01. Cellular and Molecular Pharmacology

01.001 Tachyphylaxis to serotonin in the rat corpora cavernosa. Berretta LM, Linder AE UFSC – Pharmacology

Introduction: Erectile dysfunction is characterized by the inability to obtain and/or maintain an erection for satisfactory sexual performance. The role of 5-hydroxytryptamine (5-HT, serotonin) for penile erection is not well established despite the increasing number of people using drugs that alter the homeostasis of 5-HT, such as fluoxetine. We observed that the contractile response stimulated by 5-HT is not reproducible in the erectile tissue from the penis, the corpus cavernosum (CC), suggesting tachyphylaxis. Thus, this study aimed to investigate this tachyphylactic response induced by 5-HT in the CC. We hypothesized that this phenomena is due to 5-HT receptor internalization mediated by caveolae, small invaginations at the plasma membrane rich in cholesterol. **Methods**: corpus cavernosum segments were obtained from male Wistar rats (240-300g)(CEUA PP00706) and mounted in isolated organ chambers for isometric tension recording under 0,25 g of passive tension. The following protocols were performed: The segments were stimulated four consecutive times with a 35-minute washing interval between each stimulation with 5-HT (100 μM), Phenylephrine (PE; 10 μM) or sodium chloride (KCl; 80 mM). The contraction obtained from the first stimulation was considered as 100% and the other 3 contractions were expressed as % of the first. 2) Cumulative concentration-response curves (CCRC) to 5-HT and PE. 3) CCRC to 5-HT were performed in the absence and in the presence of methyl-beta-cyclodextrin (10 mM, 60 min), a cholesterol lowering drug. Two-way ANOVA followed by Bonferroni post-test and Student's t-test were used when appropriate. **Results and Discussion**: The contractions induced by the second, third and fourth stimulation with 5-HT (n = 8) represented 32 ± 3%, 12 ± 3% and 7 ± 3% of the first one whereas those with PE (n = 9) represented 93 ± 5%, 84 ± 6% and 76% ± 6%; and those with KCl (n = 7) represented 85 ± 3%, 80 ± 3% and 69 ± 4%, respectively. These results indicate that the decreased responses to PE or KCl and may be related to the tissue loss of response with time, whereas the decreased response to 5-HT observed in the second stimulation may be due to selective agonist desensitization. The CCRC showed the ability of 5-HT and PE to contract the CC in a concentration-dependent manner. However, the efficacy of 5-HT to contract the rat CC observed in the CCRC was lower (37 ± 6 mg; n = 8) than that of PE (120 ± 10 mg; n = 8). Supporting the tachyphylactic response to 5-HT, the contraction induced by 5-HT (100 μM) during the CCRC (37 ± 6 mg) was lower than that obtained by the direct administration of same concentration (60 ± 5 mg). This was not true for PE, since the contractions induced by 10 μM alone or in the CCRC were similar. **Conclusion**: The results suggest that the tachyphylactic response to 5-HT in the rat corpus cavernosum is not mediated by receptor internalization via caveolae. However, further investigation has to be done to understand these phenomena and the implications of these results for sexual behavior resulting from the administration of selective serotonin reuptake inhibitors. Supported by FAPESC/CNPq and PPG-FMC.

01.002 Vasodilatation induced by forskolin involves cyclic GMP production. Neto MA[1], Lunardi CN[2], Rodrigues GJ[3], Bendhack LM[4] [1]UNINGÁ – Farmacologia, [2]UnB – Química, [3]FMRP-USP – Farmacologia, [4]FCFRP-USP – Química e Física

Endothelium-derived relaxing factors contribute to smooth muscle relaxation. The aim of the present study was to investigate the contribution of nitric oxide (NO) produced in the endothelial cells to the vasodilatation stimulated with forskolin in rat aorta (CEUA-USP 05.1.257.53.9). Forskolin that directly activates adenylyl-cyclase, induced complete relaxation in phenylephrine-contracted aortas. Endothelium removal reduced the potency (pEC50) of forskolin (1 μM) (intact endothelium: pEC_{50}: 7.18 ± 0.05; denuded endothelium pEC_{50}: 6.99 ± 0.06; P<0.05), without changes in the maximum effect (Emax): intact endothelium: 103.8 ± 2.5%; denuded endothelium: 100%, suggesting the contribution of endothelium in its relaxing effect. Because the inhibitor of endothelial NO-synthase (10 μM L-NG-Nitroarginine, L-NNA) reduced Emax in intact endothelium to 92.2 ± 3.8% (n=6, P<0.05) and its potency (pEC_{50}: 6.54 ± 0.06, P< 0.001), and the indomethacin (10 μM) did not alter the Emax (98.3 ± 0.9%, n=6) nor the potency (pEC_{50}: 6.93 ± 0.11) of forskolin, we verified that the additive effect of endothelium on the relaxing effect of forskolin was due to liberation of endothelial NO. The effect of association of L-NNA and indomethacin (Emax: 93.5 ± 4.0%, n=6, P<0.05; pEC_{50}: 6.41 ± 0.22, P<0.01) was similar to the effect achieved with when we associated only L-NNA with forskolin. Since the endothelial production of NO may be Ca^{+2} dependete, we verify that the forskolin stimulated an increased in the fluorescence elicited by Fura 2-AM from 0.924 ± 0.104 (basal fluorescence) to 1.164 ± 0.015 units (P<0.01), indicating an increase in the cytosolic Ca^{+2} concentration in isolated endothelial cells. Subsequently, forskolin increase significantly the fluorescence intensity elicited with dye DAF-2T from 0 to 153.3 ± 10.5% (n=4, P<0.001), indicating an increase in NO concentration ([NO]c) over the basal levels in the endothelial cells. On the other hand, in endothelial cells pre-incubated with L-NNA, forskolin decreased the fluorescence of the dye DAF-2T, related with forskolin-induced NO production from 0 to -5.9 ± 1.2% (n=3, P<0.001), below the basal level. To verify the activation of NO production stimulated by forskolin depended at least in part of PKA pathway, we used the PKA inhibitor KT5720 (10 μM), that inhibited 25,8 ± 0.4% (n=3) of the effect of forskolin in increasing [NO]c. The forskolin elicited a significant increase in cGMP production (28.0 ± 1.8 fmol of protein) as compared to the basal levels in homogenized vessel containing intact endothelium (16.2 ± 1.8 fmol of protein) (P<0.05). The production of cGMP stimulated with forskolin was significantly inhibited by L-NNA (18.8 ± 0.4 fmol of protein) (P<0.05), when its values were comparated with the basal levels. Taken together, our results indicatet that vasodilatation induced by forskolin involves cyclic GMP production, since the vasodilatation mediated by forskolin in rat aortic rings is potentiated by Ca^{+2}-dependent NO production in aortic endothelial cells, which stimulates the increases of cGMP levels in the smooth muscle cells that along with cAMP, produced via PKA pathway, contribute to the vasodilatation. **Financial Support**: FAPESP

01.003 Oxidative modifications in chronological aging and treatment with vitamin E. Costa ACC, Silva TNX, Souza-Neto FP, Terra VA, Bernardes SS, Cecchini R, Cecchini AL UEL

Introduction: The term oxidative stress refers to an unbalance between the reactive oxygen and nitrogen species production (ROS and RNS) and the antioxidants defenses. The enzymes antioxidants are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), among others. Vitamin E is the main antioxidant that protects lipids from lipid peroxidation *in vivo*. Act as a donor of hydrogen to the peroxyl radical, interrupting the radical chain reaction. Aging can be understood as the consequence of time whereby a man becomes older. During chronological cutaneous aging, chemical and enzymatic oxidations involving the formation of free radicals leads to oxidative stress, which greatest damage is fatty acids peroxidation, leading to cell death. **Methods**: This project was approved by Londrina State University ethic committee (Process n. 14/11). Were used hairless mice *HRS/J,* male or female, with the ages of 18 weeks, 70 weeks and 70 weeks inoculated i.p with vitamin E (n=4 for all groups). To evaluate the previous oxidative stress degree was used chemiluminescence method (CL) induced by tert-butyl hidroperoxide. The results were expressed in relative light units/minute/mg protein. TRAP technique measures the degree of antioxidants of total tissue, especially low mass molecular antioxidants. In this method, 2-azo-bis produces photons that are amplified by luminol and measured by luminometer. The additions of antioxidants inhibit the emission for a fixed time and this parameter is used as a measure from TRAP in µM Trolox (standard antioxidant). Catalase present in the skin homogenates was determined using H_2O_2 as standard. Results were expressed in ABS/mg protein/minute. The final lipoperoxidation products (TBARS) was analyzed by measuring the formation of thiobarbituric acid-reactive species and the results were expressed in nM/mg protein. Carbonyl proteins (CP) were assayed using the method for detecting protein hydrazones followed by reaction with dinitrophenylhydrazine and the results were expressed as nM/mg protein. The results are shown as means ± SEM. All differences between the groups were analyzed by Student T test, except for QL curves, that were qualitatively analyzed by two-way ANOVA and quantitatively by Bonferroni post-hoc test. Significant levels were defined as $p<0.05$. Were used for analysis Origin 8.5.1 and GraphPad Prism 5.0. Results: There was no significant difference between groups in relation to CL and TRAP. Catalase levels were significantly decreased in group 70 weeks (0.1233±0.01856, P=0.0312) in relation to control 18 weeks (0.2067±0.01764). This decrease was restored when using vitamin E (0.1347±0.03935). CP levels were significantly raised in group 70 weeks (4.825±0.3247, P=0.0148) in relation to control 18 weeks (3.290±0.3172). This increase was restored when using vitamin E (3.547±0.2226). MDA levels were significantly raised in group 70 weeks (0.6300±0.04163, P=0.0017) in relation to control (0.3700±0.02041) and this increase was restored when using vitamin E (0.3733±0.09939). **Discussion**: According to these results, the use of vitamin E after aging can restore oxidative stress by preventing the formation of free radicals. **Financial Support**: Londrina State University.

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01.004 Agonist driven α1A-adrenoceptor phosphorylation, desensitization and internalization: Differential recruitment of PKCα and GRK2. Akinaga J[1], Alcántara-Hernández R[2], García-Sáinz JA[2], Pupo AS[1] [1]Unesp-Botucatu – Farmacologia, [2]UNAM – Fisiologia Celular

Introduction: There are three α1-adrenoceptor (AR) subtypes and it is consensual that the α1A subtype is much less phosphorylated, desensitized and internalized upon norepinephrine (NE) exposure than are the α1B and α1D subtypes. However, here we show that the low efficacy agonist oxymetazoline (OXY) induces rapid GRK2-dependent phosphorylation, desensitization and internalization of human recombinant α1A-ARs, whereas phosphorylation of α1A-ARs activated by NE depends on PKCα and is not followed by desensitization and internalization. Importantly, OXY also desensitizes native α1A-ARs from rat tail artery and vas deferens, indicating that this property is not limited to recombinant receptors heterologously expressed in cell systems. **Methods**: Plasmids encoding human α1A-ARs and a dominant negative mutant of GRK2 (K220M, GRK2DN) were transfected into HEK293 cells and intracellular calcium increases were measured by FLUO4-NW or FURA2. HEK293 cell surface α1A-ARs expression was checked by confocal microscopy and quantified by [^{3}H]Prazosin binding assays. Receptor phosphorylation was detected by immunoprecipitation of α1A-ARs from ^{32}P-labeled cells. Studies on native α1A-ARs from rat tail artery and vas deferens (tissues whose contractions to adrenoceptor agonists result from α1A-AR activation) were performed using standard tissue bath assays. **Results and Discussion:** Pre-treatment of HEK293 cells with NE 10 μM/5 min has no effect in the potency (pEC_{50}) or efficacy (E_{max}) of NE to increase intracellular calcium (n=4). In contrast, NE was unable to increase intracellular calcium concentrations in cells pre-treated with OXY 10 μM/5 min (n=4), showing that OXY, but not NE, desensitizes human α1A-ARs. OXY 10 μM rapidly internalized human α1A-ARs (34% loss of cell surface receptors after 5min exposure, and maximal loss of 70% after 60 minutes), whereas the internalization induced by NE 10 μM was much slower (60min) and weaker (38%). NE or OXY (10 μM) induced time-dependent phosphorylation of human α1A-ARs, which was maximal after 15min of agonist exposure. In HEK293 cells co-expressing GRK2DN, human α1A-ARs were not phosphorylated upon OXY exposure, whereas receptor phosphorylation induced by norepinephrine was unaltered. Conversely, human α1A phosphorylation induced by NE was greatly reduced by the PKC inhibitors Bisindolylmaleimide and Gö6976, showing that α1A-AR activated by NE is phosphorylated by PKCα. As expected, desensitization and internalization of human α1A-ARs induced by OXY were greatly reduced in cells co-expressing GRK2DN. Pre-treatment of the rat tail artery and vas deferens with OXY, but not with NE, induced robust tachyphylaxis in contractions to a subsequent exposure to NE (n=4 to 8). **Conclusion:** To our knowledge, this is the first report of agonist-driven adrenoceptor phosphorylation resulting in differential receptor regulation. In addition, these novel data highlight that the low efficacy agonist OXY shows strong functional selectivity at α1A-ARs leading to significant receptor desensitization and internalization, which is important in view of the therapeutic vasoconstrictor effects of this drug. **Financial Support**: CAPES (9150-11-0 to JA), FAPESP (08/50423-7 to ASP)

01.005 Evaluation of the intrinsic efficacy of different ligands of the 5-HT1A receptor. Pompeu TET, Moura BC, Drummond C, Nöel FG ICB-UFRJ – Farmacologia e Química Medicinal

Introduction: The 5-HT1A receptors are G-protein coupled receptors (GPCRs). Binding assays based on the difference of affinity of agonists for G protein-coupled (higher affinity) and uncoupled (lower affinity) states of the receptor can be used for estimating their intrinsic efficacy. One assay (ratio of *K*i's) is based on the difference of *K*i´s measured when using an antagonist *vs* agonist, as a radioligand (BRANCHEK et al., *Mol. Pharmacol.* 38: 604, 1990). A *K*i ratio=1, indicates that the compound is an antagonist whereas *K*i ratios >1 or <1 indicate that the compound acts is an agonist or inverse agonist, respectively (ASSIÉ et al., *J. Pharmacol*. 386: 97, 1999). The second assay (GTP-shift) is based on the difference of affinity measured in the absence and presence of a large concentration of GTP that is capable to destabilize the ternary complex ARG (high affinity state of the receptor) formed by the agonist (A), the receptor (R) and the G-protein (G), to form the binary complex AR (low affinity state of the receptor) (DE LEAN et al., *J. Biol. Chem*. 255: 7108, 1980).The aim of this study was to compare these two different binding assays for evaluating the intrinsic efficacy of different ligands of the 5-HT1A receptor. **Methods**: Binding: All protocols were approved by the Ethics Committee of UFRJ (CAUAP; DFBC - ICB011). Competition assay: 40 or 75 µg of protein (rat hippocampus synaptosomes) were incubated with 1 nM [3H]-8-OH-DPAT (agonist radioligand) or 0.5 nM [3H]-*p*MPPF (antagonist radioligand) plus 1 mM of GTP, respectively, for 15 or 45 minutes at 37°C, in the absence and presence of increasing concentrations of 8-OH-DPAT, *p*MPPF, clozapine, WAY-100,635, serotonin or spiperone. GTP-shift experiments were performed using a neutral antagonist radioligand ([3H]-*p*MPPF) with or without 1 mM GTP. The results were analyzed by nonlinear regression to estimate the IC_{50} values necessary to calculate the K_i of the test ligands. Saturation assay: 40 or 75 µg of protein were incubated with increasing concentrations of [3H]-8-OH-DPAT (0.1-20 nM) or [3H]-*p*MPPF (0.05 – 2 nM) plus 1 mM of GTP, respectively, to estimate de Bmax and *K*d values. The reaction was terminated by adding a cold buffer (Tris-HCl, pH 7.4), followed by vacuum filtration. Radioactivity was quantified by liquid scintillation counter. **Results and Discussion**: The two assays resulted in qualitatively similar results: serotonin and 8-OH-DPAT were considered agonists (*K*i ratio = 76.8 and 57.3, respectively; GTP-shift to the right); clozapine, a weak partial agonist (*K*i ratio = 3.61; smaller GTP-shift to the right); *p*-MPPF, an antagonist (*K*i ratio = 0.20, absence of GTP-shift); spiperone and WAY-100,635, inverse agonists (*K*i ratio = 0.12 and 0.063, respectively; GTP-shift to the left). Our results show that both techniques can be used to evaluate the intrinsic efficacy of ligands at the 5-HT1A receptor. However, the GTP-shift assay has a better cost-effectiveness profile, since it needs only one radioligand and is based on a paired protocol, thus minimizing experimental bias, and does not suffer from errors performed when estimating the *K*d values for the two radioligands. We are now initiating a [35S]-GTPgammaS binding assay in order to directly define the intrinsic efficacy of these ligands in our experimental conditions. **Financial Support**: CNPq, INCT-INOFAR, FAPERJ.

