA (0,3 µM) produziram relaxamentos na musculatura longitudinal da porção proximal do duodeno de rato. Os relaxamentos produzidos pela EEc (1 Hz, 17,95 ± 2,18%; 2 Hz, 21,80 ± 3,2%; 4 Hz, 30,91 ± 3,79%; 8 Hz 36,08 ± 4,39%; n = 14) não foram modificados pela prévia incubação de Furosemida (3-10 µM) ou de Bumetanida (1 µM). Os relaxamentos induzidos pela adição de GABA foram reduzidos na presença de Furosemida (0,3-30 µM) (controle 43.49 ± 4,22% (n = 13), para 36,92 ± 3,12% (n = 3); 21,68 ± 3,12%* (n = 3); 21,94 ± 8,32%* (n = 3); 11,87 ± 3,35%* (n = 4); 11,58 ± 5,26%* (n = 3), respectivamente com IC_{50} = 3 µM. Por sua vez, a prévia incubação de Bumetanida (1 µM) inibiu os relaxamentos induzidos pelo GABA (42,5 ± 5,0% para 23,83 ±13,12%*, n = 3) e pela Nicotina (66,89 ± 9,18% para 42,41%*, n = 3). A incubação de DIOA (1-3 µM) não alterou os relaxamentos desencadeados pelo GABA (100 µM), Nicotina (100 µM) e NA (0,3 µM). Os relaxamentos induzidos para o NO não foram afetados pela incubação de Furosemida (1-3 µM), Bumetanida (1 µM) ou pelo DIOA (1-3 µM). **Discussão:** Os relaxamentos neuronais NANC induzidos pelos GABA e pela Nicotina dependem da atividade do co-transportador NKCC1. **Apoio Financeiro:** CNPq e UFPI

01.047

Piplartine reduces intracellular calcium in cultured myocytes isolated from rat aorta. Silva JLV[1], Cavalcante FA[2], Rigoni VLS[3], Nascimento TL[3], Claro S[3], Paredes-Gamero EJ[3], Oshiro MEM[3], Ferreira AT[3], Aboulafia J[3], Nouailhetas VLA[3], Silva BA[4] [1]LTF-CCS UFPB / UNINOVE - Medicina, [2]ICBS-UFAL, [3]UNIFESP - Biofísica, [4]UFPB - Ciências Farmacêuticas

Introduction: Piplartine {5,6-dihydro-1-[1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-2(1H)pyridinone} is an alkamide isolated from stems and roots of *Piper tuberculatum* Jacq. Previous studies have shown that piplartine relaxes rat aorta by indirectly modulating voltage–gated Ca^{2+} channel ($Ca_V1.2$) activity by unspecifically enhancing K^+-channel activity. Piplartine has also been known to cause significant cytotoxicity against tumor cell lines (Bezerra et al., *Toxicol in vitro*, 21, 1, 2007; BEZERRA et al., Mutation Res, 652, 164, 2008). We now investigated the effect of piplartine on intracellular calcium concentration ($[Ca^{2+}]_i$) and its possible toxicity on the cellular cycle of cultured myocytes from rat aorta. **Material and Methods:** All experiments were approved by the Ethics Committee in Animal Research from LTF/UFPB (nº 0509/05). Aorta from male Wistar rats (300 – 350 g) were isolated, opened longitudinally, and incubated in nominal Ca^{2+}-free Hanks' solution. Tissues were exposed to enzymatic solution for 2 h in a humidified incubator, gassed with 5% CO_2, at 37°C. Both the endothelium and the adventitia layer were removed. The remaining tissues were minced into small pieces and seeded in culture bottles containing 10% fetal calf serum, 1% glutamine, 11 mM HEPES, 1% streptomycin, and 1% penicillin. Cells were then treated with trypsin, centrifuged, resuspended, seeded on glass coverslips, and kept in a humidified incubator gassed with 5% CO_2. Cells were identified through the reaction with antibody against α-myosin by fluorescence microscopy. Confocal microscopy was used to assess $[Ca^{2+}]_i$. Cells were loaded with Fluo-3 and plan time-space fluorescent images were obtained (LSM 510 Confocal Laser Scanning System). Cellular cycle was analyzed by flow cytometry (FACS Calibur) after loading the cell with 5 mg/mL iodide propidium. **Results:** Piplartine (10^{-5} M) decreased fluorescence from cells stimulated with 60 mM KCl evidencing the corresponding $[Ca^{2+}]_i$ reduction. Prolonged treatment (30 min.) with piplartine (10^{-5} M) did not cause cell arrest, as similar percentage of cells were observed before (52.2 ± 13.4% and 20.8 ± 9.7%) and after piplartine treatment (37.3 ± 6.1 % and 24 ± 10%) in Gi/Go and S + G2/M cellular phases, respectively. **Discussion:** Altogether these results strongly corroborate our previous result that the relaxant effect of piplartine in rat aorta is due to intracellular calcium reduction, eliminating any possibility this effect being due to piplartine-induced cytotoxicity. **Supported by:** CAPES; CNPq; UNIFESP; LTF/UFPB.

