Resumos
Setor 01
Farmacologia Celular e Molecular

01.001

LIVER REGENERATION IN RATS: ROLE OF VERAPAMIL ON THE BIOCHEMICAL PROFILE

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Introduction: Calcium blocking drugs are referred to protect the liver tissue in some experimental damage. We studied the influence of verapamil on the functional liver regeneration in partially hepatectomized rats. **Methods:** There were used 72 Wistar rats. Groups: 1- partially hepatectomized treated with verapamil; 2 - control: *partially hepatectomized not treated*. Both were compared with not operated or treated rats. (Verapamil: 24mg/rat/day, administered from the 14th day before the surgery up to the sacrifice). The rats were divided in three groups: 10, 20 and 30 days after the surgery. It was determined in liver samples, initially and at the sacrifice: albumin, cholinesterase and γ-glutamyl-transferase. Enzymes results are given as specific activity. **Results:** Albumin was higher at the 30th day in the two groups of operated rats (verapamil treated: 49±2.2 g/L; not treated: 53±1.4g/L), as compared to not operated ones (22±1.1g/L). Cholinesterase activity was, at the 30th day after partial hepatectomia, significantly higher in the verapamil treated rats (859±62) than in control (478±27). γ-glutamyl transferase was increased at the 10th day (16.5±3.1), tending to normalization at the 30th day in the verapamil group (4.8±0.35), a result comparable to not operated rats (4.9±0.5). **Discussion:** Results indicate that the remaining liver of the verapamil treated partially hepatectomised rats undergo to functional regeneration at the 30th day after the surgery, regarding to albumin and cholinesterase synthesis; liver damage seemed to be restored, since at that time, γ-glutamyl transferase tended to normalize. In conclusion, verapamil indicated in partially hepatectomised rats to restore, after 30 days of surgery, protein and enzyme synthesis as well as an improving of the liver surgical damage. **Supported by:** CNPq, UNICAMP, UNIVAP.

01.002

INFLUENCE OF VERAPAMIL ON THE LIVER TISSUE OF PARTIALLY HEPATECTOMIZED RATS: SUGGESTIVE PROTECTIVE ACTION

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Introduction: A protector role of calcium channels blocker drugs (verapamil, isradipine) in heat or drugs experimental liver damage has been referred in the literature. We studied the histological role of verapamil on the growing liver tissue after partial surgical hepatectomia in rats. **Methods:** There were used 72 Wistar male rats. Two groups were submitted to partial hepatectomia: 1- *treated with verapamil* (24 mg/rat/day, administered by oral route, 14 days before the surgery up to the sacrifice); 2 - *control not treated*. The rats were divided in subgroups, according to the time elapsed after the surgery: 10, 20 and 30 days. At the sacrifice, a piece of the liver tissue was prepared to light microscopy and observed regarding to the presence of steatosis, necrosis and intracellular edema. **Results:** It was observed in the control group submitted to partial hepatectomia and no drug treatment cellular tumefaction that was severe at the 10th day after the surgery, moderate at the 20th day and light at the 30th day. Sinusoidal capillary were very congested from the 10th to the 30th days after the surgery. In the verapamil treated group, cellular tumefaction was moderate at the 10th day and very light at the 20th and 30th days while sinusoidal capillary were normal from the 20th day on. **Discussion:** Edema was the only hepatic tissular or cellular disturbance in the operated rats. However, tumefaction was significantly lower in the verapamil treated group, suggesting a protective action of the drug. **Supported by:** CNPq, UNICAMP, UNIVAP.