01.006 Influence of glycation on the biotransformation enzyme glutathione S-transferase. Bousová I, Trnková L, Průchová Z, Drsata J Charles University in Prague-Pharmacy – Biochemical Sciences

Glutathione S-transferase (GST, EC 2.5.1.18) is a group of intracellular enzymes involved in detoxification of xenobiotics including drugs like paracetamol, morphine, clofibrate, cisplatin, and others. Diabetes mellitus and age-related diseases are accompanied with glycation of proteins as a non-enzymatic process resulting in the impairment of protein functions. The reactive alpha-dicarbonyl compounds (e.g. methylglyoxal) and advanced glycation end products (AGEs) are formed in the glycation process. Alpha-dicarbonyls cause protein cross-linking and formation of AGEs mainly on intracellular proteins, which usually lose their biological activity and may persist in tissues. We suppose that GST may be modified by glycation *in vivo*, which would provide a rationale of its use as a model protein for studying glycation reactions. Glycation of GST by methylglyoxal, fructose or glucose was studied (37°C, for up to 28 days). The course of protein glycation was evaluated using following criteria: enzyme activity, formation of fluorescent AGEs, amino group's content, protein conformation, cross-linking and aggregation, carbonyl content, and changes in molecular charge of GST. Methylglyoxal 1 mM and 2 mM decreased the enzyme activity of GST by 12% and 22% after 180 minutes, respectively. The observed changes in GST activity were statistically significant since 60 minutes of incubation ($p < 0.01$). Effects of fructose 50 mM and glucose 50 mM on GST catalytic activity at chosen time intervals were negligible. Longer incubation of GST 0.5 mg/ml with glycating agents (in the range of 7 days) led to the considerable loss of catalytic activity (e.g. fructose 50 mM decreased the activity by 70%). Incubation of GST with glycating agents caused increase in non-tryptophan fluorescence at the wavelengths typical for AGEs. The effect of fructose was comparable to the effect of methylglyoxal 0.5 mM. Glucose exerted some glycating activity after longer incubation (28 days), when the concentration of AGEs increased by 7.7% compared to the control. Prolonged incubation of GST with fructose and glucose (28 days) led to decrease in the number of primary amino groups from 44 to 37 and 39, respectively. Glycation of GST by glycating agents caused changes in molecular charge of the enzyme, which became more anionic and its electrophoretic mobility towards the positive electrode increased. In case of methylglyoxal was this phenomenon noticeable since the day 1 of incubation, when migration of the sample containing GST + MGO 2 mM rose by 11.6% compared to GST alone. On the other hand, changes in the mobility of samples containing fructose and glucose were less pronounced (5.4% and 1.4%) and they were observable since the day 7 and day 14, respectively. The loss of primary amino groups was accompanied by changes in protein mobility during native PAGE. Formation of cross-links with molecular weight of 65 and 135 kDa was observed. Obtained results may contribute to understanding changes in metabolism of drugs during diabetes mellitus and ageing. **Acknowledgments***:* This study was realized with **Financial Support** from the Czech Ministry of Education SVV 265 004.

01.007 ***In vitro*** **tolerance to nitroglycerin following 5 minutes incubation is followed by an increased reactive oxygen species in isolated endothelial cell**. de Rezende V, Silva BR, Bendhack LM. FCFRP-USP – Física e Química

Introduction: Nitroglycerin (GTN) may cause tolerance during long-term application, which is its major therapeutic limitation [1-3]. However, the mechanisms underlying GTN-induced tolerance and the participation of endothelium to this phenomenon remain unclear [3, 4]. This work aimed to study the contribution of the nitric oxide (NO) and reactive oxygen species (ROS) produced and/or released from the endothelium to GTN-induced relaxation and GTN tolerance by evaluating the cellular mechanisms that cause the *in vitro* tolerance in rat aorta and in isolated aortic endothelial cells. **Methods**: Relaxation concentration-effect curves for NTG were constructed in intact endothelium (E+) rat aortic rings pre-contracted with phenylephrine. In addition, the effects of the concentration of GTN that produced the maximal effect (EC_{100}) was evaluated on both intracellular NO and ROS concentrations in isolated endothelial cells by using selective fluorescent dyes for NO and ROS (O_2^-). All the experimental procedures used are accordance with the Animal Ethics Commitee of the University of São Paulo (10.1.134.53.0). **Results**: The NO-synthase inhibitor L-NAME reduced the potency (pD_2) and maximum effect (ME) (pD_2: 7.83±0.15; ME: 69.8± 3.2 n=5; control: pD_2:.87.0±0.13; ME: 102.7±2.7, n=10) p<0.001. Otherwise, the ROS scavenger Tiron did not change the ME nor potency (p>0.05) induced by GTN. In order to study the tolerance *in vitro*, the E+ arteries were incubated for 5 min with GTN (EC_{100}: 100 mmol/L).The ME was reduced from 101.9 ± 1.6%; n=10 to 54.7± 3.6%, n=5 p<0.001 and pD_2 was reduced from 8.88 ± 0.20 to 7.63 ± 0.04; n=10, p<0.05), respectively. The results also showed that GTN enhances O_{2-} concentration in 5 min (54.15±1.49U when compared with the basal levels: 2.19 ± 0.58U; p<0.001) and it did not change the NO concentration when compared with the basal (p>0.05). **Discussion:** Taken together, our results demonstrate that the tolerance is induced by 5 min incubation with GTN and there is an increased production of ROS in rat aortic endothelial cells. **References:1**-Fung, H.L.; Annu Rev Pharmacol Toxicol, v.44;p. 67**;** 2004. **2**-Warnholtz, A.; J Am Coll Cardiol; v. 40(7): p. 1356; 2002. **3-**Ignarro, L.J. Proc Natl Acad Sci U S A; v. 99 (12) p. 7816; 2002. **4**-Munzel, T.; J Clin Invest, v.95(1); p. 187; 1995. **Supported by**: FAPESP and CNPq.

01.008 Consequences of chronic ethanol consumption on the reactivity and expression of components of the endothelinergic system in the rat corpus cavernosum. Leite LN[1], Côco H[1], Lacchini R[1], Tanus-Santos JE[1], Carnio EC[2], De Oliveira AM[3], Tirapelli CR[2] [1]FMRP-USP, [2]EERP-USP, [3]FCFRP-USP

Introduction: Endothelin-1 (ET-1) is a vasoconstrictor peptide that plays an important role in controlling the tone of the cavernous body. However, it has been demonstrated that this peptide is also involved in erectile dysfunction (ED) associated with diabetes mellitus and hypertension. Ethanol consumption increases plasma levels of ET-1 and the contractile response to this peptide in vascular tissues. This study aimed to investigate the cellular and functional consequences of chronic ethanol consumption on the endothelinergic system in penile circulation as well as the mediators involved in this response. **Methods**: The experimental protocols were approved by the Ethical Committee from USP (10.1.1084.53.6). Male Wistar rats were treated with ethanol (20% vol/vol) for 6 weeks. Reactivity experiments were performed on isolated cavernosal smooth muscle (CSM). Cumulative concentration-response curves for phenylephrine (PhE), endothelin-1 (ET-1), sodium nitroprusside (SNP), acetylcholine (ACh) and IRL-1620, a selective ET_B agonist, were performed. Nitrate levels were measured in plasma and supernatants from total CSM homogenates. mRNA for pre-pro-ET-1, ET_A and ET_B receptors, inducible NO synthase (iNOS), neuronal NO synthase (nNOS) and endothelial NO synthase (eNOS) was assessed by RQ-PCR. Plasma ET-1 was measured by enzyme immunoassay (EIA). Results: Blood ethanol levels in the ethanol-treated rats averaged (1.91 ± 0.21 mg/ml n=11). Chronic ethanol consumption increased plasma ET-1 levels. Body weight of the rats before beginning the treatment averaged (284 ± 5g) in control group and (289 ± 3g) in ethanol group. The treatment for 6 weeks reduced the body weight of the rats from ethanol group (477 ± 10g) when compared to control group (592 ± 9g) ($P<0.05$, ANOVA). Our findings show that phenylephrine-induced contraction in isolated CSM strips was not altered after treatment with ethanol. On the other hand, ET-1-induced contraction was significantly higher in cavernosal strips from ethanol-treated rats (32 ± 2.8% KCl 120mM; n=7) when compared to control (21.5 ± 1.5% KCl 120mM; n=6) ($P<0.05$, Student's t test). Chronic ethanol consumption reduced the relaxation induced by acetylcholine (28.4 ± 1.4%, n=7) in the rat CSM when compared to control group (39.4 ± 1.4%, n=10) ($P<0.05$, Student's t test). IRL-1620 and sodium nitroprusside-induced relaxation were not affected by ethanol consumption. It was found that chronic ethanol consumption did not alter mRNA levels for ET_A or ET_B receptors, pre-pro-ET-1, iNOS, nNOS and eNOS. Finally, ethanol consumption reduced nitrate plasma levels. **Discussion**: The present findings demonstrate that there is a link between chronic ethanol consumption and alterations on the endothelinergic pathway in the penile circulation. **Financial Support**: CAPES, FAPESP.

01.009 Establishment of an animal model to evaluate the healing of wounds.
Angeli-Gamba T[1], Santos JMP[1], Silva-Jesus AC[1], Machado DE[1], Nasciutti LE[2], Soares de Moura R[1], Perini JA[1] [1]UEZO, [2]UFRJ

Introduction: Wound healing is a complex process involving several overlapping stages that include inflammation, formation of granulation tissue, epithelial re-organization, extracellular matrix formation and remodeling (Takayama Yet al., Cell Biochem Biol., 90:497, 2012). Loss of skin integrity observed in injury or illness may result acutely in substantial physiologic imbalance and ultimately in significant disability or even death (Somboonwong J. et al., BMC Complement Altern Med., 12: 103, 2012). There are only few controlled trials that have proved the clinical efficacy of the traditional wound healing agents. The aim of the present study was to establishment of an animal model to evaluate the healing of wounds to further test new therapies.

Methodology: The project was approved by the Ethics Committee for Animal Experimentation of the Federal University of Rio de Janeiro (protocol number DAHEICB080) and was performed according to international rules considering the animal experiments. Wound healing activity was determined in rats using excision wound model. Wistar rats were randomly divided into four groups of six animals: negative control (NC), placebo (PC), treated with topical application of the Fibrinolysin (TF) and topical application of the Collagenase. Tissue samples were obtained 6 and 14 days after injury and wounds size were analyzed. Healing was assessed by the tissue morphological characteristics, molecular analysis, hydroxyproline dosage and nitrite content by the colorimetric study using the Ehrlich and Griess reagent, respectively. **Results**: Our results showed that treatment with Fibrinolysin and Collagenase significantly decreased the size of the wounds, and histological examination indicated regression of the lesions with better epithelialization and more effective re-organization of the dermis compared to the control or placebo group. A growth in the concentration of hydroxyproline and reduction the nitrite dosage were also observed in the treated groups. **Discussion**: These observations suggest that Fibrinolysin and Collagenase exhibit potent healing properties on excision wounds model. There are a few parameters which are involved in the healing of wound including epithelization, biochemical changes and antioxidant defense. Therefore, further approaches are needed to test healing potential on dermal wounds. **Supported by**: FAPERJ and UEZO

01.010 Cardiotonic steroids exhibit functional selectivity in LLC-PK1 cells. Amaral LS, Cunha-Filho GA, Nöel FG, Quintas LEM ICB-UFRJ

Introduction: Cardiotonic steroids (cardenolides and bufadienolides), considered specific Na^+/K^+-ATPase inhibitors, also promote the activation of signaling pathways via Na^+/K^+-ATPase through protein-protein interactions. One of these pathways involves activation of the cascade Src/Ras/Raf/MEK/ERK. The ERK protein participates in many cellular processes like cell proliferation. We have recently showed that the bufadienolides telocinobufagin (TCB) and marinobufagin (MBG), now considered in mammals steroid hormones, activate the ERK pathway with the same pattern and this stimulation occur at concentrations where no significant inhibition of enzyme activity is observed. However, MBG induces cell proliferation/viability and TCB cell death. Our aim is to investigate the mechanisms underlying these diverse effects. **Methods:** Sub-confluent LLC-PK1 cells (porcine proximal renal tubule) were treated 15 min with 1, 10 and 100 nM telocinobufagin and marinobufagin, with or without the presence of MEK inhibitor (U0126) and Src inhibitor (SU6656), lysed with RIPA buffer, centrifuged at 13,000g for 15 min and the supernatants were used in Western blot for evaluation of activation (phosphorylation) of MAP kinases ERK1/2. The evaluation of cell proliferation was measured by MTT analysis or counting the number of Trypan blue-viable LLC-PK1 cells treated with bufadienolides and MEK or Src inhibitors for 24, 48 and 72 h. Cell viability was measured by detection of anti- (Bcl-2) and pro-apoptotic (Bax) proteins by Western blot analysis. **Results:** TCB and MBG induced phospho-ERK1/2 in 1 (23% and 34%, $p<0,05$, n=3), 10 (26% and 58%, $p<0,05$, n=3) and 100 nM (40% and 64%, $p<0,05$, n=3) and the addition of 10 mM U0126 prevented the bufadienolides effect. MBG induced significant cell proliferation after 72 h (10 and 100 nM) (73 and 95%, $p<0,05$, n=4). This effect was prevented by addition of 10 mM SU6656 or U0126. TCB had no effect on cell proliferation (1 and 10 nM) and inhibited it at 100 nM (cell number decreased 73%, $p<0,05$, n=4). However, 10 mM SU6656 or U0126 did not affect TCB inhibition of proliferation. Preliminary data point out that while the expression of Bcl-2 was increased (72%, n=2), Bax was decreased in MBG-treated cells. On the other hand, the expression of Bax was increased and Bcl-2 was decreased in TCB-treated cells (90%, n=2). **Discussion:** Overall, these results indicate that bufadienolides present functional selectivity promoting divergent effects despite acting on the same receptor and contribute to the understanding of the existence of different endogenous cardiotonic steroids in mammals. The mechanisms underlying TCB effects are under investigation. **Financial Support:** CAPES, Faperj, CNPq

01.011 Low level laser therapy in the miotoxicity induced by *Bothrops jararacussu* snake venom on C2C12 muscle cells. Silva CAA[1], Silva LMG[1], Rocha CR[1], Ferrari RAM[1], Cogo JC[2], Zamuner SR[1] [1]Uninove – Ciências da Reabilitação, [2]UNIVAP – Fisiologia

Introduction: Local myonecrose is a common consequence in envenoming caused by snakes of the genus *Bothrops* which occurs through the action of myotoxins that acts directly in the muscle cell membrane. Antivenom therapy and other first-aid treatments do not reverse the local myonecrose. Thus, there is an urgent need to find therapies that can complement antivenoms in the neutralization of local tissue damage. The low level laser therapy (LLLT) is being considered as an alternative treatment for muscle injury situations because its bioestimulation effect. Objective: The present work was designed to investigate the effect of LLLT on muscle cells submitted to injury by *Bothrops jararacussu* venom (BjssuV). **Methods**: C2C12 muscle cell line was used. The cells were grown in culture medium DMEM supplemented with 10% fetal bovine serum, incubated at 37°C with 5% CO_2 for 24 hours for cell attachment, after that, the cells received BJssuV (12.5 µg/mL). Cells were irradiated for 13 s immediately after the venom administration with a semiconductor laser at 635 and 830 nm, dose of 4 J/cm^2 and power of 100 mW and were incubated for 15, 30 and 60 minutes. The cells that did not receive venom and irradiation served as control. The cell viability was analyzed by MTT assay and the dosage of creatine kinase (CK) was performed using the commercial kit CK-NAC using spectrophotometer at wavelength 340 nm at 37°C. Results: Our results showed that the LLLT increased cell viability by 86% and 92% in C2C12 myocytes by LLLT at 635 and 830 nm, respectively, 30 min after the venom administration. Also, results showed a decrease of CK release in all the periods analyzed, after LLLT, in both wavelengths studied. **Discussion**: The BjssuV is toxic to muscle cells and LLLT protected against this effect, probably by protecting the plasma membrane. **Conclusion**: The use of LLLT should be considered as a potentially useful therapeutically approach for treatment of the local effects of Bothrops snakebites.

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01.012 Redox profile in liver of silver catfish subjected to MS222 anesthesia.

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Introduction: MS222 is the most widely used immersion anesthetic for fish. Silver catfish (*Rhamdia quelen*) is an endemic species from South America with a relevant economic importance. This study aimed at verifying alterations in oxidative parameters in liver of silver catfish anesthetized with MS222. **Methods**: The experiment consisted of five groups (N=8; 95.63 ± 2.83 g and 22.12 ± 0.2 cm). Two concentrations of MS222 were used: 150 and 300 $mg.L^{-1}$. For each concentration, two groups were formed: anesthesia and recovery. Fish were exposed to MS222 until stage 4 of anesthesia was reached[1]. Fish in anesthesia groups were euthanized and livers dissected out. Fish in recovery groups were allowed to recuperate in aquaria free of anesthetic. When recovery was completed, fish were euthanized and livers excised. Animals in control group were transferred from one aquarium to another, both with water free of anesthetic substance, duplicating the handling described for the other groups. After that, euthanasia preceded liver dissection. Livers of all groups were homogenized and supernatants used to analyze the levels of lipid peroxide, using thiobarbituric acid reactive substances (TBARS) assay, and of the antioxidants catalase (CAT) and glutathione-S-transferase (GST). TBARS and CAT were analyzed through Anova and Tukey test, and GST was analyzed by Kruskal-Wallis test and multiple comparisons of mean ranks ($P<0.05$). Registration at the Ethics Committee on Animal Experimentation of UFSM: 46/2010. Results: There was an increase in TBARS (in nmol.mg $protein^{-1}$) in fish sampled after recovery from 300 $mg.L^{-1}$ MS222 (1.43±0.35; mean±SEM) comparing with control (0.37±0.08). CAT levels (in pmol.mg $protein^{-1}$) were higher in anesthesia groups (150 $mg.L^{-1}$: 5.62±0.35; 300 $mg.L^{-1}$: 5.24±0.45) and in 150 $mg.L^{-1}$ MS222 recovery group (6.1±0.26) compared with control (3.21±0.40). Activity of GST (in $pmol.min^{-1}$ mg $protein^{-1}$) increased in 150 $mg.L^{-1}$ MS222 recovery group (3.09±0.53), while consumption of the enzyme was observed in anesthesia groups (150 $mg.L^{-1}$: 0.47±0.10; 300 $mg.L^{-1}$: 0.90±0.17) and 300 $mg.L^{-1}$ MS222 recovery group (0.87±0.11) compared with control (1.85±0.30). **Discussion**: At both concentrations of MS222, the “preparation to oxidative stress” concept[2] could be observed in the greater CAT concentration in anesthetized fish (low oxygen availability). Nonetheless, CAT alone was not efficient to combat lipid peroxide generation in silver catfish recovered (reoxygenation) from 300 $mg.L^{-1}$ MS222. The pattern displayed by GST indicates a more conventional response to stress: an elevation of the enzyme activity as a consequence of the oxidative stress undergone during the hypoxic-normoxic transition[3]. Along with CAT, GST may have prevented elevation of TBARS in fish recovered from 150 $mg.L^{-1}$ MS222. Consumption of GST could be due to its readily inactivation by oxydation. **References**:[1]Schoettger R.A. (1967). Invest. Fish. Cont. 13, 1. [2]Buzadzic B. (1992). Comp. Biochem. Phys. B 101, 547. [3]Halliwell B. (1989) Free Radic. Biol. Med. (2nd ed.). Oxford, UK: Clarendon Press. **Financial Support**: FAPERGS/PRONEX, Capes, CNPq.