01.048

β-norlapachone reduces intracellular calcium in cultured myocytes isolated from guinea-pig ileum. Silva JLV[1], Cavalcante FA[2], Rigoni VLS[3], Nascimento TL[3], Claro S[3], Paredes-Gamero EJ[3], Ferreira AT[3], Câmara CA[4], Barbosa TP[5], Nouailhetas VLA[3], Aboulafia J[3], Silva BA[6] [1]LTF-CCS UFPB / UNINOVE - Medicina, [2]ICBS-UFAL, [3]UNIFESP- Biofísica, [4]UFRPE - Química Orgânica, [5]LTF-UFPB, [6]UFPB - DCF/LTF

Introduction: β-norlapachone is a synthetic naphthoquinone derivative from lapachol, natural naphthoquinone isolated of species from *Tabebuia* spp. (Bignoniaceae), popularly known as "ipê." We demonstrated that β-norlapachone relaxed guinea-pig ileum by inhibition of calcium influx through of the L-type voltage-gated calcium channels (Ca_V). Derivatives from lapachol have known by activity against tumor (DUKE, Hanbook of MedicinalHerbs, p. 470, 1985). In this work we investigated the effect of β-norlapachone on intracellular calcium concentration ($[Ca^{2+}]_i$) and its possible toxicity on the cellular cycle of cultured myocytes from guinea-pig ileum. **Methods:** All experiments were approved by the Ethical Committee in Animal Research from LTF/UFPB (Protocol nº 0706/06). The longitudinal layer was isolated from guinea-pig ileum (400-500 g). Tissues were minced into small pieces and seeded in culture bottles containing 10% fetal calf serum, 1% glutamine, 11 mM HEPES, 1% streptomycin, and 1% penicillin. Cells were then treated with trypsin, centrifuged, resuspended, seeded on glass coverslips, and kept in a humidified incubator gassed with 5% CO_2. Cells were identified through the reaction with antibody against α-myosin by fluorescence microscopy. Confocal microscopy was used to assess $[Ca^{2+}]_i$. Cells were loaded with Fluo-3 and plan time-space fluorescent images were obtained (LSM 510 Confocal Laser Scanning System). Cellular cycle was analyzed by flow cytometry (FACS Calibur) after loading the cell with 5 mg/mL iodide propidium. **Results:** β-norlapachone (10-5 M) decreased fluorescence from cells stimulated with carbachol (10-6 M) evidencing the corresponding $[Ca^{2+}]_i$ reduction. Prolonged treatment (30 min.) with 10^{-5} M β-norlapachone did not cause cell arrest, as similar percentage of cells were observed before (54.9 ± 1.2% and 12.8 ± 1.4%) and after β-norlapachone treatment (59.7 ± 4.6% and 12.6 ± 3.0%) in Gi/Go and S + G2/M cellular phases, respectively. **Discussion:** Altogether these results strongly corroborate our previous result that the relaxant effect of β-norlapachone in guinea-pig ileum is due to intracellular calcium reduction, eliminating any possibility this effect being due to β-norlapachone-induced cytotoxicity. **Financial support**: CNPq, CAPES, LTF/UFPB, UFAL, UNIFESP.

01.049

Análise da microdureza de tecidos mineralizados em filhotes de ratas wistar e espontaneamente hipertensas (SHR) tratadas com atenolol. Gomes WDS[1], Bertucci DV[1], Delbem ACB[2], Antoniali C[1] [1]FOA-UNESP - Ciências Básicas, [2]UNESP-Odontologia Infantil e Social

Introdução: O efeito de antagonistas b-adrenérgicos tem sido correlacionado com a remodelação óssea. Em estudo anterior demonstramos que filhotes de ratas Wistar tratadas com atenolol (AT) durante a prenhez e a lactação apresentam redução na composição mineral de fêmur (F), tíbia (T), 4ª vértebra lombar (L4), crista óssea mandibular (C) e dente incisivo. Em filhotes de SHR tratadas, não houve alteração da densidade radiográfica da maioria dos tecidos analisados, porém aumentou em L4 e C. Neste estudo avaliamos o efeito do tratamento com AT sobre a microdureza (MD) de tecidos mineralizados nos filhotes. Métodos: Ratas Wistar e SHR foram tratadas durante a prenhez e lactação com AT (100mg/Kg, v.o.). O efeito anti-hipertensivo do AT foi observado em ratas SHR desde o início do tratamento. Foram avaliadas a MD do F, da T, da L4, do esmalte (E) e dentina (D) de dentes incisivos inferiores dos filhotes (n=10, para cada grupo) aos 30 dias de vida. Para a análise de MD, as peças anatômicas foram embutidas em resina acrílica, com secção longitudinal, e analisadas no microdurômetro SHIMADZU-2000 (carga estática de 10 g para os dentes e 5 g para os ossos, 10 seg) acoplado a software para análise de imagem CAMS-WIN. Os resultados (KHN) foram expressos como média ± EPM e comparados entre os grupos (ANOVA, $p<0,05$). **Resultados**: Não houve diferença na MD de F entre SHR e Wistar (21,99±1,02 e 24,47±1,03, respectivamente). O tratamento com AT não alterou a MD do F de filhotes Wistar (24,8±0,7) e diminuiu a MD do F em SHR (17,3±1,3). Não houve diferença entre a MD da T de SHR e Wistar (22,8±0,9 e 24,6±0,6), apesar de uma redução em SHR ser bastante sugestiva. O tratamento com AT não alterou a MD da T em filhotes Wistar (26,7±0,9). O AT também não alterou a MD de T de SHR (23,97±0,7). Não houve diferença entre a MD da L4 de filhotes SHR e filhotes Wistar (60,23±1,7 e 55,87±2,9). O AT não alterou a MD da L4 em Wistar (62,7±2,8) e em SHR (64,7±2,2). Não houve diferença entre a MD de E do dente incisivo de SHR e Wistar (299,6±7,5 e 312,8±3,1). O AT não alterou a MD de E de Wistar (342,2±12,5) apesar de uma forte tendência ao aumento ser percebida. No grupo de filhotes hipertensos, observamos que o AT reduziu a MD do E (252,7±13,9). Filhotes SHR não apresentaram alteração de MD de D quando comparados aos filhotes Wistar (47,25±1,1 e 44,5±0,3). O tratamento com AT não alterou a MD de D dos dentes de Wistar (45,9±0,96) e SHR (48,9±0,5). A MD da D de filhotes SHR tratados com AT foi maior que a observada em filhotes Wistar tratados ou não com AT. Discussão: Alterações nos valores de MD poderiam sugerir diferenças na resistência de tecido mineralizado. Os resultados demonstraram que tecidos ósseos como F, T, L4, e E e D de SHR não apresentam alteração de MD. O AT não alterou a MD dos tecidos de filhotes Wistar, porém reduziu a MD do F e do E em SHR.Os resultados sugerem os receptores b1-adrenérgicos participam da formação ou desenvolvimento de tecidos mineralizados e que os mecanismos moleculares envolvidos estariam alterados em alguns tecidos de SHR. FAPESP, Comitê de Ética no. 2007-003175