01.003

A NOVEL LIPID MEDIATOR ASPIRIN-TRIGGERED LIPOXIN A_4 INDUCES HEME OXYGENASE-1 ON ENDOTHELIAL CELLS

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Introduction: Lipoxins (LX) and their aspirin-triggered 15-epimer isoforms are endogenous anti-inflammatory and pro-resolution eicosanoids. Recently, it has been reported that aspirin is able to activate heme-oxygenase-1 (HO-1) on endothelial cells (EC) in a COX-independent manner, what confers protection against pro-oxidant insults. However, the underlying mechanisms remain unclear. In this study, we investigated whether an aspirin-triggered lipoxin A_4 stable analog, *15-epi-16-(para-fluoro)-phenoxy-lipoxin A_4* (ATL-1) was able to induce endothelial HO-1. **Methods:** Confluent human umbilical vein endothelial cells (HUVEC) or imortalized cell line ECV 304 were incubated overnight at 37° C in the absence or presence of ATL-1 (1-100 nM). After treatment, HO-1 expression was detected by Western Blotting assay. **Results:** ATL-1 was able to increase HO-1 expression on EC and the phenomenon seems to be mediated by the activation of a G-protein-coupled receptor (GPCR) since Pertussis toxin (PTX) treatment (0.1 μ g/mL) significantly inhibited ATL-1-induced HO-1 expression. PD 98056 (10 μ M), an ERK-2 inhibitor, downregulated ATL-1 effect on HO-1 expression, suggesting that the MAP kinase pathway is one of the downstream targets of GPCR activation. **Conclusion:** These data point to a new mechanism for the anti-inflammatory activity of these lipid mediators, namely HO-1 expression. **Supported by:** FAPERJ, CNPq and SR-2/UERJ.

01.004

ACTIONS OF CONOTOXIN ρ-TIA AT α_1-ADRENOCEPTORS (α_1-AR)

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Introduction The19-aminoacid Conotoxin ρ-TIA (ρ-TIA) from *Conus tulipa* was recently reported to interact with α_1-ARs. ρ-TIA inhibited allosterically the binding of [^{3}H]-prazosin to membranes from COS-7

cells expressing α_{1B}-ARs (Sharpe, *JBC*, **278**, 34451, 2003). **Objective** To determine the action of ρ-TIA at functional responses mediated by α_{1A}- and α_{1D}-ARs. **Methods** The contractions of the rat vas deferens and aorta to noradrenaline were used as models of actions mediated by α_{1A}- and α_{1D}-ARs, respectively. The tissues were treated with phenoxybenzamine (POB) to minimize the effect of the hyperbolic *occupancy-response* relationship to noradrenaline and α_1-ARs on the antagonism by ρ-TIA. The potency of competitive antagonists should not change after POB while that of allosteric antagonists should be increased because of the elimination of the dextral shift effect after POB. Therefore, the potencies (pIC_{50}) of ρ-TIA and α_1-competitive antagonists (prazosin, 5-methyl-urapidil and BMY-7378) were determined against equieffective concentrations of noradrenaline before and after treatment with POB. **Results** As expected, the pIC_{50} of competitive antagonists in the vas deferens and aorta remained unchanged after POB treatment. However, the treatment of the vas deferens with POB induced a 8-fold increase in the potency of ρ-TIA (pIC_{50}: before=7.2±0.1; after=8.1±0.1, n=4). The treatment of the aorta with POB did not change the potency of ρ-TIA (pIC_{50} ≅7.2). **Conclusion** These results suggest that the action of ρ-TIA is allosteric at the α_{1A}-AR in the vas deferens but competitive at the α_{1D}-ARs in the aorta. **Supported by:** FAPESP.

01.005

AFFINITY AND RELATIVE EFFICACY OF PHENYLETHYLAMINES AND IMIDAZOLINES AT α_1-ADRENOCEPTORS (α_1-AR)

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Introduction α_1-AR agonists are much less used than antagonists for the characterization of α_1-AR subtypes. This is partially explained by the hyperbolic relationship between α_1-AR occupancy and response often observed, which complicates the analysis of the selectivity of these agents. **Objective** To compare the affinities and relative efficacies of the most commonly used phenylethylamine derivatives noradrenaline (NA), phenylephrine (PE) and methoxamine (ME) and the imidazoline oxymetazoline (OXY) at α_1-AR. **Methods** The contractions of the rat epididymal vas deferens (RVD) and aorta (RA) were used as models for actions mediated by α_{1A}- and α_{1D}-ARs, respectively. The apparent affinities (pK_A) and relative efficacies (in relation to the efficacy of NA, ε) of the agonists were calculated by null methods after partial receptor alkylation. **Results** The pK_A for PE (RVD≅5.6; RA≅5.8) and ME (RVD≅4.7; RA≅4.3) suggest that these drugs show no selectivity towards α_{1A}- or α_{1D}-ARs. Also, the efficacies were high (ε≅0.9), further indicating that these drugs poorly discriminate subtypes of α_1-AR. In the other hand, NA showed higher affinity at the α_{1D}-ARs in the RA (pK_A≅6.6) than at the α_{1A}-ARs in the RVD (pK_A≅5.5). The opposite selectivity was observed with OXY, which presented higher affinity at the α_{1A}-ARs of the RVD (pK_A≅6.4) than at the α_{1D}-ARs of the RA (pK_A≅5.3). However, OXY showed low relative efficacy in these tissues (ε≅0.07). **Conclusion** NA and OXY conveniently discriminate α_1-ARs subtypes in functional studies although the low efficacy of the latter might be a problem in tissues with poor coupling efficiency. **Supported by:** FAPESP