01.013 CXCL12/SDF-1 and histamine stimulate mice pulmonary fibroblast to produce CXCL1/KC, CXCL2/MIP-2 AND CCL3/MIP-1α. Danilucci TM, Oliveira SHP
FOA-Unesp – Pharmacology

Aim: A large number of chemokines have been identified as possible targets in the development of asthma, among them the Chemokine (C-X-C Motif) ligand 12/Stromal Cells-derived Factor-1 (CXCL12/SDF-1) has been linked to a crucial aspect in the development of the disease, due to its chemotactic action on inflammatory cells. Histamine is also an important mediator of inflammatory allergic reactions in the pathophysiology of asthma triggers responses such as vasodilatation, smooth muscle contraction, hyper-secretion of mucus and edema. Because CXCL12 and histamine influence cell types that govern immune and inflammatory reactions, its potential role in asthma has long been of interest. The aim of our study is to investigate whether CXCL12 or histamine stimulate lung fibroblasts to produce CXCL1/KC, CXCL2/MIP-2 and CCL3/MIP-1α. **Methods and Results**: Protocol number of the animal ethics committee: 003448. Male Balb/c mice were killed by halothane inhalation and their lungs were removed. The lungs were fragmented, resuspended and placed into 25 cm^2 culture flaks in DMEM supplemented with 15% fetal bovine serum (FCS), 100 U/mL penicillin, 100 µg/mL streptomycin and 0.5 mg/mL of amphotericin B. Cells were used in experiments between 4th and 7th passage. After confluence, the lung fibroblasts were placed into 24 well plates and maintained overnight to adhere at 37 °C in an incubator 5% CO_2. Then culture medium (500 µL) containing CXCL12 (1, 10, 50 and 100 ng/mL) or histamine (10, 100 and 1000 ng/mL) was added to the wells. The control group was only added culture medium. After 1, 6 and 24 h cell-free supernatant was collected. The level of CXCL1/KC, CXCL2/MIP-2 and CCL3/MIP-1α in the cell-free supernatant was analyzed by ELISA (Enzyme Linked Immunosorbent Assay). Statistical significance was determined using analysis of variance with P values <0.05. The results demonstrated CXCL-12 and histamine are able to stimulate mice lung fibroblasts to produce CXCL1/KC, CXCL2/MIP-2 and CCL3/MIP-1α. CXCL12 (10 ng/mL) and histamine (10 ng/mL) induced CXCL1 production one hour after stimulation compared with control group. At 6 to 24 hours CXCL1 production was not observed after CXCL12 or histamine stimulation. CXCL12 (100 ng/mL) induced CXCL2 production as early as one hour after stimulation. At six hour after stimulation CXCL12 (1 ng/mL and 10 ng/mL) also induced CCL2 production when compared with control group. At 24 hours the CXCL2 production was not statistically significant compared with control group. Histamine (10 ng/mL) induced CXCL2 production in relation to the group control than one hour as six hours after stimulation. At 24 hours did not observe cell stimulation. CXCL12 (50 ng/mL) and histamine (1000 ng/mL) induced CCL3 production one hour after stimulation in relation to group control. At 6 and 24 hours did not occur cell stimulation for CCL3 production induces by CXCL12 and histamine. **Conclusion**: CXCL-12 and histamine stimulate lung fibroblast to produce CXCL1/KC, CXCL2/MIP-2 and CCL3/MIP-1α. These CC and CXC chemokine can be an important mediator involved in the neutrophil and/or eosinophil recruitment to the inflammatory focus in the allergic process mediated by lung fibroblast. The mechanism involved in the process is under investigation. **Financial Support**: CAPES and FAPESP.

01.014 Pharmacologic evaluation of new multi-target α1A/D-adrenoceptors and 5-HT1A antagonists candidates to lead compounds for the treatment of benign prostatic hyperplasia. Chagas-Silva F[1], Romeiro LAS[2], Barberato LC[3], Silva RO[3], Lemes LFN[3], Nascente LC[3], Nöel FG[1], Silva CLM[1] [1]ICB-UFRJ – Farmacologia e Química Medicinal, [2]FS-UNB – Ciências Farmacêuticas, [3]LADETER-UCB – Química Bioinorgânica e Medicinal

Aim: Previously, we described that some *N*-phenylpiperazines derivatives (LDT series LDT65-LDT68) have affinity for $alpha_{1A/D}$ adrenoceptors ($a_{1A/D}$-ADR) and 5-HT_{1A} receptors, being high affinity a_{1D}-ADR and 5-HT_{1A} antagonists (Chagas-Silva et. al, Eur. J. Med. Chem., 2012; submitted). Both $a_{1A/D}$-ADR and 5-HT_{1A} receptors are implicated in the physiopathology of benign prostatic hyperplasia (BPH), therefore these new multi-target compounds could be considered as hit compounds in the search for drugs for BPH treatment. The objectives of this study were to i) evaluate the affinity of these compounds for prostate a_{1A}-ADR ii) to assess acute cytotoxicity in human prostate cell line and iii) their acute toxicity in vivo. **Methods**: All protocols were approved by the ethics committee of UFRJ (CAUAP; DFBC-ICB011). Functional studies: isometric contraction experiments were performed with strips of rat ventral prostates (a_{1A}-ADR) (37ºC, pH 7.4) (*Nanda et al. Eur.J. Pharmacol. 607:213, 2009*) that were contracted with phenylephrine (PE) (0.01 to 10000 µM), in the presence of 0.1 µM propranolol, before and after incubation with LDT65-LDT68 (3, 10 or 100 nM). Data were individually analyzed by nonlinear regression (GraphPad Prism 5.0) to obtain the EC_{50} values before and after treatment with the test compounds that were converted to K_B values using the Schild equation (Kenakin, Raven Press, New York, pp278, 1993). Acute cytotoxicity: cell viability was assessed by formazan production from MTT (3-(4,5-dimethiazol-zyl)-2-5-diphenyltetrazolium bromide) (Mosmann, J Immunol **Methods** 65: 55, 1983) by living human prostate cancer cells, DU145, after acute treatment with LDT65-LDT68 (1, 10 and 30 µM) for 24 and 48 hours. Acute toxicity: female Swiss mice (35 - 40 g) were observed at 1, 2, 4 and 8h after treatment, and up to 14 days (once a day) after administration of a single dose of LDTs (10 µg/kg, i.p.). Control groups received vehicle. **Results and Discussion**: Preliminary results from functional studies showed that there was a parallel shift of the log-concentration curve induced by PE to the right, after incubation with all LTDs at 10 nM, as typical surmountable antagonists. The K_B values of LDT65 and LDT66 in rat prostate were respectively: 1.3 and 0.6 nM, suggesting a high affinity antagonism corroborating previous data obtained with binding assays in a_{1A}-ADR. LDT65 and LDT66 showed no cytotoxicity up to 10 µM (24 and 48h). Considering the classic parameters of toxicity *in vivo*, no clinical signs, changes in behavior or mortality were detected. Thus, LDT65-LDT67 could be considered as hit compounds in the search of drugs for BPH treatment. **Acknowledgments**: Drª. Gilda Angela Neves (ICB/UFRJ) **Financial Support**: FAPERJ, CNPq and CAPES

01.015 Influence of Verapamil and exercise training on cardiac function and morphometry in rats. Signor I[1], Aguiar DH[2], Sugizaki MM[2], Gomes LFF[1], Rodrigues RWP[2], Mueller A[2] [1]UFMT, [2]UFMT – Ciências da Saúde

Introduction: The heart calcium-channel blocker like verapamil and the exercise training (ET) are commonly used in the treatment of hypertension.

Objective: Evaluate the influence of verapamil and exercise training on morphometry and cardiac function in normotensive rats. **Methods**: The experimental procedures were approved by the ethics committee for the Use of Experimental Animals from UFMT (nº 23108.019254/11-0) and are in accordance with the Guide for the Care and Use of Laboratory Animals and the Law nº 11.794/2008. Male Wistar rats with 200g were divided in groups: control (C, n = 8), verapamil (VERA, n = 7), trained (T, n = 8) and trained plus verapamil (T_{VERA}, n = 8). Verapamil was administered intraperitoneally at a dose of 5 $mg.kg^{-1}$. The ET consisted of 60 days (1 hour day, 5 days week). Cardiac function was assessed by echocardiography and data was analyzed by ANOVA, Bonferroni, $p < 0.05$. **Results and Discussion**: The verapamil did not change cardiac morphometry or function. However, the association of verapamil plus training (T_{VERA}) increased diastolic diameter of left ventricle (5.6±1.1 vs 4.6±0.7 mm), the diastolic posterior wall thickness of left ventricle (2.52±0.57 vs 1.95±0.16 mm), the cardiac output (55±22 vs 31±10 mL/min) and the cardiac index (155±77 vs 89±32 mL/min/Kg) when compared to T group. Thus, the verapamil increased morphometric and functional cardiac parameters when associated to exercise training. New experiments are being conducted to confirm these findings.

01.016 The effects of intense and exhaustive exercise in isolated uterus of C57BL/6 female mice. Costa AEA[1], Silva JLV[2], Simões MJ[3], Nouailhetas VLA[1]
[1]Unifesp – Biofísica, [2]Uninove – Farmácia-Bioquímica, [3]Unifesp – Morfologia

Regular practice of physical exercise has positive effects on the organism preventing and helping in the treatment of chronic degenerative diseases. However, intense and exhaustive exercise (IEE) without appropriate recovery can compromise health causing damage in several tissues and undesirable metabolic changes. Many women realize fatiguing training that overcame her physical limits either by sportive or esthetical goal. It is known that IEE may cause several dysfunctions like amenorrhea, oligomenorrhea, short luteal phase and ovulation absence. Although not having the same physiology the rodent animal models have been used to study exercise. In the case of reproductive system studies are mostly focused on the pregnant rather than non-pregnant uterus. The aim of the present study was to investigate the possible relationship between exercise-induced oxidative stress and morphofunctional changes in the isolated uterine smooth muscle of non-pregnant C57BL/6 female mice in response to IEE. C57BL/6 females mice (3-4 mo old) were randomly ascribed into two groups: control (CT, n=6) and exercised for two days (EX2, n=6). The estrous cycle was identified and monitored through vaginal smears stained with toluidine blue 1%. Exercise protocol was based on Rosa *et al* (2008) developed for male mice and consisted of a daily acute bout of treadmill running until exhaustion with an intensity corresponding to 85% animal's maximum velocity (IEE) which was assessed by incremental test. The exercise program was performed throughout one estrous cycle. After 24 hours of the end of last exercise session, the animals were sacrificed by cervical dislocation and uterus isolated. We analyzed: makers of exercise: exhausted time in each session and maximum velocity; morphology studies were done through 4μm thin section of uterus stained with HE; uterus contractility was assessed by non cumulative concentration-contractile response curves to KCl and carbachol (CCh) and protein oxidation by carbonyl radical level measurements. All procedures and **Methods** were approved by the Ethics Committee in Research of UNIFESP (No. 1390/10). Makers of exercises: 36% decrease in the second day exhaustion time relative to the first day of exercise and 30% reduction in the maximum velocity. Morphological studies: 10% reduction of longitudinal layer thickness; contractility: concentration-response curves to KCl was not modified, but for CCh it was shifted to the right, causing higher EC_{50} (CT (2,8 ± 1,2) x 10^{-6}M and EX2 (1,8±0,3) x 10^{-5}M) and decreased of the maximum response (CT 1,7 ± 0,3g and EX2 1 ± 0,2g); oxidative stress: carbonyl radical levels were similar in both groups. IEE protocol was effective since it caused decrease physical performance and reduced maximum velocity. IEE was deleterious to non-pregnant uterus since it led to reduced longitudinal layer thickness and decreased potency and efficacy for muscarinic signaling without causing any change in KCl-depolarization signaling. Finally, unexpectedly IEE did not alter uterus redox status since no increased in tissue carbonyl radical level was observed. All together we evidenced that non-pregnant uterus is quite sensitive to exercise so that careful studies should be done concerning exercise effects on female reproductive system. **Acknowledgments**: Rigoni,VLS

Financial Support: Capes and Fapesp

01.017 Influence of digoxin and physical training on cardiac function and morphology in rats Souza KG, Aguiar DH, Sugizaki MM, Gomes LFF, Rodrigues RWP, Mueller A UFMT

Introduction: Digoxin is a cardiac glycoside commonly used in the treatment of heart failure. The physical training (PT) has been added to pharmacological treatment in cardiac patients. **Objective**: Evaluate the influence of digoxin and physical training on morphology and function of myocardium in normotensive rats. **Methods**: The experimental procedures were approved by the ethics committee for the Use of Experimental Animals from UFMT (nº 23108.019254/11-0) and are in accordance with the Guide for the Care and Use of Laboratory Animals and the Law nº 11.794/2008. Male Wistar rats with 200g were divided in groups: control (C, n = 8), digoxin (DIGO, n = 7), trained (T, n = 8) and trained plus digoxin (T_{DIGO}, n = 6). Digoxin was administered intraperitoneally at a dose of 30 $mg.kg^{-1}$. The PT consisted of 60 days (1 hour day, 5 days week). Cardiac function was assessed by echocardiography and the morphological results were obtained by measuring the area of cardiomyocytes. Data was analyzed by ANOVA, Bonferroni, $p < 0.05$. **Results and Discussion**: The use of digoxin associated to physical training increased the left ventricular diastolic dimension (T=4.57±0.71 *vs* T_{DIGO} =5.91±0.38), the left ventricular diastolic volume (T=0.11±0.03 *vs* T_{DIGO} =0.21±0.04) and left ventricular systolic volume (T=0.017±0.007 *vs* T_{DIGO} =0.044±0.006), the cardiac output (T=30.8±10.3 *vs* T_{DIGO}=56.4±8.4) and the cardiac index (T=89.2 *vs* T_{DIGO}=173±27). Morphological data showed a cardiomyocyte area reduction in DIGO (380.3 ± 4.5) compared to C group (484.5±5.3), and a cardiomyocyte area reduction in T_{DIGO} (363.2 ± 3.0) compared to T group (414.9 ± 4.3). Although the association of digoxin and physical training promoted a reduction in cell area, the cardiac structural and functional analyzed *in vivo* by echocardiography increased. New experiments are being conducted to confirm these findings.

01.018 Sympathetic outflow and protein expression in the mouse submandibular gland: a proteomic approach. Heluany CS, Luna MS, Yamanouye N IBu – Farmacologia

Data in literature show that sympathetic outflow has only a role in stimulating synthesis and secretion of the saliva proteins in mammals. However, we have shown that the stimulation of noradrenergic innervation by extraction of venom is the key activator of venom gland of *Bothrops jararaca*, an oral exocrine gland related to salivary glands. The new function of the noradrenergic innervation was discovered just because the venom gland can assume distinct quiescent and activated stages, in contrast to the salivary gland that is constantly activated. The aim of this study is to verify whether sympathetic innervation is also important to keep submandibular gland activated and to identify the proteins that have their expression regulated by sympathetic outflow.

Adult Swiss male mice (25-30g) were divided into 3 groups: 1) control (n=3); 2) treated with reserpine (n=3) for 6 days (0,5mg/kg - i.p.); 3) treated with reserpine for 6 days (0,5mg/kg - i.p.) and phenylephrine plus isoprenaline (20mg/kg – i.p.) in the sixth day (n=3). Extracts of submandibular glands were prepared and proteins were analyzed by two-dimensional gel electrophoresis. Gels were run in triplicate, stained with Coomassie Blue G and the quantification of density of the spots and comparison among groups were made using ImageMaster 2D Platinum 7. Only specific protein spots from each group were analyzed and the proteins were identified. These spots were excised from the gels, digested with trypsin and analyzed by ESI-LTQ XL/ORBITRAP. All mass spectrometry data were analyzed using PEAKS Studio 5.3 and searches were made using NCBI non-redundant database. All information about the proteins identified is in accordance to data collected on UniProt (www.uniprot.org). Comparison among the groups pointed out that different proteins were expressed. We identified 123 specific spots of proteins in the control group, 192 in the reserpine-treated group and 214 spots in the reserpine plus adrenoceptors agonists-treated group. Interestingly, 117 spots were detected in common between the control group (active gland) and the reserpine plus adrenoceptors agonists-treated group, and only 7 spots in both the control and the reserpine-treated group. Comparison among the reserpine-treated group and the reserpine plus adrenoceptors agonists-treated group revealed just 9 spots in both conditions. The analysis of specific proteins identified after treatment with reserpine showed that the expression of cytoplasmatic proteins was increased. The expression of kallikrein, a cytoplasmic protein, increased significantly after reserpine treatment. Furthermore, after administration of adrenoceptors agonists, the expression of kallikrein decreased. These data showed that this protein is probably down-regulated by sympathetic outflow. **Conclusion**, we showed that sympathetic outflow regulates the expression of proteins of the submandibular gland. These results are in accordance to previous data from our laboratory that show the importance of the sympathetic outflow to regulate the expression of proteins of venom gland of *Bothrops jararaca* in order to activate it. Besides, this study could open promising new avenues for treatments of oral diseases. **Support**: FAPESP. Animal Ethics Committee of the Instituto Butantan: protocol 640/2009.