01.050

Androgen regulation of epidermal growth factor (EGF) and its receptor (EGFR) along rat epididymis. Patrão MTCC, Silveira-Neto, AP, Avellar MCW UNIFESP - Farmacologia

Introduction: EGF is a polypeptide classically involved in cell proliferation and growth. EGF also plays a role in the androgen-dependent male sexual differentiation and proliferation, suggesting that the stimulatory pathways activated by this growth factor and androgens are closely related. Our group has reported that EGF and EGFR are expressed in the epithelial and interstitial cells of adult rat epididymis. In these cells, basal levels of activated EGFR (pEGFR, phosphorylated receptor) suggested a potential role for this receptor in the maintenance of epididymal functions. In order to gain more insight into the androgen regulation of such proteins, our aim was to analyze EGF and EGFR (mRNA and protein) in the epididymis of rats in different stages of sexual maturation or submitted to castration. **Methods:** Epididymides from immature (40d), young adult (60d) and adult Wistar rats (120d) were used. Epididymides from adult rats submitted to sham-operation (S) or surgical castration for 7 (C7d) or 15 days (C15d), as well as C7d rats treated with testosterone propionate (10 mg/kg, sc) for 6 days (C7d+T) were also used. Epididymides (n=3 each group) were divided into initial segment (IS), caput (CP), corpus (CO) and cauda (CD) and used in semi-quantitative RT-PCR with primers against rat *Egf*, *Egfr* and *Gapdh.* Longitudinal paraffin sections (n=3 each group) were used in IHC with anti-EGF, -EGFR and -pEGFR antibodies. Total epididymides from S, C7d and C15d (n=4 each) were incubated *in vitro* in the absence or presence of EGF (100 ng/mL; 1-15 min) and total protein extracts tested by Western blot (WB) with anti-EGFR or -pEGFR. Negative controls were performed using primary antibody preadsorbed with excess of its repective blocking peptide. Statistical comparisons were performed by ANOVA (sexual maturation) or Student´s *t* test (castration); $p<0.05$. **Results:** *Egfr*, but not *Egf*, mRNA levels were increased by sexual maturation (~20% in CP, CO and CD of 120d when compared to 40 and 60d). In contrast, castration for 7 or 15 days altered both *Egf* (decrease of ~20% in CO and increase of ~30% in CD) and *Egfr* (increase of ~30% in CO and ~50% in CD) mRNAs when compared to S. Sexual maturation and castration induced qualitative changes in EGF, EGFR and pEGFR immunolocalizations mainly in the epithelial and smooth muscle layer. Epididymis from immature, C7d and C15d presented cytoplasmatic EGF, EGFR and pEGFR staining in smooth muscle cells, a staining not observed in S and C7d+T. Epithelial staining changes were dependent on the epididymal region, antibody and experimental group analyzed. In S epididymis, WB detected the expected band of 175 kDa for EGFR and pEGFR. Within 1 min of *in vitro* EGF stimulation, an increase in pEGFR levels was already observed. A band of 130 KDa, probably related to the non-glycosylated EGFR, was also observed and was not activated by EGF. When compared to S, epididymis from C15d presented higher levels of this 130 KDa band. Expression of the 175 KDa EGFR and its activation by EGF, however, was identical among S, C7d and C15d. **Discussion:** Our results indicate that, in the rat epididymis, EGF and EGFR (mRNA and protein) are regulated by androgens. Furthermore, immunolocalization of EGF, EGFR and pEGFR in the epithelium and smooth muscle cells was dependent on the androgenic status of the animal, which indicates that the male hormone can direct EGFR signaling to different cell types along this tissue. **Support:** CNPq, FAPESP, Fogarty. **CEP number:** 0921/6 (UNIFESP).