01.006

CHARACTERIZATION OF A FUNCTIONAL CaM KINASE IN RAT VAS DEFERENS

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Introduction: In a previous work we demostrated that CaM kinase phosphorylates and increases the total Ca^{2+} transport in rat vas deferens. **Objective:** Characterize and investigate the effect of CaM kinase on SERCA pump activity present in RVD. **Methods:** The tissue was washed, homogenized and centrifuged to obtain the nuclear fraction (N). The $^{45}Ca^{2+}$ accumulation was performed with 25μM ruthenium red (RR) in the presence or absence of 3μM Thapsigargin (Tg), 10μM W7, 2μM calmodulin (CaM) and 1-5μM KN-93, a CaM kinase inhibitor. Phosphorylation assays were taken place with or without 10μM Ca^{2+} or 2μM CaM. The reaction was started with 0.8 mM ATP in ice. After 2 minutes, Western blotting assays were performed using polyclonal antibodies anti residues *Ser-Pi*. Western blotting was also performed in N fraction using a polyclonal antibody anti CaM kinase. **Results:** Western blotting assays suggested monomeric and heterodimeric forms of CaM kinase II with about 45, 65 and 105 kDa in N fraction, and an increase of immunolabbled band (about 105 kDa) after the addition of Ca^{2+} and CaM in phosphorylation assays. The total $^{45}Ca^{2+}$ accumulation was increased by CaM (1.1 to 3.6 nmolCa^{2+}. mg^{-1}, n = 4), but was not inhibited by KN93. **Discussion:** These data indicate the presence of different isoforms of CaM kinase in RVD and that this enzyme directly phosphorylates the SERCA pumps in this tissue. The lack of inhibition of total Ca^{2+} uptake by KN-93 suggests that the excess of free CaM interacts and activates PMCA pumps. **Supported by:** FAPERJ,CAPES.

01.007

CULTURED SKELETAL MUSCLE FIBERS EXPRESS THE CYCLIC AMPADENOSINE PATHWAY

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Introduction: Adenosine (ADO) regulates many physiological processes of skeletal neuromuscular system via pre- and postsynaptic receptors. The source of ADO on synaptic cleft has been related to ATP metabolism, however, extracellular cAMP (cAMP***e***), metabolized by ecto-PDEs and ecto-5-nucleotidases (cAMP-ADO pathway), might be an alternative source of ADO. Considering that cAMP is secreted from muscle fiber after stimulation of adenylyl cyclase (AC; Godinho, *Br J Pharmacol* 138:995, 2003), the aim of this work was to asses whether the skeletal muscle expresses the cAMPADO pathway. **Methods:** Rat cultured skeletal muscle fibers were treated for 30 min with 1-100μM forskolin (FK) or 0.1-100μM isoproterenol (ISO) + 1mM IBMX to

inhibit the PDEs. cAMP was quantified by radioassay. The effect of 100μM probenecid, an inhibitor of organic anion transporters, on ISO-induced cAMP accumulation was also evaluated. The extracelullar metabolism of cAMP was evaluated by incubation of 30nM-100μM cAMP in the medium. The subsequent production of ADO was determined by HPLC. **Results:** FK and ISO increased by 27.4 and 2.13 folds the basal intracellular cAMP (cAMP***i***) (26 pmol/mg protein, n=4), respectively. The FK-dependent increase in cAMP***i*** was followed by a proportional increase in cAMP***e***. Treatment of cells with probenecid reduced by 40% the ISO-dependent-cAMP***e*** accumulation. Finally, incubation of cells with 100μM cAMP for 30 and 60 min was followed by the extracellular generation of ADO (28.5 ± 4.9 and 23.5 ± 3.4 nmol/dish, respectively; n=3) and 30% decrease of incubated cAMP. **Discussion:** Our results show that activation of skeletal muscle fiber AC results in the efflux of cAMP to the extracellular space and its conversion to ADO. The physiological significance of cAMP-ADO pathway in skeletal muscle may be related to the regulation of pre- and postsynaptic events such as quantal ACh release and muscle metabolism. **Supported by:** FAPESP, CNPq