01.019 Intrinsic activity determination of the derivatives N-phenylpiperazinics for 5HT1A receptors. Carvalho AR[1], Nascimento Viana JB[1], Romeiro LAS[2], Nascente LC[3], Lemes LFN[3], Nöel FG[1], Silva CLM[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]LADETER-UnB, [3]LADETER-UCB

Introduction: 5-Hydroxytryptamine (5-HT)-1A receptors stimulate prostate cellular proliferation and the use of antagonists of 5-HT1A receptors has been considered in the treatment of benign prostatic hyperplasia (Abdul et al., J. Urol. 154:247, 1995). Previously, N-phenylpiperazinics derivates (LDT series) was reported to exhibit high affinity for $5HT_{1A}$ receptors but we did not know their intrinsic activity. The affinity of an agonist for G protein-coupled receptors, but not of an antagonist, varies with the receptor conformation due to GTP-binding (Assié et al., *Eur. J. Pharmacol.* 386:97, 1999). The objective of this study was to determine the intrinsic activity of LDT3, LDT5 and LDT 8 at $5HT_{1A}$ receptors. **Methods**: All protocols were approved by the Ethics Committee of UFRJ (DFBCICB011). Wistar rats were anesthetized and killed. Membrane preparations containing $5HT_{1A}$ receptors were obtained from hippocampus (Hall et al., J. Neurochem., 44:1685, 1985). Binding assays: 50 µg of the preparation were incubated with [3H]8-OH-DPAT 1 nM (agonist) or [3H]*p*-MPPF 0.5 nM (antagonist), $CaCl_2$ 1 mM, $MnCl_2$ 1 mM, pargiline 10 µM, with or without GTP 1 mM, Tris 50 mM, (pH 7.4), for 15 or 45 min in the presence and absence of LDT3,5 (10^{-10}M – $3x10^{-7}$M) and LDT8 (10^{-12}M – 10^{-7}M). The non-specific binding was determined in the presence of 5-HT (10 mM). The reaction was stopped with the addition of cold Tris-HCl buffer (5 mM) followed by filtration under vacuum and the radioactivity was quantified in a liquid scintillation counter. The results were analyzed by non-linear regression (GraphPad Prism 5, USA) to calculate the parameters IC50 and Ki of LDTs in each receptor conformation. **Results and Discussion**: In the presence of GTP the low affinity state of the receptor predominates, and in the absence of GTP there is a mixture of high and low affinity states (Lahti et al., *Mol. Pharmacol.,* 42:432, 1992). It was used an agonist radioligand to define the affinity of LDTs for the high affinity state of the receptor and an antagonist radioligand to define the affinity of LDT for the low affinity state of the receptor. The *K*i (nM) values followed by their 95% confidence intervals for LDT 3, 5 and 8 were: 1.7 [1.3-2.2]; 3.9 [3.2-4.6] and 0.02 [0.00-0.54], respectively, in the first condition (n=4) and 2.0 [0.6-6.3]; 8.1 [3.1-21.1] and 0.62 [0.38-0.99] in the second condition (n=3). The *K*i ratios of LDT 3, 5 and 8 were, respectively, 1.2 [0.6-2.2]; 2.1 [1.3-3.5] and 35 [3.7-186.2]. Therefore, LDT 3 and 5 can be considered as high affinity antagonists and LDT 8 as a partial agonist of $5\text{-}HT_{1A}$ receptor. In **Conclusion** the LDT 3 and 5 could represent prototypes of new drugs for the treatment of benign prostatic hyperplasia. **Acknowledgement**: FAPERJ (**Financial Support**, fellowship) and CNPq (fellowship).

01.020 Quantifying ligand bias signaling at human αlpha1A- and αlpha1b-adrenoceptors. Lima V, Pupo AS Unesp – Farmacologia

Introduction: It has been proposed that a ligand induces or stabilizes different conformations of 7 transmembrane domain receptors leading to differential modulation of receptor activities. For example, according to this model a ligand preferentially activates the G protein or β-arrestin signaling pathway, or activates both indistinctly. This phenomena is known as collateral/pluridimentional efficacy, functional selectivity or ligand bias signaling (Kenakin, *TIPS*, 28, 407, 2007; Kenakin, *JPET*, 336, 296, 2011). This study investigates if some ligands commonly used as α_1-adrenoceptors (ARs) agonists (the phenylethylamines: noradrenaline, dopamine, phenylephrine and methoxamine; and the imidazolines: A-61603, oxymetazoline and naphazoline) present biased signaling for G protein or β-arrestin in human recombinant α_{1A}- and α_{1B}-ARs expressed in HEK293 cells. **Methods**: The bias factors β (Rajagopal *et al*, *Mol Pharm*, 80, 367, 2011) and $\Delta\Delta\log \tau/K_A$ (Tschammer *et al*, *Mol Pharm*, 79, 575, 2011) of α_1-AR ligands were measured by the Operational Method (Black and Leff, *Proc R Soc Lond B Biol Sci,*220, 141, 1983) to analyze the efficacies of these ligands in two different signaling pathways, the G protein-dependent increase in intracellular calcium and the β-arrestin dependent receptor internalization in HEK293 cells expressing human recombinant α_{1A}- and α_{1B}-ARs. Further investigation of the efficacies of oxymetazoline and noradrenaline at the β-arrestin pathway were assessed by ERK 1/2 phosphorylation assays in HEK293 cells co-expressing human recombinant α_{1B}-ARs and a dominant negative mutant (DN) of β-arrestin1 (319-418). **Results and Discussion**: Although presenting different efficacies, all phenylethylamines and imidazolines increased the intracellular calcium concentrations in HEK293 cells expressing α_{1A}-ARs, but only the phenylethylamines were able to increase intracellular calcium in cells expressing α_{1B}-ARs. All ligands induced internalization of α_{1A}-ARs and surprisingly, the imidazolines were able to internalize α_{1B}-ARs despite being unable to increase intracellular calcium in cells expressing this receptor subtype. The bias factors β and $\Delta\Delta\log \tau/K_A$ revealed that all imidazolines are biased agonists in α_{1B}-ARs towards the β-arrestin pathway. On the other hand, the bias factors β and $\Delta\Delta\log \tau/K_A$ for phenylephrine and A-61603 in α_{1A}-ARs revealed that these ligands are biased agonists for the G protein pathway, whereas dopamine is biased for the β-arrestin pathway. The phosphorylation of ERK 1/2 induced by noradrenaline and oxymetazoline in HEK293 cells expressing α_{1B}-ARs was biphasic and composed by an early phosphorylation dependent on G protein activation and a late phase due to β-arrestin recruitment. In HEK293 co-expressing α_{1B}-ARs and DN β-arrestin (319-418), the late peak of phosphorylation of ERK 1/2 disappeared, confirming the recruitment of β-arrestin; in agreement, the internalization of α_{1B}-ARs in these cells was also greatly inhibited. **Conclusion**: Both **Methods** for quantification of biased signaling discriminated similar profiles of biased agonism in α_{1A}- and α_{1B}-ARs. In addition, our findings show that ligands commonly used as subtype selective α_1-AR agonists are biased towards the G-protein or β-arrestin signaling pathway and this is important to determine the net pharmacological effects of these ligands. **Financial Support**: CAPES, FAPESP (08/50423-7 to ASP)

01.021 Androgen deprivation unravels plasticity of functional α_1-adrenoceptors mediating cauda epididymal contraction to noradrenaline. Kiguti LRA, Pacini ESA, Pupo AS Unesp-Botucatu – Farmacologia

Introduction*:* Cauda epididymal duct (CE) expresses equal proportions of α_{1A} and $_{1D}$-adrenoceptors (-AR) at mRNA level and binding site densities, although functional contraction studies shows only an α_{1A}-AR subtype mediated component in the contraction of CE in response to noradrenaline (NA). Due to the high dependence of epididymal structure and function on gonadal steroid hormones we sought to evaluate the contribution of androgen and estrogen in the regulation of the α_1-AR subtype mediating contractions of rat distal cauda epididymis (CE). **Methods***:* All experimental procedures were approved by the local Ethics committee for the use of experimental animals (Protocol nº 297/2011). Male Wistar rats (120days old) were castrated by bilateral orchidectomy and were treated with corn oil (CAST+VEH), 17-β-estradiol benzoate (CAST+E2; 20ug/kg/day) or dihydrotestosterone (CAST+DHT; 5mg/day) for 30 days. A group of non-castrated rats treated with vehicle for 30 days was taken as control group (CONTROL). At the end of the treatments the rats were killed by decapitation and segments (1.0-1.5cm length) of distal CE were isolated to record of *in vitro* contractions to NA or A61603 (an α_{1A}-AR selective agonist). The α_1-AR subtypes mediating contractions to NA or A61603 were identified by Schild analysis of the antagonism displayed by prazosin (α_1-selective), RS 100329 and WB 4101 (α_{1A}-selective) and BMY 7378 (α_{1D}-selective). Antagonist affinities (pK_B) or potencies (pA_2) were evaluated. **Results**: Contractions of CE to NA from all groups were competitively antagonized with high affinity by prazosin ($pK_B \approx 9.50$), RS 100329 ($pK_B \approx 9.50$) and WB 4101 ($pK_B \approx 9.50$) showing activation of α_{1A}-ARs by the NA. The α_{1D}-AR selective antagonist BMY 7378 displayed a low affinity competitive antagonism against functional receptors from CONTROL and CAST+DHT rats (pK_B=7.02±0.05 and 6.98±0.07, respectively). In contrast, BMY 7378 showed a complex antagonism of NA-induced contractions in CE from CAST+VEH and CAST+E2 rats (Schild slopes=0.67 and 0.52, respectively). The complex antagonism of NA-induced contractions by BMY 7378 was caused by a significant reduction in the agonist potency at 10 and 30nM BMY 7378. These low concentrations of BMY 7378 were ineffective against contractions of CE from CAST+VEH and CAST+E2 rats to A61603. pA_2 values calculated with BMY 7378 10nM against NA-induced contractions in CE from CAST+VEH and CAST+E2 resulted pA_2 values of 8.44±0.07 and 8.51±0.06, respectively. These high potencies of BMY 7378 are similar to the pK_B of BMY 7378 at α_{1D}-ARs of rat aorta (pK_B=8.00±0.03). **Discussion**: The rat CE expresses a heterogeneous population of α_1-ARs composed by similar densities of α_{1A}- and α_{1D}-ARs. In the course of our research effort towards pharmacological characterization of functional α_1-ARs in the rat CE only the α_{1A}-ARs were shown to mediate contractions to NA whereas the roles of CE α_{1D}-ARs were unknown. After castration a α_{1D}-AR-mediated contraction became evident as a high-potency component of BMY 7378 antagonism against the non-subtype selective agonist NA. Therefore, our results shows plasticity of the α_1-AR subtype mediating contraction of CE and characterizes the androgen, but not estrogen, as an important factor mediating the functional balance between CE α_{1A}/ α_{1D}-ARs. **Financial Support**: CAPES and FAPESP (08/50423-7 to ASP).

01.022 Importance of the Arginine1 residue of bradykinin in the activation of the kinin B2 receptor in mouse stomach fundus. Silva RF, Rodrigues ES, Martin RP, Oliveira L, Shimuta SI Unifesp – Biofísica

Introduction: Kinins are important mediators of cardiovascular homeostasis, nociception and inflammation and cause contraction and relaxation of several vascular and nonvascular smooth muscles. The major kinin effects are ascribed to bradykinin (BK, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), which performs its actions activating the B_2 receptor, a GPCR. Due to the physiological importance of BK, our aim was to study the biological activity of BK analogs to verify the role of Arg1 in the binding of BK into B_2 receptor. **Methods**: To analyze the importance of the N-terminal Arg1 of BK, the biological activities of Ala1BK, Gly1BK, Glu1BK and Lys1BK were evaluated. Pharmacological studies were carried out in the stomach fundus isolated from C56/BL7 mice, in agreement of Ethics and Research Committee of UNIFESP, under 2009/00841 protocol. For the isometric contraction recordings, the Compact Organ Bath, Thermostat LE 13206, Force Transducers TRI201, Coupler Module Amplifier ISO5010 (Panlab, CA, ESP), Powerlab Analogical Digital Conversor 4/30 and Labchart7 Data Acquiring Software (ADInstruments, NSW, AUS) were used. Cumulative dose response curves (10^{-10}M-10^{-6}M) for all peptides were obtained, and normalized with 1μM carbachol induced response. From these curves, the efficacy (maximum response, Emax) and potency (pD2, -log of the concentration that induces 50% of the Emax) were determined using the GraphPad Prism 3.02 (GraphPad, CA, USA). Tissues were calibrated with 0.5g of tension and 80mM KCl were applied at 45min intervals to follow the tissue stabilization. Results: BK induced contractile responses were: Emax: 66±3% and pD2: 7.9±0.3. Reduced responses were observed to the analogs Ala1BK, Gly1BK, Glu1BK and Lys1BK. Stimulations of tissue with the concentrations from 10^{-10}M-10^{-7}M caused no significantly effect, but an increasing effect was observed at 10^{-6}M. This response, which was not considered the maximal effect induced by each analog, was: Gly1BK, 25±3; Ala1BK, 23±3; Glu1BK, 12±3; Lys1BK, 35±3. Therefore the values of Emax and pD2 could not be determined for the analogs. **Discussion**: The substitution of Arg1 residue, a positive aminoacid (aa), for Ala1, a small residue without charge, caused a drastic reduction in the response, similarly to that observed with another small and nonpolar residue, Gly1. It is in agreement with the previous observation by Regoli (1980) that analyzed Ala1BK in several tissues obtaining pD2 values about 5.5. This finding could be ascribed to the lack of a charge or to the small size of the substitute aa. The replacement of a positive to a negative charge of the first aa of BK (Glu1), almost abolished the contractile response, where only at 10^{-6}M a very low effect was observed. This finding showed a requirement of the positive charge for B_2 receptor activation. However, the replacement of Arg1 for Lys1, another positive aa, caused a significant reduction in the responses, indicating that the protonated N-terminal amino group, the guanidinium group of Arg1 side chain, is essential for the interaction of BK to B_2 receptor. The marked low contraction, where increasing responses were observed from 10^{-6}M Lys1BK, as occurred for the other analogs, suggests that Arg1 exerts an important role for the binding affinity of BK and activation of B_2 receptor. **Supported by** FAPESP and CNPq.

01.023 Molecular dynamics of angiotensin AT1 receptor: **The effect of site-directed C18S mutation**. Martin RP, Rodrigues ES, Silva RF, Oliveira L, Shimuta SI
Unifesp – Biofísica

Introduction: Angiotensin II is the main agent of the renin-angiotensin system to regulate blood pressure and hydro-electrolytic homeostasis. Its physiological actions are mainly mediated by the AT1 receptor which belongs to the G protein coupled receptors family (GPCR), bearing seven alpha helices connected by 3 extracellular and 3 intracellular loops and an extracellular N-terminal and an intracellular C-terminal segments. To predict the structures of these receptor, the solved CXCR4 chemokine receptor structure could be used as a template. Since our previous results showed that the lack of the second SS bound changed the binding properties and caused the constitutive activation it was our interest to evaluate the effect of this mutation on the AT1 receptor using the molecular dynamics with the generated AT1 receptor modeled on the CXCR4 structure. **Methods**: The AT1 model was generated by the Nest routine of Jackal package based in the alignment of GPCRs using the CXCR4 model as the template. After that, the agonist peptide was inserted into the AT1 receptor with the YASARA program, the model was evaluated by PROCHECK from PDBsum server. Then the models were submitted to the molecular dynamics in the YASARA with initial steps of energy minimization and the dynamics until 5ns long. After each dynamics the models were re-submitted to the PROCHECK evaluation. The mutated receptor was generated using the AT1 receptor as template just like described before. Results. The obtained data showed that the side chains distribution in the Ramachandram graph-plot the AT1 receptor before the dynamics was: about 83.4% in the most favored regions, 12.4% in the additional allowed regions, 2.5% in the generously allowed regions and 1.8% in the disallowed regions. After the dynamics reaction the distribution of side-chain in the most favored region was increased to 87.6% and the disallowed region decreases to 0.4%. To evaluate the effect of mutation, both wild and mutant receptors were superposed in a single scene and aligned with the Align tool from YASARA where it was possible to observe that the N-terminal segment, which contains several important residues to binding, was totally displaced keeping the N-terminal far from the EC3 loop. **Discussion and Conclusion**s: Since the displacement of the N-terminal segment carries several binding residues to opposite side of the receptor, the changes in the binding properties, which were observed in our previous results, could be ascribed to these conformation changes. Beside this, the use of chemokine CXCR4 receptor is a good template to predict the structure of GPCRs containing structural elements that are specific to a few receptors as the angiotensin AT1 and AT2 receptors. As well as in other GPCRs as the kinin B1 and B2 receptors and the endothelin ETA and ETB receptors, since the single mutation of the receptor in molecular dynamics presents similar result to the already reported results. The molecular dynamics is an important step to a better accommodation of side-chains and important to evaluate the structural changes in different molecules. This study will be useful to better understand the structural changes of receptor due to its activation and to the development of antagonists as treatments of hypertension and cardiovascular diseases. Supported by CAPES, CNPq and FAPESP.

01.024 Evidence for the interaction between the ASP301 B1 receptor´s residue and the ARG1 DES-ARG9-bradykinin peptide. Rodrigues ES, Martin RP, Silva RF, Oliveira L, Shimuta SI Unifesp – Biofísica

Introduction: The Kinin B1 receptor (B1R) belongs to the family of the G protein coupled receptors GPCR. It has seven transmembrane domains and is involved in the inflammatory and nociception processes in response to its agonist des-Arg9-bradykinin (DBK, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe). Recent studies on the structure-activity of the B1R revealed that important residues for its binding to DBK are homologous to those in the interaction between angiotensin II (AngII) and its type I receptor (AT1R). It has been reported that both, AT1R and B1R, present a conserved Asp located on the top of the helix III. Previous study has described that in the AT1R the negative charge of Asp281 should interact with the positive charge of the N-terminal Arg side chain of AngII. Therefore we decide to study the B1R bearing the mutant Asp301Ala (D301A) which removes the negative site from the top of helix III in the B1R in order to verify the hypothesis that similar interactions may occur between B1R and DBK and between AT1R and AngII. **Methods**: The D301A mutant receptor was produced according to a commercially mutagenesis kit which was expressed in the CHO cells. Similarly the wild type B1R was transfected into the CHO cells. The both cell lines were submitted to the binding competition test using the wild agonist DBK and its two analogues previously obtained. Both analogues resulted from the substitution of the Arg residue, bearing a positive charge at position 1 of DBK to Ala1, analogue lacking the positive charge and Lys1, another amino acid residue which is protonated at N-terminal side of DBK. In addition to the binding assays, the IP3 production and intracellular calcium levels were measured. Results. It was found that the absence of the negative charge in the top of the helix III affected the DBK binding affinity since the IC50 affinity was 3.1 ± 1.6 nM (3) to the wild receptor and 370.5 ± 1.1 nM (3) to the mutant D301A. The binding affinity of the Ala1DBK to the receptor was 152 ± 0.6 nM (3) to wild type and 171.9 ± 1.1 nM (3) to the mutant. Concerning IC50 value for Lys1DBK was 3.2 ± 1.4 nM (3) to wild type receptor and 387.6 ± 1.5 nM (3) to the mutant. The functional expression of the mutant D301A was assessed by the IP3 production: 5.5±1.1 nM (3) for the wild type and 362.3±2.2 nM (3) for the mutant, whereas the intracellular calcium concentration measurements were: 4.3 ± 1.8 nM (3) to the wild receptor and 191.5 ± 1.4 nM (3) to the mutant. **Discussion** and **Conclusion**s: The reduction in the binding affinity of DBK to D301A indicated that the negative charge of Asp301 residue located in the top of the helix III of the B1R is essential for its interaction with DBK. The finding that the binding affinity of Lys1DBK to wild type B1R was similar to that obtained for DBK whereas the affinity of Lys1DBK to the mutant D301A was drastically reduced indicated that the positive charge of the N-terminal residue plays an important role for the interaction between DBK to its receptor. Our findings provide perspective in the development of new antagonists as effective anti-inflammatory agents acting through the B1R. Supported by FAPESP and CNPq.