01.051

Effect of the PDE-4 inhibitor compound LASSBio-448 on the experimental silicosis in mice. Jurgilas PB[1], Arantes ACS de[1], Ferreira TPT[1], Santos TPO[1], Pires ALA[1], França TG[1], Azevedo RB[1], Lima LM[2], Barreiro EJ[2], Cordeiro RSB[1], Martins MA[1], Silva PMR[1]
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Introduction: Silicosis is a chronic occupational disease caused by inhalation of free crystalline silica particles and it is characterized by an intense inflammatory response followed fibrosis and granuloma formation. Phosphodiesterase type 4 (PDE-4) plays a major role in modulating the activity of all cells involved in the inflammatory process. Since PDE-4 is an enzyme that metabolizes cAMP, a signal molecule known to attenuate cell activation, its inhibition causes elevation of intracellular cAMP levels and subsequently down-regulation of a variety of inflammatory cell functions. Taking into account that there is no effective treatment for silicosis, in this work we investigated the effect of a PDE-4 inhibitor compound LASSBio-448 on lung fibrosis and airways hyperreactivity in a murine model of silicosis. **Methods:** Anesthetized Swiss-Webster mice received intranasal instillation of silica (10 mg/50 µL) or vehicle (saline). Treatment consisted of intranasal administration of the LASSBio-448 (50 mg/kg, p.o.) every day, starting on day 21 up to day 28 post silica provocation. Twenty four hours later, lung function (resistance and elastance) and airways hyperreactivity to aerosol with the bronchoconstrictor agent metacholine (3-27 mg/mL) were measured by invasive whole body plestimography (Buxco System). Morphological alterations were analyzed by histological techniques including staining with Hematoxylin-Eosin and Picrus-Sirius for granuloma quantification and collagen deposition, respectively. Cytokines and chemokines were quantified by ELISA. All experimental procedures involving animal were approved by the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (License number- CEUA 0213-4). **Results:** We noted that silicotic mice showed an increase in basal levels of lung resistance and elastance as well as airways hyperreactivity after stimulation with aerosolized methacholine. They also exhibited a very intense inflammatory response followed by collagen deposition and granuloma formation. An increase in the levels of chemokines (KC and MIP-2) and cytokines (TNF-α, TGF-β and IL-6) was detected in the lung tissue as compared to those of control group. After treatment of silicotic mice with the compound LASSBio-448 we observed as significant decreased of basal lung resistance, but not elastance, and airways hyperreactivity to methacholine. LASSBio- 448 significantly suppressed granuloma formation and collagen deposition. The generation of the chemokines as well as of the cytokines was reduced in 66% (KC and MIP-2) and 48, 27 and 58% for TNF-α, TGF-β and IL-6, respectively, in the lung tissue of silicotic mice as compared with the treated group with LASSBio-448. **Conclusion:** Our results show treatment with PDE-4 inhibitor LASSBio-448 effectively inhibited important features of silicosis including failure of lung function and fibrotic response, thus indicating that this compound may constitute a promising antifibrotic therapy in the case of chronic fibrotic diseases such as silicosis. **Financial support:** FIOCRUZ, PRONEX, CNPq, FAPERJ.

01.052

Eugenol blocks native tetrodotoxin resistant Na^+ channels on DRG neurons. Carvalho-de-Souza JL[1], Leal-Cardoso JH[2], Cassola AC[3] [1]ICB-USP - Fisiologia e Biofísica, [2]UECE - Ciências Biomédicas

INTRODUCTION: Eugenol is a phenylpropene synthesized by many angiosperms, in which it acts as antimicrobial toxin and as pollinator attractant. Eugenol is used as analgesic and antimicrobial agent in dentistry. Previous studies have shown that eugenol blocks action potential firing in peripheral nerves, in a dose-dependent manner. Besides, we have shown that eugenol reversibly blocks voltage-gated Na^+ channels (Na_v) in in dorsal root ganglia (DRG) neurons. Here are results of a detailed analysis of EUG blockade of tetrodotoxin-resistant (TTX-R) Na_v in DRG neurons. **Methods:** The effects of EUG and lidocaine (LID) were thoroughly compared. Currents were recorded in DRG neurons from newborn rats, with patch-clamp technique, whole-cell configuration. The experiments were done in the presence of 100 nM tetrodotoxin to block all TTX-sensitive Nav. Results**:** EUG blocked TTX-R Nav channels fast and reversibly, in a concentration-dependent manner. The IC50 for EUG was of 2.27±0.22 mM and 0.44±0.08 mM for LID and inhibition is due mostly to binding to the channel resting state. EUG and LID did not shift the steady-state activation curve along voltage axis. The steady-state inactivation curve was displaced to more negative voltages, reflecting some binding to the inactivated state, by both agents. EUG affects the kinetics of inactivation recovery, increasing the weight of the slow component from 21.3% to 27.8%. EUG effect is smaller than the LID effect (from 18.0% to 30.7%). Both inhibitors prolonged the half-times of the slow component of inactivation. In concentrations around IC_{50} the frequency-dependent blockade was less conspicuous for EUG. The ratio of a remaining current peak for the 20th /1st pulse, frequency of 5 Hz, was 0.86 for EUG and 0,58 for LID. **Discussion**: In conclusion, EUG is a fast and reversible blocker of tetrodotoxin-resistant Na^+ currents, with affinity 5 times lower than that of LID for the same channel isoforms. Compared to LID, EUG has a higher relative affinity for the resting state and lower relative affinity for the open/inactive channel state, as unveiled by low dependence on voltage and frequency of the blocking action. **Support**: CAPES, Fapesp