01.008

A METHOD FOR SIMULTANEOUS MEASUREMENT OF cAMP AND cGMP PHOSPHODIESTERASES ACTIVITY BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

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Introduction: Cellular cAMP or cGMP concentrations are determined by the balance between their synthesis by specific enzymes such as adenylyl or guanylyl cyclases and its breakdown to AMP or GMP by cyclic nucleotide phosphodiesterases (PDEs). **Objective:** Development of a selective and rapid method to measure the simultaneous activity of cAMP and cGMP-PDEs using LC-MS/MS. **Method:** Enzymatic reaction was conducted in a mixture containing 50 mM Tris-HCl buffer (pH 8.0), 100 mM $MgCl_2$, 20 μM cGMP or cAMP and partially purified PDE from human washed platelets. Cyclic and non cyclic nucleotides were analyzed by LC-MS/MS operating in positive ion mode by MRM, monitoring the ions m/z 346.0 and 152.2, (cGMP), m/z 363.9 and 152.1 (GMP), m/z 330.0 and 136.1, (cAMP), and m/z 347.9 and 136.3 (AMP) **Results:** The increase in GMP or AMP and the decrease in cGMP or cAMP concentrations showed a direct correlation to the amount of platelet protein (6.0-130.0 μg/ml). Calibration curves showed a very good linearity (r^2=0.999) at the range of 0.25 ng/ml to 10 ng/ml. Previous incubation with sildenafil also showed a dose response curve and 50% of PDE inhibition was obtained with 0.3 μM sildenafil. **Discussion:** A selective, fast and reproducible method to measure PDE enzymatic activity was developed. This method has been successfully used to screen PDE inhibitors obtained by combinatory chemical synthesis. The high selectivity of this method allows the simultaneous determination of different PDEs activity, like those specific for cGMP and cAMP. **Supported by:** FAPESP

01.009

INHIBITON OF PLATELET ADHESION BY LIPOPOLYSACCHARIDE (LPS) DOES NOT INVOLVE NITRIC OXIDE PRODUCTION.

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Introduction: Biological effects of LPS are commonly associated with iNOS activation in most cells examined, but studies about the presence of this enzyme in platelets are controversial. The present work was designed to investigate if the NO-cGMP signaling pathway is involved in the inhibition of platelet adhesion by LPS. **Methods**: The human washed platelet adhesion was evaluated using fibrinogen-coated 96-well microtiter plates. The platelets were maintained in the plate for 15 min. After that, the adherent platelets were incubated with the acid phosphatase substrate (p-Nitrophenyl phosphate disodium) for 1h. The plate was read by a microplate reader set at 405nm. Levels of cAMP and cGMP as well as Nitrate/Nitrite were also measured. **Results:** LPS (0.01-300 μg/ml) dose- and time-dependently inhibited spontaneous platelet adhesion (for 0.1 μg/ml: 15±3 and 45±10% inhibition at 5 an 60 min, respectively; for 10 μg/ml: 25±3 and 58±5% inhibition at 5 an 60 min, respectively). In thrombin (50 mU/ml)-stimulated platelets, LPS also time- and dose-dependently inhibited adhesion. Inhibition of activated platelets by LPS was smaller compared with spontaneous adhesion. LPS-induced platelet inhibition was not accompanied by increased levels of cAMP and cGMP, except at 100 μg/mL of LPS where a 20% increase ($P<0.05$) in cGMP was observed. No enhancement of nitrate/nitrite concentration was detected in LPS-treated platelets. **Conclusion:** The inhibitory effect of LPS on platelet adhesion is not dependent on nitric oxide production. **Supported by:** FAPESP