01.025 Pulmonary fibroblast spheroids from silica-stimulated mice: **Establishment of a 3D cell culture system**. Guimarães-Silva A M[1], Trentin PG[1], Dalzy DV[1], Barbosa HS[2], Martins MA[1], Silva PMR[1] [1]Fiocruz – Inflammation, [2]Fiocruz – Structural Biology

Introduction: Silicosis is a restrictive occupational disease characterized by chronic inflammation associated with intense fibroblast proliferation and accumulation of extracellular matrix components. Fibroblasts are the main targets of this disease and the development of systems that the search for anti-fibrotic therapies is quite relevant. It is well known that cells grown in a 3D cell culture more closely mimic natural tissues and organs than cells grown in 2D. Thus, this study was undertaken to establish a 3D cell culture using lung fibroblasts from silica-stimulated mice. **Methods**: Adult Swiss-Webster mice (20 - 25g) were intranasally instilled with silica particles (10 mg) and 7 days later, lungs were removed and enzymatically dissociated with collagenase A. Saline-instilled mice were used as controls. 3D fibroblast culture was used to evaluate morphological and functional parameters. For morphology (length/diameter/constitution) inverted light and scanning electron microscopy was used. For cell function, proliferation (^{3}H-timidine incorporation) as well as activation (quantification of MCP-1 and TGF-β by ELISA) were analyzed. In some set of experiments, the spheroids were stimulated with rmIL13 (40 ng/mL). All experimental procedures were approved by Ethics Committee of Animal Use of FIOCRUZ (License 034/09). **Results**: By means of inverted light microscopy, we showed that spheroids with fibroblasts from normal and silicotic mice exhibited a progressive reduction in size/diameter and increased density, over a 4 day analysis. Spheroids from silicotic animals were always bigger than those from the normal group. The scanning electron microscopy revealed important morphological differences regarding shape and constitution. Normal and silicotic spheroid populations showed round shape/smooth surface and helicoidal shape/rough surface, respectively. Stimulation with IL-13 increased the extracellular matrix content in both experimental groups. Concerning functional parameters, spheroids from silicotic mice exhibited basal levels of cell proliferation and TGF-β release higher than those from the normal group. IL-13 clearly increased length/diameter as well as proliferative response and TGF-β secretion in normal and silicotic spheroid populations. **Conclusion**: Altogether, our findings show that lung fibroblasts from adult mice can be grown in 3D culture system and that spheroids recovered from silicotic mice exhibited clear features of activation as compared to the normal ones. This model proved to be reproducible and promising concerning the possibility of future use in the search for compounds with anti-fibrotic activity. **Financial Support**: FIOCRUZ, CNPq, FAPERJ and CAPES.

01.027 Pharmacologic evaluation of new alpha-1 adrenoceptor and 5-HT1A antagonists. Nascimento Viana JB[1], Carvalho AR[1], Romeiro LAS[2], Nascente LC[3], Lemes LFN[3], Nöel FG[1], Silva CLM[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]LADETER-UnB, [3]LADETER-UCB

Introduction: Alpha1-adrenoceptor (AR) antagonists are commonly used in the treatment of hypertension and benign prostate hyperplasia (BPH). Recently, 5-HT_{1A} receptors antagonists have also been considered for the treatment of this condition since they have a prostatic anti-proliferative effect. Previously, a new N-phenylpiperazine was synthesized and the pharmacological evaluation unveiled a potent alpha1-adrenoceptor (AR) blockage, being selective for alpha-1A and alpha-1D AR (Romeiro et al., *Eur. J. Med. Chem*. 2011). New N-phenylpiperazine analogues (LDT series) with substitutions at the main pharmacophoric subunit showed high affinity for $alpha_{1A}$ and $alpha_{1D}$-AR subtypes and also for 5-HT_{1A}. The objective of this study was to assess the affinity for $alpha_2$-AR, D2-like and muscarinic receptors. Furthermore the toxicity *in vitro* and *in vivo* was evaluated for these compounds. **Methods**: All protocols are approved by the Ethics Committee of UFRJ (CAUAP; DFBC - ICB011). Animals were anesthetized and killed. Binding assays: 150 ug of membrane proteins enriched in $alpha_{2A}$-AR and muscarinic receptors (rat cortex), D2-like receptors (rat striatum) were incubated with 1 nM [3H]-RX821002, 0.1 nM [3H]-QNB or 0.1 nM [3H] YM-09151-2, respectively, for 60 min at 37oC in the absence and presence of LTD 3, 5 and 8 (1 - 30,000 nM). The reaction was stopped with the addition of cold Tris-HCl buffer (pH 7.4), followed by filtration under vacuum. The radioactivity was quantified in a liquid scintillation counter. The results were analyzed by non-linear regression to calculate the parameters IC50. The KB value was calculated using Schild equation. Cytotoxicity assays: DU-145 cell cultures were established and treated with 1-30 µM of LDT 3, 5 and 8 in order to evaluate its effects on cytotoxicity and viability by MTT assay 24 and 48h after treatment. Acute toxicity test: LDT 3, 5 and 8 (10 µg/Kg i.p.) were administered in a single dose to mice (25-30 g) in order to perform the Hippocratic Screening for 14 days. **Results and Discussion**: All LDTs showed low affinity (*K*i in the µM range) for $alpha_{2A}$-AR and muscarinic receptors. The *K*B values of LDTs 3, 5 and 8 at D2-like receptors were respectively: 53.7, 23.6 and 4.1 nM. Previous data of our group showed that LDT3 has high affinity for alpha1A/D-AR and 5-HT1A receptors acting as a multi-target antagonist. In addition, this compound showed low affinity for the receptors analyzed (present data). In the cytoxicity or acute toxicity assays none of the compounds proved to be toxic. In **Conclusion**, LDT3 is a potential new drug candidate for the treatment of BPH.

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01.028 Dual regulation of the glucocorticoid system by histamine H1 receptor signaling. Role of canonical and non-canonical pathways. Zappia D[1], Granja-Galeano G[1], Fernandez N[1], Fitzsimons C[2], Monczor F[1] [1]UBA – Pharmacy and Biochemistry, [2]University of Amsterdam – Life Sciences

Introduction: Previous studies have indicated a possible interaction between G-protein coupled receptors (GPCRs) signaling and glucocorticoid receptor (GR) activation. The histamine H1 receptor (H1R, a GPCR coupled to Gαq/11) and the GR are within the most common pharmacological targets. Furthermore, under many circumstances, ligands of these two receptors are coadministered as antiallergic in current human prescriptions. In this context, the present work aims to study the possible modulation of GR activity by ligands of the histamine H1 receptor. **Methods and Results**: we cotransfected HEK293 cells with plasmids expressing the H1R, the GR, and an artificial glucocorticoid reporter gene containing 3 repetitions of the GRE present in the TAT promoter (TAT3-Luc). In this cell system, histamine significantly increased dexamethasone-induced transcriptional response in a dose-dependent manner by a 300% without affecting dexamethasone pEC50. To investigate whether Gβγ subunits could be involved in the effects induced by H1R activation, we overexpressed Gα tranducin as a Gβγ scavenger. Intriguingly, under this conditions histamine potentiation of dexamethasone response was first abolished and then switched to inhibition. This result suggests that histamine exerts a dual effect on GR activity, a potentiating effect dependent on Gβγ, and an inhibitory effect that becomes apparent when Gβγ subunits are sequestered. Cotransfection of cells with several combinations of Gβ and Gγ subunits resulted in the identification of Gβ2γ5 and Gβ2γ11 as responsible for the boosting effect of histamine on GR-dependent transcriptional activity. On the other hand, using specific pharmacological blockers and a dominant negative mutant of the small G protein Rac (RacN17), we show that the inhibitory effect induced by histamine is mediated by PLC but not PKC, and Rac. **Conclusion**s and relevance: we identified a novel molecular mechanism of interaction between the H1R and the GR. H1R and GR ligands are among the most widely prescribed and over the counter-sold drugs in the world. Because of their widespread use to treat non-life-threatening conditions, there is a trend to use them as long-term therapy for atopic diseases, allergic asthma, and allergic rhinitis. Considering that these ligands are coadministered and sometimes coexist in pharmaceutical formulations (e.g. dexamethasone+loratadine), the assessment of potential interactions is of vital importance to prevent serious side effects. This work was financially supported by grants of ANPCyT (PICT 2010-1672) and University of Buenos Aires (20020090200141). DZ is a CONICET doctoral fellow and GGG is a CIN undergraduate fellow.

01.029 Participation of cytosolic glucocorticoid receptor and Annexin-A1 on neutrophil traffic from bone marrow into blood: **Adhesion molecule expression and SDF-1alpha/CXCR4 axis**. Machado ID[1], Santin JR[1], Ferraz-de-Paula V[1], Perretti M[2], Farsky SHP[1] [1]USP – Pharmaceutics Science, [2]William Harvey Institute – Immunopharmacology

Introduction: The traffic of neutrophils is a complex process, dependent on coordinated interaction of chemical substances and their receptors, besides perfect cell interactions. We have shown that endogenous glucocorticoids (EG) and Annexin-A1 (ANXA1), a protein induced by glucocorticoid actions, modulate the neutrophil traffic from the bone marrow, but the mechanisms involved are not fully elucidated. Therefore, this study investigated the role of the glucocorticoid cytosolic receptor (GCR) and ANXA1 participation on neutrophil mobilization from bone marrow (BM) into blood (PB), focusing SDF-1α/CXCR-4/CXCR-2 axis and adhesion molecules (CD18, CD49d and CD62L) expressions. **Methods**: ANXA1-null (ANXA1$^{-/-}$) and Balb/C wild-type male were employed. Balb/C mice were treated with vehicle or with the antagonist of GCR (RU38486; RU; 10 mg/Kg, i.p., 28 e 6 h). PB and BM perfusate were collected to quantify the numbers of leukocytes, expressions of CXCR-2, CXCR-4, CD62L, CD49d and CD18 (flow cytometry) and SDF-1α levels (ELISA). Phagocytosis of senescent neutrophils by BM macrophages obtained from all groups of animals was *in vitro* assessed by optical microscopy. Results: RU treatment and ANXA1 deficiency accelerated the granulocyte maturation in BM and caused neutrophilia in PB, but only ANXA1$^{-/-}$ animals showed increased number of mature granulocytes in BM ($p<0.05$). Both GCR blockade decreased the number of granulocytes CD62L^{+} ($p<0.01$) in BM, but GCR blockade ($p<0.01$) and ANXA1 deficiency ($p<0.05$) increased the number of neutrophils CD62L^{+} in PB and the expression of molecule CD62L *per* neutrophils. No alterations on CD49d and CD18 were detected in all groups of animals. GCR blockade increased and reduced the number of granulocytes CXCR-4^{+} ($p<0.001$) and CXCR-2^{+} ($p<0.05$) in BM, respectively, and deficiency of ANXA1 enhanced both number of cells CXCR4^{+} ($p<0.05$) and number of CXCR-2^{+} ($p<0.05$) and expression of this latter receptor *per* cell. In the PB, GCR blockade enhanced the number of CXCR4^{+} neutrophils ($p<0.01$) and reduced the number of neutrophils CXCR-2^{+} ($p<0.05$) and its expression *per* cell. Differently, ANXA1$^{-/-}$ animals only presented reduced number of neutrophils CXCR2^{+} ($p<0.01$) and expression *per* cell in the circulation. SDF-1-a levels in the bone marrow was only reduced in ANXA1$^{-/-}$ animals ($p<0.05$). BM macrophages collected from RU treated (20.3 ± 1.4 %) or ANXA1$^{-/-}$ (32.5 ± 2.5 %) phagocyted less number of senescent neutrophils than macrophages collected from animals treated with vehicle (48.8 ± 3.2 %). **Conclusion**: Data here obtained show that EG modulation on neutrophil traffic from BM into PB is dependent on its interaction to GCR and ANXA1 secretion. Nevertheless, the mechanisms of control on SDF-1α/CXCR-4/CXCR-2 axis is different, suggesting that other pathways besides ANXA1 is involved in the EG modulation of neutrophil traffic. Sources of research support: FAPESP (2010/08402-2; 2010/16828-0).

01.030 Protein expression during snake venom gland activation. Luna MS[1], Valente RH[2], Perales J[2], Yamanouye N[1] [1]IBu – Farmacologia, [2]IOC-Fiocruz – Toxinologia

In previous studies, we have shown that the protein composition of venom gland changes during the venom production cycle. Noradrenaline, released just after venom removal, regulates the activation of transcription factors and consequently regulates the synthesis of proteins of the gland which is important to activate this gland for venom production. The aim of this study is to analyze venom gland proteins during venom production cycle and proteins whose expression is regulated by alfa and beta adrenoceptors stimulation. Venom glands were obtained from female *Bothrops jararaca* snake in quiescent stage and in activated stages (4 and 7 days after milking). Some snakes were treated with reserpine (20mg/kg, sc, 24h before milking, followed by daily injections of 5mg/kg, during 4 days) and some reserpine-treated snakes received phenylephrine and isoprenaline (100mg/kg, sc) just after milking (N=3 for each group). Proteins of venom glands were analyzed by two-dimensional gel electrophoresis (2-DE). Spot density was quantified using ImageMaster 2D Platinum 7. Highest abundant specific spots from each group were digested with trypsin and their protein content identified by MALDI-TOF/TOF and/or nESI-LTQ XL/Orbitrap mass spectrometry. The expression of some proteins was further confirmed by Western blot (WB). Analysis of the 2-DE of venom gland extracts revealed that different proteins were expressed. The overall analysis showed that proteins expression of the cytoplasm, cytoskeleton, endoplasmic reticulum, membrane and nucleus were up-regulated in activated stage. After treatment with reserpine the expression of actin, a cytoskeleton protein, was down-regulated. Administration of alfa and beta-adrenoceptors agonists restored the expression of actin, suggesting that stimulation of these receptors up regulates the expression of this protein. Although the expression of total secreted proteins did not change during venom production cycle, analyzing only the specific spots, we detected more glycoprotein IB-binding protein (GPIb-BP) in quiescent venom gland than in activated venom gland and more metalloproteinases (SVMPs) and phospholipases A2 in activated venom gland than in quiescent gland. After treatment with reserpine, detection of GPIb-BP and SVMPs was similar to the quiescent gland and after administration of alfa and beta-adrenoceptors agonists the detection of these toxins is similar to the activated gland. Since noradrenaline is released only after venom extraction, our data suggest that the expression of GPIb-BP is down-regulated and SVMPs is up-regulated by sympathetic outflow. Expression of actin and SVMPs was confirmed by WB. In **Conclusion**, our data showed a great variation of proteins expression during venom production cycle. Besides, sympathetic outflow regulates the expression of actin and some toxins. We also showed for the first time that the synthesis of toxins occurs not only in activated stage, but also in quiescent stage. The identification of these proteins will give us new insides to understand the mechanism of venom gland activation and venom production. **Supported by**: FAPESP, CNPq, FAPERJ and IOC-FIOCRUZ. Animal Ethics Committee of the Instituto Butantan 555/2008 and IBAMA 01/2009

01.031 Implication of purinergic P2X7 receptor in the immune profile of *Mycobacterium tuberculosis*-infected mice. Santos Jr AA[2,1], Rodrigues-Junior VS[1], Zanin RF[3], Borges TJ[3], Bonorino C[3], Coutinho-Silva R[4], Santos DS[1], Campos MM[5,6], Morrone FB[2,6,7] [1]INCT-TB-PUCRS, [2]PUCRS – Biologia Celular e Molecular, [3]IPB-PUCRS, [4]ICCBF-UFRJ, [5]PUCRS – Medicina e Ciências da Saúde, [6]PUCRS – Toxicologia e Farmacologia, [7]PUCRS –Farmácia

Introduction: It was previously demonstrated that treatment of Bacillus Calmette-Guérin (BCG)-infected human macrophages with ATP induced P2X7 receptor-mediated killing of intracellular mycobacteria (Biswas, BMC Immunol. 9:35, 2008). In the present study, we have investigated the immune profile of P2X7 receptor (P2X7R) knockout mice infected with the laboratorial *M. tuberculosis* H37Rv strain, by means of an extensive flow cytometry analysis. **Methods**: Male P2X7R knockout (P2X7R$^{-/-}$) and C57BL/6 wild-type (WT) mice (8 per group, 25-30 g) were used. All the experimental protocols were approved by the Local Animal Ethics Committee (CEUA 10/00203-PUCRS). The infection model was accomplished according to the methodology described before (Chambers, Antimicrob. Agents Chemother. 49:2816, 2005). The animals were anesthetized and received an intravenous injection of 200 μl of a *M. tuberculosis* suspension (H37Rv strain; 5 x 10^8 CFU/ml). Control mice received the same volume of saline. After 28 days of infection, the animals were euthanized. Following euthanasia, the spleens and lungs were excised and disrupted against a nylon screen in media containing Collagenase D. We investigated the following population subsets of immune cells: CD4+, CD8+, CD4+Foxp3+, CD4+CD25+Foxp3, CD4+CD25+, CD8+CD25+, CD11b+, CD11c+ and B220+. Cells were analyzed in a flow cytometer. Data was corrected using the correspondent (non-infected) control group and expressed in fold variations between P2X7R$^{-/-}$ and WT mice. Results: In spleens obtained from infected P2X7R$^{-/-}$ mice, there was a significant decrease in the populations of Treg cells (CD4$^+$CD25$^+$Foxp3$^+$), which corresponded to 0.54-fold (P<0.05) when compared to WT *M. tuberculosis*-infected mice. T cells (CD4$^+$; CD8$^+$CD25$^+$; and CD4$^+$CD25$^+$) from infected P2X7R$^{-/-}$ mice decreased by 0.36-fold, 0.63-fold and 0.93-fold, respectively (P<0.05). For dendritic cells (CD11c$^+$), there was a decrease of 0.69-fold in P2X7R$^{-/-}$ mice, when compared to WT mice (P<0.001), whilst a reduction of 0.59-fold was seen in B220$^+$ cells (P<0.001). However, a significant increase in CD11b$^+$ cells (0.36-fold) (P<0.01) was observed in P2X7R$^{-/-}$ mice when compared to WT mice. Concerning the lungs, P2X7R$^{-/-}$ *M. tuberculosis*-infected mice exhibited pulmonary infiltrates containing an increased number of Treg cells CD4$^+$Foxp3$^+$ (4.56-fold) (P<0.01). T cells (CD4$^+$ and CD8$^+$) increased by 7.52-fold and 3.47-fold, respectively (P<0.01), whereas B220$^+$ cells decreased in 0.87-fold (P<0.05), when compared to WT *M. tuberculosis*-infected mice. **Discussion**: Our results demonstrate that P2X7R$^{-/-}$ *M. tuberculosis*-infected mice present a different profile of immune cells when compared to WT *M. tuberculosis*-infected mice, which may be responsible for an ineffective control of this infection. This data provides novel evidence on the relevance of P2X7R as target for the pathogenesis of tuberculosis. **Financial Support**: CNPq-INCT-TB, CAPES, BNDES and FINEP/PUCRSINFRA #01.11.0014-00.