01.053

Chronic ethanol during pregnancy and breast-feeding: consequences on mothers and development of infant rats. Verde LF, Jurkiewicz NH, Caricati-Neto A, Jurkiewicz A
UNIFESP - Farmacologia

Introduction: Studies in both humans and animals have extensively demonstrated the deleterious effects on the infants of maternal alcohol ingestion (Vaglenova & Petkov, Alcohol Clin. Exp. Res. 2293:697-703, 1998). Our objective was to check if alcohol ingestion during pregnancy and breast-feeding depresses food consumption and body weight of mothers and pups. **Methods:** Wistar rats, 4-month old, were treated from the 1st day of pregnancy until the 28th day post-partum with up to 30% oral alcohol *ad libitum*. Controls received drinking water. Food and ethanol intakes and body weights were measured throughout the pregnancy and breast-feeding. Pups were weighed between 5 to 45 days of age. **Results:** Daily food consumption (means ± SEM) of treated group (T) was 20.0 ± 1.6 g as compared to control (C) value of 27.9 ± 1.0 g during pregnancy. Litter size was decreased in treated group: (C=8,5 ± 0,22 and T=5,7 ± 0,41 (n=11)). Daily consumption of ethanol was 2.7 ± 0.6 g/day during pregnancy and during breast-feeding 6.6 ± 0.4 g/day. The values of ethanol group food consumption was 23.2 ± 1.4 g as compared to non-treated control value of 65.5 ± 6.3 g/day during breast-feeding. Weight gain was significantly lower in treated group (C=23,0 ± 3,1 and T=16,5 ± 2,1 (n=11)) as well as the corresponding body weights (C=245,5± 6,0 and T=228,2 ± 4,3 (n=11)). Female pups weighted less (C=107,0 ± 1,7 and T=66,2 ± 1,4 (n=16)) as well as males (C=116,0 ± 8,0 and T=78,6 ± 3,3 (n=16)). **Discussion:** The present study shows that ingestion of ethanol attenuates food consumption, gain in body weight and litter size of mothers and decreased of body weight of litters. CEP n. 0245/06 Supported by Fapesp, CNPq and Capes.

01.054

Changes on Ca^{2+} translocation in rat vas deferens of young rat descendants of mothers treated with ethanol during pregnancy and breast-feeding. Verde LF, Lopes GS, Jurkiewicz NH, Caricati-Neto A, Jurkiewicz A UNIFESP - Farmacologia

Introduction: It is known that ethanol affects smooth muscle contractility by interfering with Ca^{2+} translocation (Dillon et al., Alcoholism: Clin. Exp. Res. 7, 349, 1983). We have checked if chronic treatment of rats with alcohol during pregnancy and nursery causes alteration on translocation of Ca^{2+} and tension induced by KCl and norepinephrine (NE) in rat vas deferens (RVD) of descendants. **Material and Methods:** Wistar rats, 4-month old, were treated from the 1st day of pregnancy until the 28th day post-partum with up to 30% oral alcohol *ad libitum*. Controls received drinking water. Strips of prostatic portion of RVD of 40-day old litters were loaded with fura-2 and mounted in a PTI system (USA) for the simultaneous measurement of intracellular Ca^{2+} and corresponding contractions. The mean changes on values (± epm) of the fluorescence ratios ($R_{340/380}$(%) as indicative of calcium translocation (Ctr%), and of the respective tension (T%) were evoked after KCl (80mM) or NE (10^{-4}M). **Results:** Calcium mobilization and tension after KCl were significantly lower in treated (CTr= 45,8 ± 9,47 and T= 0,28 ± 0,05 (n=5)) than in controls (CTr= 105,7 ± 33,9 and T= 1,025 ± 0,27(n=4)), but was not different under NE treatment (CTr= 93,3 ± 40,55 and T= 0,48 ± 0,13 (n=3)) and (CTr= 118,5 ± 81,5 and T= 0,75 ± 0,15 (n=2)) in treated and controls respectively. **Discussion:** Our results showed that the use of alcohol during pregnancy and nursery induces an alteration on translocation of Ca^{2+} in RVD of young descendant males. CEP n. 0245/06 Supported by Fapesp, CNPq and Capes.

01.055

Cell-specific localization of the quiescin sulphydryl oxidase (QSOX) in rat testis and epididymis: influence of androgens. de Andrade CR, Patrão MTCC, Avellar MCW
UNIFESP - Farmacologia