01.010

ATL-1, A STABLE ANALOG OF LIPOXIN A_4, INDUCES MONOCYTE CHEMOTAXIS VIA MAPK/MLCK PATHWAY

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Lipoxins (LX) are lipid mediators, arachidonic acid metabolites, able to induce monocyte chemotaxis *in vitro* and *in vivo*. Nonetheless, the signaling pathways mediating this process are yet unclear. In this work, we investigated the different mechanisms involved on monocyte activation by 15-epi-16-(*para*-fluoro) phenoxy-lipoxin A_4 (ATL-1), a stable analog of 15-epi-Lipoxin A_4. Monocytes were isolated from human peripheral blood by Ficoll/Percoll gradients. ATL-1-induced chemotaxis was inhibited (≅ 60%) by pertussis toxin (1μg/mL), suggesting an effect via the G-protein-linked LXA_4 receptor. Monocyte migration depends on actin polymerization, a highly regulated process controlled by surface receptors. ATL-1 (1-1000 nM) promoted actin cytoskeleton reorganization in a dose-dependent manner. The pre-treatment of these cells with PD98059 (10μ M), a specific inhibitor of the MEK pathway, partially decreased ATL-1-induced chemotaxis (≅ 60% of inhibition). Also, an immunocytochemistry assay showed an increase on ERK-2 phosphorylation in the cells stimulated with ATL-1, suggesting the involvement of this signaling pathway in this process. ERK-2 nuclear translocation

was not observed after ATL-1 treatment, pointing to a cytoplasmatic action of this kinase, possibly myosin light chain kinase (MLCK) activation. We observed that ATL-1 (100 nM) induced a MLCK phosphorylation (1.5 fold of increase) in a MAPK-dependent manner. Together, these results indicate that the LX analog is a potent monocyte chemoattractant, acting via MAPK/MLCK pathway. **Supported by:** FAPERJ, CNPq, SR-2/UERJ.

01.011

LIPOXIN, LEUKOTRIENE AND VEGF RECEPTORS CROSS-TALK ON ENDOTHELIAL CELLS

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Introduction: Lipoxygenase products of arachidonic acid exert pleiotropic effects in biological systems. Leukotrienes are significant regulators of leukocyte trafficking and cell growth. We have shown that lipoxins (LX), which are generated by the sequential action of lipoxygenases, inhibit vascular endothelial growth factor-(VEGF) induced endothelial cells (EC) proliferation and migration in a MAP kinase-dependent manner. Furthermore, EC express G-protein-coupled receptors (GPCR) for LXA_4 and LTD_4 and both agents can activate MAP kinases. In this work, we sought to investigate a cross-talk between GPCR and tyrosine kinase receptors in EC. **Methods:** Human umbilical vein endothelial cells (HUVEC) were treated with different concentrations (0.1-100 nM) of 15-epi-16-(*para*-fluoro)-phenoxy-lipoxin A_4 (ATL-1) and after 72h cells were enumerated using the MTT assay (n=4, triplicates). VEGF receptor (KDR) phosphotyrosine content was assessed by anti-phosphotyrosine immunoblotting of KDR immunoprecipitates after LTD_4 (10nM) or ATL (100nM) treatment (n=3). **Results:** The mitogenic action of LTD_4 (10 nM) on EC is antagonized by exposure of the cells to ATL-1, with an IC_{50} of ~ 3 nM, suggesting that LX can modulate LT actions on EC *in vitro*. Binding of VEGF to KDR leads to transphosphorylation of several cytoplasmic signaling proteins and this activation was significantly inhibited by pre-exposure of EC to ATL-1 (100nM). **Conclusion:** The data suggest that LX receptor activation is coupled with inactivation of KDR and possibly LT receptor on EC and point to a complex cross-talk between different mediators of the angiogenic process. **Supported by:** FAPERJ, CNPq and SR-2/UERJ

01.012

TISSUE DISTRIBUTION OF α_{1a} ADRENOCEPTOR SPLICE VARIANTS IN HUMAN AND RHESUS MALE REPRODUCTIVE TRACT

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Aim: Several α_{1a} adrenoceptor (α_{1a}-AR) splice variants, differing in their C-terminal regions, have been identified in human and other species. Our laboratory has also recently characterized the nucleotide sequence of different α_{1a}-AR variants in rhesus monkey (Patrão MTCC et al., Anais do Congresso SBFTE, p.173, 2003). The aim of the present study was to characterize the tissue distribution of α_{1a}-AR splice variants in human and rhesus male reproductive tract. **Methods and Results:** RT-PCR was performed using specific primers against different α_{1a}-AR splice variants and total RNA (5 μg) from adult rhesus (10-12-year old) and human (58-83-year old) testis (T), seminal vesicle (SV), epididymis (E) and prostate (P). All total RNAs were kindly obtained from Dr. F.S. French (UNC-Chapel Hill). The following tissue distribution was observed:

Tissues	Human α_{1a} splice variants	Rhesus α_{1a} splice variants
T	$\alpha_{1a\text{-}1}$, $\alpha_{1a\text{-}2a}$, $\alpha_{1a\text{-}2c}$, $\alpha_{1a\text{-}3a}$, $\alpha_{1a\text{-}3c}$	$\alpha_{1a\text{-}1}$, $\alpha_{1a\text{-}2a}$, $\alpha_{1a\text{-}2c}$, $\alpha_{1a\text{-}3}$ (variants 1 and 4)
E	$\alpha_{1a\text{-}1}$, $\alpha_{1a\text{-}2a}$, $\alpha_{1a\text{-}2c}$, $\alpha_{1a\text{-}3a}$, $\alpha_{1a\text{-}3c}$	$\alpha_{1a\text{-}1}$, $\alpha_{1a\text{-}2c}$, $\alpha_{1a\text{-}3}$ (variants 1 and 4)
SV	$\alpha_{1a\text{-}1}$, $\alpha_{1a\text{-}2a}$, $\alpha_{1a\text{-}2c}$, $\alpha_{1a\text{-}3a}$, $\alpha_{1a\text{-}3c}$	$\alpha_{1a\text{-}1}$, $\alpha_{1a\text{-}2a}$, $\alpha_{1a\text{-}2c}$, $\alpha_{1a\text{-}3}$ (variants 1, 2, 3 and 4)
P	$\alpha_{1a\text{-}1}$, $\alpha_{1a\text{-}2a}$, $\alpha_{1a\text{-}2c}$, $\alpha_{1a\text{-}3a}$, $\alpha_{1a\text{-}3c}$	$\alpha_{1a\text{-}1}$, $\alpha_{1a\text{-}2a}$, $\alpha_{1a\text{-}2c}$, $\alpha_{1a\text{-}3}$ (variants 1, 3 and 4)

Conclusion: The results indicate that α_{1a}-AR splice variants are expressed throughout the primate male reproductive tract. The expression of different variants in a same tissue may suggest important physiological role for α_{1a} splicing mechanisms.

Supported by: FAPESP, CNPq, TW Fogarty International.

01.013

HEME INHIBITS HUMAN NEUTROPHIL APOPTOSIS MODULATING THE EXPRESSION OF BCL-2 FAMILY PROTEINS

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Objective: Our group have previously shown that heme is a proinflammatory molecule able to delay human neutrophil spontaneous apoptosis involving a number of signaling pathways (Graça-Souza *et al*, 2002, Arruda *et al*, 2004). These features may corroborate for the development of local or systemic chronic inflammation during hemolytic episodes, which are characterized for high levels of free heme in circulation. In this study, we aim to evaluate the effect of free heme upon the expression the Bcl-2 family members. **Methods and Results:** Human peripheral blood neutrophils incubated wilth heme (3 μM) displayed a decrease in the Bad/Bcl-X_L ratio as accessed by Western blot analysis. The expression of Bcl-X_L was maximal after 4 hours of incubation (358% of control). Bad levels, however, dramaticaly decreased, reaching lower levels after 30 minutes (5% of control) and being restored after 60 minutes. These events seems to be modulated by NADPH oxidase-derived reactive oxygen species, as well as PI-3 kinase and MAP kinase signaling cascades. The involvement of heme oxygenase activity and NF-kB pathways on these phenomena are under investigation. **Conclusion:** These results points to the relevance of anti- and pro-apoptotic proteins balance on heme anti-apoptotic effects upon human neutrophils.

Supported by: SR-2/UERJ, CNPq, CAPES, FAPERJ.