01.032 Extracellular cyclic AMP-adenosine pathway regulates skeletal muscle proteolysis. Figueiredo LB[1], Godinho RO[1] [1]Unifesp – Pharmacology

Previous studies from our lab have shown that increment of cyclic AMP (cAMP) production induced by β_2-adrenoceptor (β_2-AR) agonists is followed by efflux of cyclic nucleotide and the extracellular generation of adenosine (Chiavegatti et al., *Br J Pharmacol*, 153:1331, 2008), which may result in autocrine activation of adenosine receptors (A_1, A_{2a}, A_{2b}, and A_3). Taking into account that β_2-AR agonists are able to reduce skeletal muscle proteolysis via activation of G_s protein/ adenylyl cyclases and increased generation of intracellular cAMP, in the present study, we evaluated a possible role of extracellular cAMP on skeletal muscle proteolysis. The effects of β_2-AR agonist clenbuterol, cAMP, adenosine, probenecid (inhibitor of organic anion transporter) and the non-selective adenosine receptor antagonist CGS-15943 on muscle proteolysis were evaluated by measuring tyrosine release from lumbrical muscle of adult male Wistar rats. All animal procedures were approved by the Institutional Research Ethics Committee (protocol 0034/12) at Escola Paulista de Medicina - Universidade Federal de São Paulo. Rates of tyrosine were expressed as nmol of tyrosine/ mg of wet mass/ h. Incubation of lumbrical muscles with clenbuterol (10-1000 nM) for 1 to 4h reduced by up to 33% the basal tyrosine release (0.16±0.01 nmol tyr/ mg/ h to control; n=3). The effect of β_2-AR agonists was mimicked by incubation of muscles with either 3-30 µM cAMP or 1-100 µM adenosine. Interestingly, probenecid alone (100 µM, n=4) reduced by 17% the basal muscle proteolysis. In order to investigate the possible contribution of extracellular cAMP-adenosine pathway on muscle proteolysis, the effect of adenosine receptor antagonist CGS-15943 was evaluated on muscle tyrosine release. While CGS-15943 alone increased by up to 20% the basal tyrosine release, it prevented the inhibitory effect of cAMP on muscle proteolysis. Considering that exogenous cAMP does not readily cross plasma membrane, but is converted to adenosine by sequential action of ecto-phosphodiesterases and ecto-5'-nucleotidases, our results demonstrate that extracellular cAMP reduces tyrosine release from muscle fibers via activation of adenosine receptors, indicating a physiological role of extracellular cAMP-adenosine pathway on the regulation of skeletal muscle proteolysis. **Financial Agencies**: CNPq Fellowship 143459/2009-0; CNPq Grant 302275/2011-8; Fapesp Grant 2011/01519-4.

01.033 ***In vitro* effects of the PhTx3-3 toxin obtained from the Brazilian spider *Phoneutria nigriventer* on glioma cells**. Nicoletti NF[1], Erig TC[2], Gomes MV[3], Souza AH[3], Campos MM[4], Morrone FB[1,2,5] [1]PUCRS – Biologia Celular e Molecular, [2]PUCRS – Farmácia, [3]UFMG – Medicina Molecular, [4]FO-PUCRS – Toxicologia e Farmacologia, [5]PUCRS – Toxicologia e Farmacologia

Aim: Gliomas are highly invasive brain tumors. Recent studies have suggested a possible implication of voltage-gated calcium channels (VGCC) in the mechanisms of proliferation, angiogenesis and invasion of glioma tumor cells (Wen, PY et al; N Engl J Med 359: 492, 2008; Pinheiro, AC et al; Hippocampus 19:1123, 2009). This study was designed to evaluate the effects of pharmacological inhibition of P/Q-type VGCC on glioma cell proliferation, by using animal-derived toxins. **Methods**: Human glioma U138MG and M059J cell lines obtained from ATCC were cultured in DMEM supplemented with 10% FBS. Cells were seeded in 24 or 96-well plates at densities of 5×103 to 2x103 cells/well. The cells were treated for 24 h with the selective P/Q-type VGCC blockers PhTx3-3 (0.3 pM to 300 pM; obtained from *Phoneutria nigriventer*) or ω-conotoxin MVIIC (0.3 pM to 300 pM; from Conus magnus). After 24 h, the cells were counted in a hemocytometer. Moreover, the cell viability was assessed by MTT assay at the same time-point. The experiments were carried out three times, in triplicate. Data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey test. **Results**: Our results show that treatment with PhTx3-3 toxin (1 pM to 100 pM) significantly diminished the proliferation of either M059J or U138MG cell lines, in a concentration-dependent manner. The maximal inhibitory effects were 49.8 ± 9% and 52.3 ± 1%, respectively at 1 pM and 3 pM. Furthermore, PhTx3-3 also displayed a significant inhibitory effect on viability of both cell lines, with maximal inhibitory percentages of 32.6 ± 1% and 15.1 ± 3% at 10 pM and 3 pM, respectively. Otherwise, the treatment with ω-conotoxin MVIIC failed to significantly affect the proliferation rates of M059J and U138MG cells, in either tested concentration. **Discussion**: Our results show that treatment PhTx3-3 toxin obtained from the venom of the Brazilian spider P. nigriventer was able to decrease the proliferation and the viability of human glioma cell lines, according to assessment at pM concentrations in vitro. Our data indicate a potential protective role for PhTx3-3 toxin on growth and invasiveness gliomas and point out P/Q-type VDCC as therapeutic targets for this tumor type. Further studies are being carried out to assess the *in vivo* effects of this toxin on glioma progression. **Financial Support**: PRPPG/PUCRS, CAPES-AUX-PE Toxinologia, CNPq and FINEP/PUCRSINFRA #01.11.0014-00.

01.034 The cardiotonic steroid bufalin induces endocytosis and promotes change in LLC-PK1 cells morphology. Martins-Ferreira J, Cunha-Filho GA, Quintas LEM, Nöel FG ICB-UFRJ

Introduction: Na^+/K^+ pump performs a pivotal role in epithelia by driving transepithelial Na^+-dependent transport of various solutes and water. The driving force of Na^+/K^+-ATPase depends on tight junctions to maintain polar distribution of basolateral and apical distribution of transporters, and those in turn depend on adherent junctions to stabilize intercellular contacts and trigger signaling pathways. Na^+/K^+-ATPase is inhibited by cardiotonic steroids (cardenolides and bufadienolides), and these inhibitors promote pump endocytosis. Bufalin promotes endocytosis in NT-2 cells and marinobufagenin promotes an epithelial-to-mesenchymal transition in LLC-PK1 cells. Taking together these information we aim to describe bufalin´s effect in LLC-PK1 cells endocytosis as well as in cell morphology in long term treatment and how endocytosis and morphological changes can be related. **Methods**: LLC-PK1 cells (porcine proximal renal tubule) were treated 24 hours with 1, 5 and 10 nM bufalin (BFL) or 5 and 10 mM ouabain (OUA), and photographed in bright field in order to visualize vesicles in the cytoplasm. Cells were randomically selected and intracellular vesicles counted in order to measure BFL and OUA effect in endocytosis. Phosphorilation of caveolin (CAV) induces it's endocytosis, we then treated LLC-PK1 cells with 10, 20 and 50 nM bufalin (BFL) or 10 and 50 mM ouabain (OUA) for 48 hours, fixed with 4% paraformaldehyde, permeabilized with 0.2% triton X-100 and immunostained cells with antibody against phospho-CAV, which was visualized using a green fluorescent Alexa Fluor® 488 anti rabbit IgG. In order to observe changes in cells morphology we treated LLC-PK1 cells with BFL and OUA with the concentrations cited above and photographed them at 24, 48 and 72 hours after treatment. Since we observed a retraction in cell membrane we thought that adherence proteins could be diminished at membrane and to test this possibility we split control and treated cells to new plates and accompanied the kinetics of cells adherence. We photographed cells 1, 2, 3, 12 and 24 hours after the seeding of the cells. **Results and Discussion**: In cells treated with BFL and OUA for 24 hours the number of intracellular vesicles is around 50 folds higher compared to control cells. Immunofluorescence for intracellular phosphoCAV is visually higher in BFL treated cells, it points to increase in endocytosis. LLC-PK1 cultures treated with BFL show an increasing number of morphologically different cells according to time of treatment compared to OUA treated and control cells. And when cells treated with BFL 20 or 50 nM were split to new plates we observed a different kinetics for adherence compared to OUA treated and control cells. We are performing cell surface biotinylation in order to detect decrease in Na^+/K^+-ATPase and adherent junction protein cadherin and will look for correlation with the late change in morphology of the cells. **Financial Support**: CAPEs; CNPq; FAPERJ.

01.035 ***In vitro* cytoxicity of benzoquinoline isolated from *Mitracarpus baturitensis* (Rubiaceae)**.

Costa MP[1], Bomfim IS[1], Cavalcanti BC[1], Rodrigues FAR[1], Albuquerque MRJR[2], Santos HS[2], Bandeira PN[2], Souza EB[2], Muniz FL[2], Moraes MO[1], Pessoa C[1] [1]UFC – Fisiologia e Farmacologia, [2]UVA – Química/Biologia

Introduction: Benzoquinoline benz[g]isoquinoline-5,10-dione (BsQ-d) is a benzoquinone isolated from the aerial parts of *Mitracarpus baturitensis* (Rubiaceae). Previous studies have shown that benzoquinoline and other benzoquinone's derivatives have bactericidal, antiviral and antiprotozoal activities. Benzoquinone analogues have also shown antiproliferative effects in cancer cells. The present study aims to describe the cyto- and genotoxicity of BsQ-d isolated from *M. baturitensis*, collected on the Northeast region of Brazil. **Methods**: The cytotoxic activity was measured on different cell lines (HL60, HCT-8, MDAMB-435, SF-295) through the MTT assay at 24 and 72h. Subsequent tests were done in HCT-8 cells incubated with BsQ-d (2.5, 5, 10 and 15 µg/ml) for 24h. Cell *viability* was analyzed using *trypan* blue and the proportion of necrotic/apoptotic cells was assessed by acridine orange/ethidium bromide (AO/EB) coloration. The genotoxicity of the compound was evaluated using the comet assay. **Results and Discussion**: BsQ-d demonstrated cytotoxic activity against all cancer cell lines at 24h with IC_{50} values of 2.37, 2.50, 0.53 and 3.11 µg/mL in HL60, HCT-8, MDAMB-435, SF-295, respectively. IC_{50} values at 72h were 0.34, 0.07, 0.25, 0.42 µg/mL in HL60, HCT-8, MDAMB-435, SF-295, respectively. Apoptosis, evaluated by the AO/EB assay, was observed after 24h of incubation on HCT-8. The compound *showed DNA damage, when* evaluated by the comet assay, in concentrations above 10 µg/ml after 24h. BsQ-d displayed considerable cytotoxic activity against cancer cell lines that seemed to be related to an effect on apoptosis induction. Further studies are already in development to elucidate the BsQ-d cytotoxicity mechanism against the tumor cells. Supported by: CNPq, CAPES, FUNCAP and PRONEX.

01.036 ***In vitro* effects of kinin receptors on glioma cell proliferation**. Erig TC[1], Nicoletti NF[2], Campos MM[3,4], Morrone FB[1,2,4] [1]PUCRS – Farmácia, [2]PUCRS – Biologia Celular e Molecular, [3]FO-PUCRS, [4]PUCRS – Toxicologia e Farmacologia

Introduction: Gliomas are among the most deadly and prevalent brain tumors (Wen, PY et al; N Engl J Med 359: 492, 2008). This study evaluated the relevance of B1 and B2 receptors (B1R and B2R) in the proliferation and viability of the human glioma U138MG and M059J cell lines. **Methods**: Glioma cell lines obtained from ATCC were cultured in DMEM supplemented with 10% FBS. Cells were seeded in 24 or 96-well plates at densities of 5×103 to 2x103 cells/well and treated for 24 h with B1R and B2R ligands, as described below. After 24 h, the cells were counted in a hemocytometer, to determine the proliferation rate. Furthermore, the cell viability was assessed by MTT assay at the same time-point. The experiments were carried out three times in triplicate. The cells were treated with the selective B1R des-Arg9-BK or B2R BK agonists (1 to 100 nM), or B1R SSR240612 or B2R HOE-140 antagonists (1 to 30 μM). Data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey test. **Results**: Our results show that treatment with the selective B1R agonist des-Arg9-BK did not alter the cell counts of the radiosensitive M059J cells. Otherwise, the incubation with the B2R selective agonist BK (at 10 nM) significantly increased the cell counts of the same cell line (192.5 ± 10%). Of note, the treatment with the either the selective B1R des-Arg9-BK or B2R BK agonists significantly increased the cell counts of the radioresistant U138MG cell lineage (10 nM and 30 nM or 3 nM to 30 nM, respectively), in 162.8 ± 10% and 171.6 ± 8% or 210.2 ± 5%, 201.6 ± 14% and 212.5 ± 6%, respectively. Furthermore, the cell viability was not altered when the human glioma cell line M059J was treated with the selective B1R antagonist SSR240612 at different concentrations, whereas the B2R antagonist HOE-140 (100 μM) markedly decreased the viability of this cell line (24.5 ± 3%). Interestingly, both HOE-140 (1 to 30 μM) and SSR240612 (30 μM) significantly diminished the cell viability of U138MG, with maximal inhibitions of 45.9 ± 7% and 69.7 ± 7%. **Discussion**: Our results suggest that both glioma cell lines display functional B2R, whereas B1R are likely relevant for the proliferation of the radioresistant cell line U138MG, but not for the radiosensitive M059J cells. Molecular studies are currently in progress to confirm this hypothesis. **Financial Support**: CAPES, CNPq, FAPERGS and FINEP/PUCRSINFRA #01.11.0014-00.

01.037 Contribution of the extracellular cyclic AMP- adenosine pathway to dual coupling of β2-adrenoceptors to Gs and Gi proteins in mouse skeletal muscle.

Duarte T, Menezes-Rodrigues FS, Godinho RO Unifesp – Pharmacology

The primary action of β_2-adrenoceptor (β_2-AR) agonists depends on activation of stimulatory G (Gs) protein and increased generation of cyclic AMP. However, in many tissues, β_2-AR-mediated responses are affected by promiscuous coupling of these receptors to other Gα subunits, especially the inhibitory Gαi subfamily (Daaka et al. Nature., 390:88, 1997). In the skeletal muscle, activation of β_2-AR induces a positive inotropic effect as result of Gs/adenylyl cyclase activation and increased generation of cAMP (Andrade-Lopes et al. *Respir Physiol Neurobiol*., 175:212, 2011), which is followed by the efflux of cAMP to the extracellular space (Godinho and Costa-Jr, *Br J Pharmacol*, 138:995, 2003) and extracellular generation of adenosine (Chiavegatti et al., *Br J Pharmacol*, 153:1331, 2008). In the present study we investigate the additional coupling of β_2-AR Gi proteins at skeletal muscle and the influence of the β_2-AR/Gs-Gi/cAMP signaling cascade on muscle contraction. The contribution of the extracellular cAMP-adenosine pathway on the β_2-AR inotropic response was also addressed. The effects of isoproterenol (3-1000 nM; non selective β-AR agonist), clenbuterol (10-1000 nM; β_2-AR agonist), forskolin (1 μM; adenylyl cyclase activator), cAMP (1-100 μM) and adenosine (1-100 μM) were evaluated on the isometric contractility of adult mouse diaphragm muscle induced by transmural electrical stimulation (0.1 Hz, 2 ms duration). Isoproterenol, clenbuterol and forskolin increased by 51%, 43% and 15% the basal contraction, 30 min after stimulation onset. The positive inotropic effect of β-AR agonists was followed by a descending phase that resembles the negative inotropic effects of cAMP (10 μM, n=4) or adenosine (10 μM, n = 3). Pre-incubation of diaphragm with pertussis toxin (PTX; Gi signaling inhibitor, n=3-4) or the organic anion transporter inhibitor probenecid (100 μM, n=3) abolished either the late descending phase of forskolin/β2-AR-induced inotropic effects or the negative inotropic effect of cAMP/ adenosine. Remarkably, the PTX-sensitive β_2-AR inotropic effect was inhibited by the A_1 adenosine receptor antagonist DPCPX (50 μM, n=3), indicating that β_2-AR coupling to Gi is indirect and dependent on the following sequential events: cAMP efflux, extracellular generation of adenosine and autocrine activation of A_1 adenosine receptor. In summary, our results show the involvement of extracellular cAMP-adenosine pathway in the regulation of the β_2-AR inotropic response at skeletal muscle. This extracellular arm of cAMP signaling cascade provides a negative feedback loop, which may limit Gs protein-coupled receptor response and possible harmful exacerbation of muscle contraction. Keywords: cyclic AMP, skeletal muscle contraction, β_2-adrenoceptors, adenosine. Ethical Committee: Unifesp 0022/12. **Financial Agencies**: CNPq Fellowship 556690/2010-9; CNPq Grant 302275/2011-8; Fapesp Grant 2011/01519-4.