Introduction: QSOX (quiescin sulphydryl oxidase) protein family has been studied in humans and other species. The first member reported, *QSOX1* gene, is abundantly expressed in rat seminal vesicle. The QSOX1 expression and secretion by rat testis, seminal vesicle and epididymis was reported, suggesting possible role in spermatogenesis and sperm maturation. Two *Qsox1* mRNA splice variants have been described: *Qsox1 variant 1 (Qsox1_v1)*, encoding the full length protein and *Qsox1 variant 2 (Qsox1_v2)*, which encodes a truncated protein. Additionally, another member of the QSOX family, named *QSOX2* gene, has been identified with high homology to classical QSOX1, and was only described in human neuroblastoma cells and, based on protein structure; it can be possibly involved in functions similar to those observed for QSOX1. The proteins encoded by *QSOX1* mRNA variants have important functions on protein folding, redox homeostasis and cell cycle control. However, the tissue distribution and physiological function for each QSOX1 isoform (QSOX1_i1 and QSOX1_i2) and QSOX2, especially in the male reprodutive tract, is still unclear. To gain insights into the role of QSOX in sperm and male reproductive tissues, QSOX immunolocalization and its androgen dependence in rat epididymis, as well as in adult testis and in maturing sperm was investigated. **Methods:** RT−PCR with total RNA from caput (CP), corpus (CO) and cauda (CD) from adult control rats was performed with primers against *Qsox*. Testis and different epididymal regions (initial segment, IS/CP, CO and CD) from immature (40 days), young adult (60 days) and adult Wistar rats (90 days) and surgically castrated (7 and 15 days) adult rats were used. Longitudinal paraffin sections (n=3 each group) were used in immunohistochemical (IHC) studies with antibody against rat QSOX1 and 2 isoforms (EDPQFPKVQWPPRE). Immunofluorescent studies in sperm isolated from testis and different epididymal regions were also performed. Proper negative (pre-absorbed antibody) and positive (rat seminal vesicle) controls were used. **Results**: RT-PCR analysis confirmed the presence of *Qsox1_v1*, *Qsox1_v2* and *Qsox2* in epididymis (CP, CO and CD), testis and seminal vesicles from adult rats. As expected, IHC showed that the apical surface of epithelial cells from adult seminal vesicles was abundantly stained. Immunoreaction was identified in sperm present in the lumen of seminiferous tubules and epididimal ducts from adult rats. In fact, immunofluorescence studies confirmed differential QSOX distribution along sperm isolated from testis (head and midpiece) and epididymal regions (CP and CO: head, neck and midpiece; CD: head, neck, middle and principal piece). QSOX was diffusely distributed in the cytoplasm of epithelial and some interstitial cells along adult rat epididymis. A prominent apical staining was also evidenced when CO and CD were analyzed. QSOX immunodistribution in IS and CP was unaltered with sexual maturation and castration. However, apical epithelial staining in CO and CD was reduced with age, but increased with castration, suggesting an androgen effect on QSOX expression in these epididimal regions. **Conclusions:** The constitutive QSOX expression in rat testis and epididymis, as well as its differential immunolocalization in isolated testicular and epididimal sperm, point out to possible role(s) of this protein in events leading to sperm maturation and, consequently, to male fertility. **Support:** CNPq, Fogarty International Center. **CEP:** 082/09 (UNIFESP-EPM).

01.056

Hypertrophy induced by short-term diaphragm denervation is associated with increased G_s protein expression and adenylyl cyclase activity. Andrade-Lopes AL, Bergantin LB, Chiavegatti T, Godinho RO UNIFESP - Farmacologia

Introduction: Short-term diaphragm denervation induces a pronounced, although transient, hypertrophy that precedes the classical skeletal muscle atrophy induced by chronic denervation, as seen in extensor digitorum longus (EDL) muscle. Although chronic denervation differently affects adenylyl cyclase (AC) activity of fast- and slow-twitch muscles during atrophy process and agonists of receptors coupled to stimulatory G protein (Gs protein) are able to delay muscle atrophy and to induce muscle hypertrophy, it is still unclear if components of cAMP signaling pathways are actively modulated during muscle hypertrophy process. So, we evaluated Gs protein expression and AC activity at hypertrophic denervated-diaphragm. **Material and Methods:** The effect of unilateral phrenicectomy and tibial nerve section of 3-month old Wistar rats was evaluated on muscle weight, Gs protein expression and AC activity of diaphragm and EDL muscles, respectively (n=3-4). Functional binding assay of [^{35}S]GTPγS (non-hydrolysable GTP analogue) was performed to quantify G proteins using membranes (50 µg) from control or denervated diaphragm and EDL. In order to analyze if Gs protein expression was modulated during hypertrophy process, [^{35}S]GTPγS binding assay was followed by immunoprecipitation with specific antibody against Gαs isoform. AC activity was measured using ATP as substrate and forskolin as enzyme activator in control and denervated muscles (10 µg), and cAMP content was measured by radiometric assay. **Results:** One-week denervation induced a 40% transient hypertrophy of diahragm (control: 332,0 ± 28,7 mg), returning to control values after 14 days, whereas EDL progressively atrophied, reaching 70% of control value (122,4 ± 5,8 mg) after 14 days. One-week denervation also increased by 123% diaphragm AC activity (control = 2,6 ± 0,4 pmol cAMP/ h/ µg protein). The specific [^{35}S]GTPγS binding sites increased by 115% after 7-day-denervation (control = 32.6 ± 1.2 fmols/mg protein) and the amount of Gαs subunit increased by 40% (control = 1.7±0.5 fmols/mg protein). Conversely, EDL AC activity and total amount of G proteins remained unchanged during muscle atrophy (control EDL AC: 2,7 ± 0,15 pmol cAMP/ h/ µg protein; control EDL G protein = control = 8.9 fmol/mg). **Discussion:** Our results showed that Gs protein expression and AC activity are actively upregulated at hypertrophied diaphragm, a nerve-dependent hypertrophy model, which completely abolishes the participation of neurotrophic factors. The correlation between upregulation of cAMP signaling cascade and muscle hypertrophy make components of Gs protein/AC/cAMP pathway important pharmacological targets for development of new anabolic therapies. **Supported by:** FAPESP and CNPq. **Ethical committee:** 0057-8

01.057

L-carnitine supplementation increases the tolerance to physical exercise and time to fatigue of the gastrocnemius muscle in rats fed with hyperlipidic diet. Gómez-Campos RA[1], Priviero FBM[2], Valgas da Silva, CP[2], Rojas-Moscoso, JA[2], Cossio-Bolaños, MA[3], Antunes E[2], Zanesco A[1] [1]UNESP – Educação Física, [2]UNICAMP - Farmacologia, [3]UNICAMP – Educação Física

Background: Tolerance to physical exercise is reduced in obesity because the energetic metabolism is impaired, including β-oxidation of the free fatty acids, which depends on the enzyme carnitine palmitoyltransferase I. Obese people who start an exercise program, usually quit it due to their reduced aerobic capacity. Supplementation with L-Carnitine (L-Car) improves tolerance to exercise in non-obese sedentary and trained rats. We aimed to investigate whether the oral supplementation with L-Car improves the tolerance to exercise and time to fatigue and maximal force of the gastrocnemius muscle of rats fed with a hyperlipidic diet (HD) submitted or not to regular physical exercise (EX). **Methods:** Experimental procedures were approved by the Animal Care and Use Co mmittee of the State University of Campinas (Protocol # 1307-1). Male Wistar rats were divided into 4 groups: 1) HDSD: hyperlipidic diet, sedentary; 2) HDEX: hyperlipidic diet, exercised; 3) HDSD L-Car: hyperlipidic diet, sedentary and supplemented with L-Car; 4) HDEX L-Car: hyperlipidic diet, exercised and supplemented with L-Car. After 4 weeks of HD intake, rats were orally supplemented and submitted to an EX program of running on a treadmill, in sessions of 60 min, 5 days/week, during 4 weeks. Afterwards, rats were submitted to an exercise tolerance test, which consisted in an acute session of running using incremental loads, each 3 minutes, until exhaustion. After 48h of rest, muscle fatigue was evaluated in anaesthetized rats. The gastrocnemius muscle and the sciatic nerve were exposed. The muscle was connected to a force transducer and changes were recorded in a Powerlab acquisition data system. The sciatic nerve was electrically stimulated during 3-4 minutes with a supra-threshold frequency (5 Hz) until the stabilization of the contractions. Then, the frequency was increased to 50 Hz and the stimulation was kept until the contractile force was decreased to zero. The maximal force and time to fatigue were recorded. **Results:** In rats fed with HD, tolerance to exercise was increased in EX animals (15.4 ± 0.44 min) compared to SD animals (7.08 ± 1.41 min). Supplementation with L-Car improved the tolerance to exercise in both EX and SD animals (20.32 ± 1.79 min and 12.35 ± 0.93 min, for EX and SD, respectively) compared with their age matched non-supplemented rats. Similarly, time to fatigue was higher in HDEX rats (at 31 min, contractile force was 0.73 ± 1.07 g) compared with HDSD rats (at 21 min, contractile force was 0.62 ± 0.13 g). Supplementation with L-Car caused a significant increase in the time to fatigue in either HDSD L-Car (at 81 min, contractile force was 0.04 g ± 0.09 g) or HDEX L-Car (at 84 min, contractile force was 0.1 ± 0.1 g). However, neither the supplementation nor the physical exercise changed the maximal contractile force of the gastrocnemius muscle. **Discussion:** Our findings showed that supplementation with L-Car increased the tolerance to exercise and time to fatigue of the gastrocnemius muscle without changes in its maximal contractile force. These data suggest that supplementation with L-Car increase the time to exhaustion and might be an useful tool to obese people in the attempt to adopt a healthy lifestyle. Financial Support: **Capes and FAPESP**

01.058

Estudo da neurotransmissão periférica na musculatura lisa do ducto deferente de ratos periadolescentes tratados agudamente com uma dose simples e dupla de anfetamina.