01.038 Influence of age on the responsiveness of vas deferens from Wistar rats stimulated by adrenergic and purinergic agonists. Peña MG, Miranda-Ferreira R, Caricati-Neto A, Jurkiewicz NH, Jurkiewicz A Unifesp – Farmacologia

Introduction and Objectives: As one of the most densely innervated peripheral organs by the sympathetic nervous system, the vas deferens (VD) of a rat is the best model for studying the mechanisms involved in sympathetic neurotransmission. It has long been known that the contractile action of noradrenaline (NA) in VD is mediated by α-adrenoceptors, especially the α1-adrenoceptor (Jurkiewicz & Jurkiewicz, 1976; Westfall & Westfall, 2001) while the contractile action of ATP is mediated by different subtypes of P2-purinoceptores, in particular by the P2X-purinoceptores (Burnstock, 1988). Although it is clear the role of these neurotransmitters in the contractile response of the VD little is known about the influence of age on the responsiveness of the VD against these stimuli. In this sense, the objective was to study the contractile response of the Wistar-EPM rats of 8, 12, 16 and 20 weeks.

Materials and Methods: The vas deferens (VD) of NWR and SHR of 8,12,16 and 20 weeks were mounted in isolated organ bath with LNV nutrient solution and subjected to 1 g of tension for the testing of isometric contraction. Were performed concentration-response curves with the adrenergic agonist norepinephrine (NA 10^{-9}, $3x10^{-9}$, 10^{-8}, $3x10^{-8}$, 10^{-7}, $3x10^{-7}$, 10^{-6}, $3x10^{-6}$, 10^{-5}, $3x10^{-5}$, 10^{-4}, $3x10^{-4}$), and also was done a single stimulus with NA ($10M^{-4}$), ATP (10^{-3} M) and KCl (40 mM). The number of animals (n) used for every group was 5 or 6. The results were normalized by the weight in grams of VD. The pharmacological parameters pD2 (apparent affinity) and Emax (maximum effect) were evaluated. Statistical analysis was performed ANOVA test. **Results**: The NA phasic response (10^{-4} M) was significantly lower with increase of rat age (8 weeks: 44.9 ± 1.7, 12 weeks: 34.2 ± 1.9, 16 weeks: 33.7± 2.3 and 20 weeks: 26.3 ± 1.4, - p <0.05 ANOVA with Bonferroni post-test). The same happened for the tonic response of NA (10^{-4}M) except at 12 weeks compared with 8 weeks. The contractile response of ATP (10^{-3} M) also was significantly lower (8 weeks: 21.6 ± 1.0, 12 weeks: 14.8 ± 0.5, 16 weeks: 14.1 ± 0.8 and 20 weeks: 11.7 ± 0.4, - p <0.05 ANOVA with Bonferroni post-test). The phasic response of the depolarizing agent KCl (40 mM) also was significantly lower (8 weeks: 40.9 ± 2.2, 12 weeks: 32.3 ± 2.2, 16 weeks: 27.3 ± 2.2 and 20 weeks 20.8 ± 1.4, - p <0.05 ANOVA with Bonferroni post-test). The same happened for the tonic response of KCl (40 mM). The NA concentration-effect curve also was significantly lower especially comparing 8 weeks to 20 weeks. The E max of 8 weeks was 37.7 ± 0.7 and 20 weeks was 27.1 ± 0.8 g while the pD2 for 8 weeks was 5.9 ± 0.04 and for 20 weeks was 5.6 ± 0, 06 g (p <0.05 ANOVA with Bonferroni post-test). **Conclusion**: The results show that age interferes in the responsiveness of the VD to stimulation of neurotransmitters of the sympathetic nervous system. These differences suggest changes in density and affinity for these receptors (corroborated by pD2 data), and even differences in cell signaling pathway (eg, by calcium) involved in activation of these receptors. **Financial Support**: Capes, CNPq, FAPESP.

01.039 Pharmacologic evaluation of LASSBio-998 analogues designed as p38 inhibitors. Guimarães JPD, Berto-Júnior C, Soares RA, Lopes RO, Barreiro EJ, Souza AM, Lima LM LASSBio-FF-UFRJ

Introduction: The mitogen activated protein kinases (MAPKs) comprise a family of enzymes which form an integrated network required to reach specialized cell functions, such as differentiation, proliferation and cell death. Among the known MAPKs, p38 has a crucial role in inflammation, mainly by regulate the levels of TNF-α and cytokine production by immune cells, and are responsible for the activation of transcription factors like ATF-2, MEF-2 and NFκB (ZARUBIN, Cell Res, v. 15, p. 11, 2005). Accordingly, the rational design of new compounds capable of inhibiting the activity of p38 is very interesting from the standpoint to treat inflammatory diseases. In this way, our group realized the design and synthesis of novel p38 inhibitors planned by structural modification at the prototype LASSBio-998, aiming to improve their pharmacological profile. . In this context, the objective of this study was to evaluate the comparative effect of LASSBio-998 and its synthetic analogues on the p38 MAPK activity. **Methods**: Balb/c mice were challenged with thioglicolate three days before the collection of peritoneal exudate rich in macrophages. The macrophages collected were cultured on a six-well plate and challenged with LPS. Incubation with the compounds was performed as described and p38 activity was measured by immunoprecipitation of phospho-p38, followed by incubation with ATF2 substrate and immunobloting for phospho-ATF2, using SB 203580 as standard. All procedures were performed as described in the “Principles of Laboratory Care” (NIH 85-23, 1985) and approved by Animal Ethics Committee (DFBCICB013) **Results**: We observe that in macrophages previously challenged with LPS, MAPK p38 activity was increased and that cells pretreated with tested compounds present a reduced p38 activity. The same inhibition was observed when the tested compounds were added after the immunoprecipitation of phopho-p38. All compounds are tested in a 10mM concentration. **Discussion**: These data allow us to conclude that the compounds LASSBio-1494, LASSBio-1495, LASSBio-1496 and LASSBio-1497 block the MAPK p38 pathway by direct modulation of the MAPK p38 activity. In other words, we described in this work a new series of direct MAPK p38 inhibitors. **Financial Support**: CAPES, CNPq, FAPERJ,INCT-INOFAR.

01.040 Lipoxin A4 inhibits mediators releasing in mouse mesothelial pleural cells stimulated with *Mycobacterium bovis* (BCG). Candea ALP, Menezes-Lima-Júnior O, Henriques MGMO Fiocruz – Farmacologia Aplicada

Introduction & Aim: Tuberculous pleural effusion is the most important extra pulmonary manifestation of tuberculosis, resulting in an accumulation of fluid and inflammatory cells in the pleural space. Moreover, pleural mesothelial cells are often stimulated by mycobacterial bacilli, probably due to the activation of many receptors, as Toll Like Receptors (TLR), that enter into the pleura after rupture of a granuloma or from other subpleural tuberculous focus. Nowadays, we know that distinct mediators, as Lipoxin A4, play an important role in containing this inflammatory process. However, the mechanisms of their regulation in mycobacterium infection are poorly understood. In this study, we investigated the mechanism by which mesothelial cells become activated in response to M. bovis-BCG infection and we studied the role of Lipoxin A4 in this process. **Methods** & Results: Firstly, mesothelial cells were infected with BCG ($4x10^5$ CFU/well) for 18h or 24h. After some time, the free cells supernatant has been collected for the analysis of cytokine production and intracellular signaling pathways. Once stimulated, mesothelial cells were able to produce NO, CCL2, CXCL1, TNF-α and IL-6, parallel to activation of p38 MAPK and p-JNK, but not ERK-2 pathway. This process, apparently, occurred independently of MyD88 and TIRAP signaling (intracellular signaling pathways of many TLR). After, mesothelial cells were previously incubated with different concentrations of Lipoxin A4 for 1h and later they were infected with BCG ($4x10^5$ CFU/well). The pretreatment of Lipoxin was able to reduce cytokine releasing and the intracellular signaling pathways. **Conclusion**: Our results show that mesothelial cells release mediators that may contribute to control pleural inflammation induced by bacterial agents and that Lipoxin A4 has an anti-inflammatory activity profile in this process.

01.041 Impact of systemic administration of bacterial endotoxin on vascular permeability and inflammatory genes in the rat epididymis: **modulation by glucocorticoids**. Pinto T, Denadai-Souza A, Honda L, Avellar MCW Unifesp – Farmacologia

Introduction: Infection diseases affecting organs from the male reproductive tract are one of the major causes leading to infertility. However, the cellular and molecular processes taking place within these organs during an inflammatory response to microbial agents are poorly understood. Herein, we have investigated the impact of the systemic administration of bacterial endotoxin on vascular permeability and expression of inflammatory genes in the rat reproductive tract organs, with an emphasis on the epididymis, as well as the modulation of these parameters by endogenous and exogenous glucocorticoids. **Methods**: Adult male Wistar rats (90 d) were treated with LPS (0.01 - 1.0 mg/kg; 2, 6 and 24h; i.v.). Control animals received sterile saline. Changes of endogenous glucocorticoids were assessed by surgical adrenalectomy (ADX, 7 d) followed or not by treatment with a synthetic glucocorticoid (dexamethasone, DEX 7 mg/kg; i.p.; 4 h). Vascular permeability status was analyzed by Evans Blue dye tissue accumulation (50 mg/kg, i.v., 1 h prior to sacrifice). RT-qPCR was used to assess mRNA expression of the following genes: *Il1b* (interleukin 1 beta); *Tsc22d3* (glucocorticoid-induced leucine zipper or Gilz, a GR modulator); *Cish* (cytokine inducible SH2-containing protein); Nuclear factor Kappa b inhibitor alpha (*Nfkbia*); Prostaglandin-endoperoxide synthase 1 and 2 (*Ptgs1/2*); Toll-like receptor 4 (*Tlr4*); Cluster of differentiation 14 (*Cd14*) and Cyclophilin A (*Ppia;* a reference gene. Results: Control testis and caput epididymis exhibited an intense Evans Blue dye staining in comparison to corpus and cauda epididymis. Testis and cauda from 2h LPS-treated rats presented a significant reduction in vascular permeability when compared to control and ADX. This reduction was maintained in testis, but reversed in cauda epididymis, after LPS 6h. Only cauda epididymis presented a decrease in the plasma extravasation after 24h LPS. Control and LPS-treated caput and corpus epididymis presented similar vascular permeability values, although ADX induced differential changes in this parameter when control and LPS-treated were compared. Constitutive mRNA expression of all genes analyzed was observed in epididymis (caput, corpus, cauda), testis, seminal vesicle, prostate and vas deferens. In the caput region, upregulation of *Il1b*, *Ptgs1/2*, *Nfkbia*, *Cish*, *Tlr4* and *Cd14* mRNA was observed in ADX rats treated with LPS 2h. DEX treatment significantly up- and down-regulated *Tsc22d3* and *Cish* mRNA levels, respectively, when compared to control or LPS-treated. **Discussion**: The data suggest the existence of a high degree of fenestrated microcapillaries in testis and cauda epididymis, where blood flow/vascular permeability are impaired by acute LPS treatment, in the presence or absence of endogenous glucocorticoids. At least in the caput epididymis, the response of inflammatory genes to LPS treatment exacerbated in the absence of endogenous glucocorticoids. Collectively our data provide valuable information on the mechanisms underlying the vascular permeability and gene regulation in the epididymis under inflammatory and non-inflammatory conditions. **Financial Support**: Fogarty Foundation, CAPES, CNPq, FAPESP. Ethics Committee Approval: 0318/11

01.042 ATL-1, a synthetic analog of 15-epi-lipoxin A4, modulates key function of tumor-associated macrophage: A potential anti-tumoral tool. De Brito NM, Simões RL, Fierro IM, Barja-Fidalgo TC UERJ – Biologia celular

Aim: In the later years, the relationship between inflammatory response and cancer has been extensively investigated. Macrophages, important effectors cell in inflammatory response, have key roles in different steps of the tumor progression, such as migration, angiogenesis, invasion and metastasis. Macrophages classically activated (M1 profile) possess pro-inflammatory and antitumoral activities. In the tumor microenvironment, these M1 macrophages acquire an anti-inflammatory and pro-tumor profile, now being known as tumor-associated macrophages (TAM), with a M2-like profile, characterized by an increase of CD200 expression and the immunosuppressive cytokine IL-10 release, for example. Lipoxins (LX) are bioactive lipid mediators that exhibit anti-inflammatory and pro-resolution activities in mononuclear cells, but its effects on TAM are not well investigated. In this study, we investigated the effects of 15-epi-16-(para-fluoro)phenoxy-LXA_4 (ATL-1), a synthetic analog of 15-epi-lipoxin A_4, in tumor-associated macrophages (TAM). **Methods and Results**: Human blood monocytes were cultured for 7 days to macrophage maturation (MO). After, cells were incubated for 3 days with the conditioned medium of MV3, a human melanoma lineage cell, to differentiate in TAM. First, we have shown that TAM, as seen in MO, express greater levels of ALX/FPR2, a specific receptor of LX. Also, ATL do not alter ALX expression. Treatment with ATL-1 (10nM) decrease CD200, and IL-10 gene expression, both characteristic markers of TAM, suggesting that lipoxin can modulate the shift of macrophage phenotype from M2 to M1. ATL-1 does not alter viability of TAM, as demonstrated by Trypan Blue exclusion and MTT assays. Then, we investigated the possible effects of ATL in key events in tumor progression. In the tumor microenvironment, TAM exhibit low cytotoxic properties, as nitric oxide (NO) and reactive oxygen species (ROS) production, a mechanism induced by cancer cells to possibility tumor progression. By an increase of iNOS/Arginase-1 ratio, the treatment of TAM with ATL-1 induced NO production in a dose-dependent manner (1-100nM). Also, ATL-1 triggers ROS production by TAM, which was inhibited in the presence of DPI, an NADPH oxidase inhibitor, suggesting that lipoxin may restore the antitumoral activity of TAM activating NADPH oxidase system. The recovery of cytotoxic properties of TAM could control tumor progression trough promotion of tumor cell apoptosis. Here, we observed that ATL-1 (10nM) reverts the anti-apoptotic effect of TAM on MV3, as demonstrated by cell cycle analysis. Additionally, we also observe that the conditioned medium of TAM, but not MO conditioned medium, increased endothelial cell (EC) tubulogenesis, a crucial step in angiogenesis process. The treatment of TAM with ATL-1 significantly decreases EC tubulogenesis, suggesting that lipoxin negatively modulate tumor angiogenesis. ATL-1 do not alters VEGF release by TAM, suggesting that this analog could inhibit angiogenesis by others factors. **Conclusion**: Together, our results demonstrate that lipoxin has inhibitory effect on the tumor progression stimulated by the presence of TAM, inducing the shift from M2 to M1 profile, leading to tumor cell apoptosis. **Supported by**: CAPES, Faperj, CNPq, SR-2/UERJ

01.043 Snakebites envenomation and alternative serotherapy by camelid nanobodies. Prado NDR[1], Pereira SS[1], Morais MSS[1], Silva SCG[1], Braum DT[2], Pereira da Silva LH[1], Soares AM[1,3], Stabeli RG[1,3], Fernandes CF[1,2] [1]Fiocruz, [2]Cepem, [3]CEBIO-UNIR

Envenomation by snakebites represent a relevant public health problem in tropical countries due to high morbidity and mortality its can cause (1). The *Bothrops* genus represents 73.5% of reported accidents by snakes in Brazil, and its venom can induce the release of active substances with systemic effects as blood coagulation and shock or local effects such as bleeding, edema and necrosis that can lead to permanent loss of tissue function, requiring amputation of the affected limb (2). From the *Bothrops jararacussu* venom were isolated two myotoxic phospholipases, called Bothropstoxin I and II (BthTX-I and BthTX-II), which BthTX-I has no enzymatic activity, and BthTX-II is enzymatically active(3). Nowadays, the treatment used for snakebites is the sorotherapy produced by immunized horses with sublethal doses of venom (4). This treatment is not efficient for local effects and is associated with several adverse reactions. Camelids produce, in addition to conventional antibodies, IgGs composed exclusively of heavy chains, in which the antigen binding site is formed only by the single domain, called VHH or nanobody. Besides their small size and high solubility, nanobodies are not affected by variations in temperature and pH, which are important advantages in field treatment (5). So, Nanobodies have become important tools for the manufacture of diagnostic kits or passive immunotherapy. This work proposes the use of camelids nanobodies as an alternative in the treatment of snakebites. To this end, phage display technology was employed. So, after monitoring of the camelid immune response by ELISA, VHHs regions were isolated by PCR using peripheral lymphocyte cDNA obtained from one camelid previously immunized with the BThTX antigens (Animal Ethic Committee: 03/2012). Then, amplicons were cloned into a phagemid using TG1 *E. coli* strain to construct a phage antibody immune library with a titer of $4x10^{13}$ cfu/mL. After infection by M13K07 helper phage, VHHs were displayed fused to phage coat protein III and the selection step was performed on immobilized BThTX antigens. Therefore, ELISA immunoassays experiments were carried out to verify the capacity of selected clones to recognize the antigen. After the second enrichment round of biopanning, 80 clones shown to be positive for VHH sequence by PCR. About 26 clones recognized specifically BThTX antigens by ELISA. To characterize the molecular interaction between the selected nanobodies e BThTX antigens, SPR-based biosensors are being used. Further experiments are being carried out in aim to purify, and verify the neutralization capacity of selected nanobodies. These findings support the idea that selected VHHs could be a powerful strategy to the treatment of *Bothrops jararacussu* snakebites. **References**: 1. Cardoso, J. L. C. Mem Inst Butantan, 52, 43, 1990. 2. Nishioka, S. A. *Am. J. Trop. Med. Hyg.*, 47, 1992, 805. 3. Andrião-Escarso, S. H. Biochimie, 82, 2000, 755. 4. Chippaux, J. P. *Toxicon, 36,* 1998, 823. 5. Wesolowski, J. Med Microbiol Immunol, 198, 2009, 157. **Financial Agencies**: FINEP, CNPq. **Acknowledgments**: CAPES