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Introdução: A anfetamina é utilizada no tratamento de várias desordens, tais como obesidade, déficit de atenção e narcolepsia. Seus principais alvos moleculares são os tranportadores vesiculares e neuronais de noradrenalina, dopamina e serotonina, além de inibir a monoamino oxidase A (MAO-A), tendo como resultado o aumento dos níveis extracelulares de monoaminas (Sulzer, 2005; Robertson, 2009). As ações de doses baixas e altas de anfetamina são bem conhecidas no sistema nervoso central (Ellinwood, 2000). No entanto, no sistema nervoso autônomo poucos estudos foram realizados. Desta forma, o objetivo do presente trabalho foi estudar a transmissão adrenérgica na musculatura lisa do ducto deferente de ratos periadolescentes ao tratamento agudo com dose simples e dupla de anfetamina. **Métodos:** Utilizamos ratos Wistar de 35 dias de idade, os quais foram divididos em dois grupos, o primeiro grupo (GRUPO A) foi tratado, via subcutânea, com anfetamina em dose simples (3mg/Kg) e o segundo grupo (GRUPO B) com uma dose dupla (7mg/Kg). Quatro a três horas e meia depois do tratamento os animais foram sacrificados, os ductos deferentes removidos e montados em banhos de órgão isolado; foram realizadas curvas dose-efeito para a noradrenalina, bário, fenilefrina e dopamina (10^{-10} - 10^{-3} M), dose única de tiramina (10^{-4} M) e curvas freqüência-resposta (0,1 - 20 Hz, 1ms, 60V). Para cada grupo tratado houve um grupo controle. Os resultados foram expressos em média ± EPM e a resposta do tecido foi dada em tensão em gramas/gramas do tecido. **Resultados:** Nos animais do GRUPO A observou-se um aumento do efeito máximo (Emax) para a noradrenalina e para o bário (89,42 ± 7,93, n=4; 112,52 ± 7,13, n=4; respectivamente) quando comparados aos seus controles (65,91 ± 5,18, n=4; 82,64 ± 5,29, n=4; respectivamente), fato não ocorrido no GRUPO B. A fenilefrina e a dopamina, para ambos os grupos, não demonstraram alteração quando comparadas com seus respectivos controles. A liberação indireta de noradrenalina pela tiramina foi mais acentuada no GRUPO A (64,71 ± 8,53, n=6) que no GRUPO B (58,18 ± 3,88, n=4) quando comparadas com seus respectivos controles (56,68 ± 9,85, n=6; 55,42 ± 1,68, n=4; respectivamente). Este último grupo demonstrou na estimulação elétrica uma resposta fásica mais acentuada quando comparado ao seu controle para todas as freqüências. Por outro lado, no GRUPO B ocorreu o inverso. Já para a resposta tônica, observou-se uma maior resposta no GRUPO A. Não houve alterações estatisticamente significativas entre os grupos para os demais parâmetros farmacológicos estudados (pD_2 e ρ). **Discussão:** Os resultados encontrados indicam, possivelmente, que doses mais baixas de anfetamina podem atuar numa maior produção de catecolaminas ou numa maior responsividade dos receptores adrenérgicos pós-sinápticos. Referências: Sulzer, D*. Progress in Neurobiology* 75:406–433, 2005. ROBERTSON, S. D. Mol Neurobiol 39:73–80, 2009. Ellinwood, E. H. *Neuropsychopharmacology: The Fifth Generation of progress*, 2000. Aprovado pelo CEP-UNIFESP: Protocolo n° 1650/04 Apoio Financeiro: Capes.

01.059

Pre- and post-sinaptic effects of acetilcholinesterase inhibitors on smooth muscle neurotransmission of wistar rat compared to mouse. Pereira JD[1], Caricati-Neto A[1], Godinho RO[1], Garcia AG[2], Jurkiewicz A[1], Jurkiewicz NH[1] [1]UNIFESP - Farmacologia, [2]UAM - Farmacologia

Aims: Our objective was to compare the effects of of some cholinesterase inhibitors on pharmacological reactivity and neurotransmission of the vas deferens of Wistar Rats and Swiss mouse. Newly synthesized anticholinesterasic drugs, brought from the Laboratory of Prof. Antonio Garcia, from Autonomic University of Madrid, were used. One of the drugs, 12118, has a hybrid structure derived from the anticholinesterasic compound Tacrine and from Nimodipine, a L-calcium channel inhibitor (Marco-Conteles et al., J. Medic. Chem. 49, 7607-7610, 2006). The activity of the compound will be compared with that of Physostigmine and Tacrine. **Materials and Methods**: Dose-response curves will be described for barium chloride and acetylcholine (ACh), in the presence (10 or 60 minutes) or absence of the cholinesterase blockers. The parameters maximum effect (Emax), apparent affinity of the agonists (pD_2) and Dose Ratio (DR) were evaluated. To study the effect of the compounds in relation to calcium, cumulative curves for calcium in free-calcium solution were made in the presence of the drugs. Electrical field stimulation (EFS) was made (60 V, 3ms duration in 0.1, 0.5, 1, 2, 5 10 and 20 Hz), for the study of nerve-mediated contractions. The evaluation of activity of the acetylcholinesterase (AChE) and butirilcholinesterase (BuChE) was made *in vitro* according the method of Ellman modified in microplate for confirm the inhibition by the compounds. **Results:** Some differences between the two species were found: In mouse, the % Emax for calcium curves in presence of the anticholinesterase 12118 (A) was decreased when compared to the control curves (C= 100 ± 5.4%, A = 52.4± 6,8%) as well as in the presence of Nimodipine (C= 100 ± 7.0%, N= 17.5 ± 6,9%). For rat, only Nimodipine induced a blockade of calcium entry (C= 100 ± 6.8%, N= 27.0 ± 5,0%). In mouse, Phasic and Tonic contractions produced by EFS at frequencies of 5Hz and 10Hz were decreased in presence of A and Tacrine. On the other hand, for Physostigmine the effects were increased in EFS. For the rat, the EFS presented a decreased response for Tonic contraction for A in 5Hz (C= 33.4 ± 1.1g; A = 12.1 ± 0.7g) and 10Hz (C= 33,4 ± 1,3g; A = 9,7 ± 0.44g). For rat, the curves for ACh were potentiated for Physostigmine and Tacrine (800 and 150 times, respectively) and Emax decreased. In mouse, non significant potentiations were observed. The activity for BuChE in rat compared with mouse was higher for *in vitro* assays. Differences were not detected for AChE. **Conclusions:** The compound 12118 presents a higher activity as a L-calcium channel blocker. The stiking difference for the activity of BuChE between both species, and the differences between rats and mice in relation to the response for Nimodipine and 12118 need more investigation. **Aproved by CEP-UNIFESP: Protocol nº 1650/04 Financial Support:** CAPES, CNPq and FAPESP.