01.044 Analysis of protein-protein interaction by yeast two-hybrid system in the search of protein partners for sperm associated antigen 11 C isoform. Pelosi PAJ[1], Ribeiro CM[1], Luz JS[1,2], Avellar MCW[1] [1]Unifesp – Pharmacology, [2]FCFAr-UNESP

Introduction: Sperm-associated antigen 11 (SPAG11) gene encodes several androgen-dependent secretory proteins (SPAG11A-SPAG11W) in different species, including rodents and human. SPAG11C, one of the SPAG11 isoforms, is abundantly expressed in the male reproductive tract, particularly in the epididymis, and contains in its C-terminal region a domain with similarity to beta-defensins, a family of antimicrobial peptides. An antimicrobial activity for SPAG11C has been reported *in vitro*. Little is known, however, about its mechanism of action and biological role. Charting the interactions among proteins is essential for understanding biological processes. In order to clarify SPAG11C molecular mechanism of action, here we present the results from yeast two-hybrid assays used as a tool to identify interacting partners for human SPAG11C (hSPAG11C). **Methods**: Yeast two-hybrid system was performed by Hybrigenics (France) using full length hSPAG11C as bait and a cDNA library from human testis/epididymis as prey. From the 171 cDNA clones isolated, 7 were selected for a second round of yeast two-hybrid system in our laboratory. SPAG11 isoforms (hSPAG11C, D and E; full length, N- or C-terminal region) were fused to the pBTM-116 vector with the Gal4p transcription activation domain. The preys (seven different full length cDNA, N or C-terminal region of glutaredoxin 3, GLRX3) were fused to the pGAD-C1 vector with lexA DNA binding domain. Positive interactions were observed by the yeast β-galactosidase and HIS3 markers. Appropriate positive and negative controls were also performed. Spag11c and interacting partner mRNA expression was analyzed by RT-PCR in adult rat (Wistar, 90 days old) and human tissues, while their protein expression was verified by immunohistochemistry in adult rat epididymis. Results: Yeast two-hybrid screening revealed a specific interaction between full-length and C-terminal domain of hSPAG11C (but not with full length hSPAG11D, hSPAG11E or hSPAG11C N-terminal region) with full length hGLRX3, an oxireductase with unknown function in the epididymis. RT-PCR indicated the presence of GLRX3 transcripts in rat reproductive- and non-reproductive tissues as well as in human testis and epididymis. Immunohistochemistry localized SPAG11C and GLRX3 specific immunostaining in epithelial cells along the adult rat epididymis. **Discussion**: These findings are consistent with the existence of a potential functional network between SPAG11C and GLRX3 and may give new insights into the biological function of SPAG11C. Mechanistic investigations will be now necessary to validate the physiological relevance of SPAG11C/GLRX3 interaction in the epididymis and the role of these proteins for male fertility. **Financial Support**: Fapesp, CNPq, CAPES, Fogarty International Center (Unifesp/UNC Chapel Hill). Ethics Committee Approval: 1928/11, UNIFESP-EPM

01.045 Anti-tyrosinase, anti-collagenase and cytotoxic activity of Kojic acid derivatives. Pedrosa TN[1], Carvalho ASC[2], Santos AS[2], Lima ES[1], Vasconcellos MC[1]
[1]UFAM, [2]UFPA

Introduction: The continuous exposure to sunlight is a major factor causing spots and skin aging. This exposure leads to overproduction and accumulation of melanin can induce pigmentary disorders in the skin, including melasma and freckles, as well as lead to production of reactive oxygen species in the skin, promoting oxidative stress and inflammatory responses in epidermal or dermal layer, resulting photoaging and damage to cell membranes, lipids, proteins and DNA. The growth of the cosmetics market in the world, the growing interest of people for youthful skin, free of wrinkles and blemishes, has prompted researchers to search for new assets for this purpose. Kojic acid is a well known and widely used depigmenting mainly produced by fungi of the genus *Aspergillus* has also been reported as an antioxidant and rejuvenating. However, Kojic acid (AK) has disadvantages such as low stability and poor inhibition at low concentrations which stimulates researchers in the search for more active compounds or changes in the molecule in order to search for more active derivatives, stable and secure. Objective: To evaluate the potential cytotoxic, hemolytic, anti-tyrosinase and anti-collagenase Kojic acid derivatives, Zinc kojato (KZ) Kojato copper (KC) Oleilkojato copper (OC). **Methods**: For both tests were performed by alamar blue cell viability, hemolytic potential in erythrocytes of mice and evaluation of tyrosinase and collagenase enzyme inhibition *in vitro*. Results: The activity of tyrosinase inhibition *in vitro* of the kojic acid derivatives KZ (IC_{50}= 30,4 ± 0.89 μM) is upper than the standard AK (IC_{50}= 60.2 ± 1.83 μM); KC (IC_{50}= 133.3 ± 6.59 μM) and OC (IC_{50}= 77.6 ± 4.31 μM) had a lower activity *in vitro* than AK. The derivatives showed no inhibitory activity on collagenase and no hemolytic activity at a concentration of 250 μg/mL. The compounds also showed no cytotoxicity at concentrations below 25 μg/ml in cell line B16F10 (murine melanoma). **Acknowledgments**: The FAPEAM and CNPq for **Financial Support**

01.046 Testosterone induces vascular smooth muscle cells apoptosis by mechanisms involving activation of caspase 3 and caspase 8. Lopes RAM[1], Chignalia A[2], Neves KB[3], Zanotto CZ[1], Pestana C[3], Curti C[3], Tostes RC[1] [1]FMRP-USP, [2]InCor-HC-FMUSP, [3]FCFRP-USP

The mechanisms by which testosterone (testo) affects the cardiovascular system are not fully elucidated, but may involve generation of reactive oxygen species (ROS). Mitochondria are important source and also a target for ROS effects. Increased ROS generation activates signaling pathways that culminate in cell death. Our laboratory recently showed that testo induces ROS generation in vascular smooth muscle cells (VSMCs). We hypothesized that testo induces ROS generation via mitochondria, culminating in the activation of caspase 3 and VSMCs' apoptosis. Cultured VSMCs (from the mesentery of male Wistar rats) were pre-incubated for 30 min with flutamide (flu, 10-5 mol/L, androgen receptor antagonist), CCCP (10-6 mol/L, mitochondrial uncoupler agent), MnTmPyP (3x10-5 mol/L, SOD mimetic) or vehicle and then stimulated with testo (10-7 mol/L, 2-6 h). ROS generation was measured by lucigenin and 5-(and-6)-chloromethyl-2,7 -dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA) assays, apoptosis by 4'-6-diamidino-2-phenylindole (DAPI), calcein and annexin V fluorescence; protein expression of procaspases 3 and 8 and caspase 3 and 8 by immunoblotting. Cytochrome c, Bax and Bcl-2, expression was determined by immunoblotting of proteins from the cytoplasmic and mitochondrial fractions. The results are expressed in arbitrary units and $p < 0.05$ was considered as statistically significant. Testo increased ROS generation [162.60 ± 15.95 (2 h) vs. 100.00 ± 0.02 n = 7], which was abolished by CCCP (90.43 ± 14.94 n = 4), MnTmPyP (26.24 ± 11.61 n = 4) and flu (122.70 ± 10.60 n = 4). Testo induced cleavage of caspase 3 (166.2 ± 19.11 (6 hs) vs 100.0 ± 0.02 n = 9), an effect abolished by flu (122.70 ± 10.60 n = 4), MnTmPyP (61.78 ± 2,52 n = 3) and CCCP (71.58 ± 13,19 n = 3). Testo increased florescence to DAPI [146.9 ± 8,23 (2 h), 192.1 ± 10.12 (6 h) vs. 100.0 ± 0.01 n = 4-5] and annexin V [197.6 ± 21,47 (6h) vs. 100.00 ± 0,01 n = 4] and decreased calcein fluorescence [34.42 ± 6.41 (2 h) vs. 100.0 ± 0.01 n = 7]. Although testo induced cleavage of caspase 3 and VSMCs' apoptosis, the Bax (apoptotic)/Bcl-2 (anti-apoptotic) ratio in the mitochondrial fraction was reduced [65.10 ± 8.78 (6 h) vs. 100.0 ± 0.01 n = 3] and testo did not induce translocation of cytochrome c from the mitochondria to the cytoplasm [70.05 ± 26.48 (2 h), 110.60 ± 46.28 (6 h) vs 100.0 ± 0.02 n = 4]. Testo induced cleavage of caspase 8 [161.1 ± 13:47 (6 h) vs 100.0 ± 0.01 n = 6]. In **Conclusion**, testo induces VSMCs apoptosis by mechanisms involving androgen receptor activation, mitochondrial ROS generation, and possibly activation of the extrinsic apoptotic pathway. **Financial Support**: CNPQ. Protocol of Animal Use Ethic Committee: 018/2011.

01.047 Effects of the *Bothrops moojeni* venom (VBm) on the integrity and viability of endothelial Cells (EC). Zamuner SF[1], Adamo KB[1], Figueiredo TCS[1], Zamuner SR[1], Teixeira CFP[2] [1]Uninove –Ciências da Reabilitação, [2]IBu – Inflamação

Introduction: The venom of snakes from the *Bothrops* species promotes systemic manifestations such as nausea, vomiting, sweating, bleeding, hypotension, renal failure and even can lead to shock. Local hemorrhage is part of the complex pathological alterations at the bite site characteristic of *Bothrops* envenoming, and contributes to tissue damage and impaired muscle regeneration. The bleeding in *Bothrops* envenomation is due to extensive damage to the tissues and vessel walls by the action of metalloproteinases which degrade collagen IV, leading to the collapse of the capillary structure. In this study the effect of VBm was examined on the viability and integrity of EC monolayers. Objective: The present work was designed to investigate the effect of viability and integrity submitted of endothelial cells to injury by Bothrops moojeni venom (VBm). **Methods**: In this study the effect of VBm was examined on the viability and integrity of EC monolayers. Human umbilical cord EC (ECV-304) were cultured in F12 medium-10% FBS and seeded in 96 well plates. After reaching confluence (48h) EC monolayers were incubated with VBm (5, 10, 25 ug/mL) or F12 (control) for 30, 60 and 120 min before evaluation of EC injury and viability. Integrity of monolayers was quantified by staining with crystal violet, lysis with methanol and determination of O.D. at 630 nm. EC viability was determined by three procedures: trypan blue exclusion method; lactate dehydrogenase (LDH) activity and MTT assay. Results: Our results showed that VBm 5 and 10 ug//mL, caused detachment of EC only at 120 min of incubation (15 and 83% respectively) , however incubation with 25 ug/mL VBm caused a marked detachment of EC within 30-120 min (38, 93 and 93% respectively). VBm did not affect EC viability. **Conclusion**: This effect is not related to interference of this agent on cell viability or metabolism, but may be due to effects of vemon on extracellular matrix. Key words: endothelial cells, viability, integrity, *Bothrops* venom

01.048 Ontogeny of the SPAG11C expression in male rat: could it be involved in Wolffian duct morphogenesis? Ribeiro CM, Queiróz DBC, Silva EJR, Denadai-Souza A, Avellar MCW Unifesp – Endocrinologia Experimental

Introduction: *Sperm-associated antigen 11* (*Spag11*) is a beta-defensin-like gene expressed in the male reproductive tract and regulated by androgens. SPAG11C, one of the several isoforms encoded by this gene, is abundantly expressed in the epididymal epithelia, is found in association with epididymal and ejaculated sperm and the recombinant protein acts *in vitro* as an antibacterial agent. Its physiological function, however, remain unknown. Here, we characterized the ontogeny of SPAG11C expression (protein and mRNA) through rat life focusing on the development of the wolffian/epididymal duct. **Methods**: Whole embryos were collected at embryonic days E14.5, 16.5, 18.5 and 20.5 and epididymides isolated from 1, 5, 10, 20, 40 and 120-day-old rats. *Spag11c* mRNA was evaluated by RT-PCR and *in situ* hybridization while the protein localization was assessed by immunohistochemistry. The SPAG11C immunodistribution was compared with the well-known immunohistochemical distribution pattern of the androgen receptor (AR). Ethical approval: UNIFESP-EPM 1563/09. Results: Prenatally, *Spag11c* mRNA was widely distributed in sites of different embryonic origins, including reproductive tract and extragenital tissues at all time-points analyzed. The transcript was observed in the testis, Wolffian duct, kidney, muscle (smooth, skeletal and cardiac), chondrocytes, adrenal gland, lung, liver, pancreas and in the nervous system. On the other hand, at adult life, *Spag11c* mRNA expression was almost confined in tissues from the male reproductive tract, been mainly expressed in the caput epididymis. In the postnatal epididymis, *Spag11c* mRNA was developmentally regulated and peaked at adulthood. *In situ* hybridization revealed the presence of *Spag11c* mRNA in the same sites immunopositive for SPAG11C, confirming their ability to synthesize the protein. Regarding the protein localization, SPAG11C and AR immunodistribution overlapped in the developing Wolffian/epididymal duct. Both SPAG11C and AR immunoreaction were predominantly distributed in the mesenchymal cells of the Wolffian/epididymal duct, specially surrounding the duct epithelium. Following birth, however, the predominant localization of SPAG11C and AR immunostaining switched from embryonic mesenchymal to epithelial cells of the developing epididymis. **Discussion**: The SPAG11C expression in embryo broadens the potential biological roles of this protein within and beyond the male reproductive tract. Besides, the overlapping distribution of SPAG11C and AR could indicate *Spag11* gene as a target for direct effects of androgens. Due to SPAG11C expression in the Wolffian/epididymal duct we are now testing the hypothesis that this protein is a mesenchymal factor involved in duct morphogenesis. **Financial Support**: Fapesp, CNPq, CAPES, Fogarty International Center (Unifesp/UNC Chapel Hill).

01.049 Ouabain stimulates rat sertoli cell proliferation through ERK1/2 pathway.

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Introduction: Na^+/K^+-ATPase, besides its ion pumping role, is now considered a receptor that interacts with many membrane and cytosolic proteins and mediates the activation of different downstream signaling pathways after bound to cardiotonic steroids. These events involve the activation of protein kinases and a complex set of cell type-dependent acute and chronic effects. In testicular cells, particularly Sertoli cells, Na^+/K^+-ATPase function has been poorly investigated and, although steroidal hormones have remarkable physiological and pharmacological importance, the effect of cardiotonic steroids like ouabain have not been well characterized. The aim of the present study was to investigate the cellular effects of ouabain in Sertoli cells obtained from immature rats and the related molecular mechanisms. **Methods**: Primary cultures of Sertoli cells were obtained from 15-day old male Wistar rats and were treated with increasing ouabain concentrations (10^{-10}-10^{-6} M) and at different times (2-30 min) to evaluate p-ERK1/2 and p-Akt by Western blot. Cells were also treated for longer periods (24 h) with ouabain plus inhibitors of MEK (U0126), PI3K (wortmannin), CREB (KG501), NF-κB (SN50) to evaluate cyclin D1 expression by Western blot and proliferation by [methyl-^{3}H]thymidine incorporation. In some experiments, the intracellular estrogen receptor antagonist ICI-182,780 was used. Western blot for the detection of Na^+/K^+-ATPase α isoforms was also performed. **Results**: Treatment of Sertoli cells with 1 μM ouabain, but not lower concentrations, induced a rapid (5 min) and transient significant increase in ERK1/2 and Akt phosphorylation. Only the ouabain-resistant Na^+/K^+-ATPase α1 isoform was detected in Sertoli cells. Upregulation of cyclin D1 expression and increased [methyl-^{3}H]thymidine incorporation were also evidenced with 1 μM ouabain, and were fully dependent on ERK1/2 but not Akt. Moreover, ouabain effect on these proliferation parameters was completely prevented when CREB function was disrupted and only partially when NF-κB nuclear translocation was impaired. ERK1/2 activation by ouabain did not involve the classical estrogen receptor. **Discussion**: We postulate that endogenous cardiotonic steroids may play a role in the normal testis homeostasis and this process might be important for fertility. **Financial Support**: Fapesp, Faperj, CAPES, CNPq.

01.050 The role of HO-1 on the adipogenic development of murine bone marrow-derived mesenchymal stem cells. Vargas da Silva S[1], Quirino AS[1], Gonçalves R[1], Renovato Martins M[1], Citelli M[2], Simões RL[1], Pereira CR[1], Barja-Fidalgo TC[1] [1]UERJ – Biologia Celular, [2]UERJ – Nutrição

Mesenchymal stem cells (MSCs) derived from bone marrow are multipotent cells that differentiate and proliferate into many different cells types. Adipogenesis is the developmental process by which MSCs differentiate into pre-adipocytes and adipocytes. The aim of this study was investigate the role of heme oxygenase -1 (HO-1) on differentiation to adipocytes of murine MSCs derived from bone marrow (CEA/045/2009). MSCs cultures were incubated with adipogenic medium (ADIPO medium - containing insulin - 500 nM, dexamethasone – 100 nM and methylisobutylxanthine – 500 µM) for ten days and several adipogenic parameters were evaluated. First, we characterized the MSCs identity using flow cytometry analysis. Measurements revealed a uniformly negative cell population for CD14 and CD45, and positive for CD90.2 and CD105. The analysis of cell-morphology showed that ADIPO medium altered the cell shape by induce a rounded phenotype, formation of lipid droplets and alterations in the actin cytoskeleton. Preliminary data demonstrated that adipogenic medium also induced the HO-1 expression and concomitantly promoted the mRNA expression for adiponectin and C/EBP-a and PPAR-γ protein expression. Our results suggest that HO-1 may be a target molecule in the modulation of adipogenesis from MSCs. Supported by: CAPES, CNPq and FAPERJ.