01.014

EFFECTS OF SODIUM NITROSSIDE (SNP) ON PLATELET ADHESION TO FIBRINOGEN

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Introduction: Nitric oxide (NO) inhibits human platelet adhesion by cGMP-dependent mechanisms, but cGMP-independent mechanisms may also play a role. In this study, we investigated the inhibitory mechanisms of SNP on platelet adhesion to fibrinogen. **Methods:** Human washed platelet adhesion was evaluated using fibrinogen-coated 96-well microtiter plates. Platelets were maintained in the plate for 15min. Adherent platelets were incubated with the acid phosphatase substrate (p-nitrophenyl phosphate disodium) for 1h. The plate was read by a microplate reader set at 405 nm. Cyclic GMP levels were measured using an enzyme immunoassay kit. Nitrated proteins were analyzed by immunobloting. **Results:** SNP (0.11.0mM) significantly inhibited platelet adhesion to fibrinogen in both non-stimulated and thrombin-stimulated platelets. In both of these conditions, soluble guanylate cyclase inhibitor ODQ (10μM) reversed the inhibition by SNP at lower concentration (0.1mM), without affecting the response at higher SNP concentration (1mM). The elevations of cGMP levels caused by both concentrations of SNP were decreased by ODQ. Pre-incubation of platelets with superoxide dimutase (SOD, 100U/mL) reversed by 33% and 66% the effect of SNP at 0.1 and 1mM, respectively. Epigalocatechin gallate did not affect the effect of SNP at 0.1mM, but reduced by 50% the effect at 1mM SNP. Immunobloting indicated the presence of nitrated protein in SNP (1mM) treated platelets. **Conclusions:** Cyclic GMP-independent mechanisms contribute to inhibition of platelet adhesion by SNP at high concentrations. **Supported by:** CAPES and FAPESP

01.015

STRUCTURAL ANALYSIS OF PHOSPHOLIPIDS OF SMOOTH MUSCLE CELLS OF AORTA AND MESENTERIC ARTERIES OF SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS.

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Introduction: Mutiple cell membrane alterations have been described in humans and other animals with various forms of hypertension. The spontaneously hypertensive rat (SHR) is an interesting model to study the correlation between cell membrane fluidity and the activity of transport systems, such as Na+/K+ATPase and different K+ channels, since it has been shown that the activities of these systems are different in conductance (aorta) and in resistance (mesenteric) arteries of SHR. **Objectives:** To investigate the phospholipid composition and structure of smooth muscle cell membranes of aorta and mesenteric arteries of spontaneously hypertensive (SHR) and normotensive Wistar (NWR) and Wistar-Kyoto (WKY) rats by a combination of HPTLC and GC-MS techniques. **Results and Conclusions:** The lipid compositions of the cell membranes of aorta and mesenteric arteries of SHR, NWR and WKY were not significantly different, containing the following phospholipids, which were isolated and analyzed: phosphatidylethanolamine, phosphatidylcholine, sphingomyeline, phosphatidylserine and phosphatidylinositol. This indicates that differences in membrane fluidity are not responsible for the previously reported differences in the activities of the transport mechanisms in the vascular smooth muscle cell membranes from resistance and conductance vessels of the three animal strains. **Supported by:** FAPESP, CNPq, CAPES and CEFET AM.

01.016

VLO5, A HETERODIMERIC VGD/MLD-PEPTIDE LIGAND OF a_9 / a_4b1 INTEGRIN INHIBITS NEUTROPHIL APOPTOSIS

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During inflammation, different cytokines and adhesion molecule interactions can accelerate or delay PMN survival, interfering in the resolution of this process. Integrin-mediated downstream signals modulate survival in different cells, activating intracellular pathways, as PI3K and MAPK, which interfere with the balance between BclxL and Bad. We have shown that disintegrins, peptides ligands of a2 or a9 integrins, activate integrin-coupled signaling in PMN, interfering in the apoptotic processes. VLO5 is a MLD/VGD disintegrin that was shown to activate integrin signaling pathways in PMN, inducing FAK activation, cytoskeleton mobilization and chemotaxis. In this study, we evaluated the effect of VLO5 on human PMN apoptosis and the involvement of PI3K and MAPK pathways and superoxide (O^{-2}) production. PMN were incubated with VLO5 (1mM) and apoptosis was evaluated morphologically (18h-microscopy), DNA fragmentation (8h-agarose gel) and Bad degradation (30 min-blotting). VLO5 potently inhibited spontaneous apoptosis and induced PI3K activation and ERK2 nuclear translocation. In agreement, PI3K and ERK2 inhibitors reverted VLO5 effect, accelerating PMN apoptosis. Although, discrete O^{-2} production induced by VLO5 might contribute to its anti-apoptotic effect since DPI, an inhibitor of oxidative burst, partially reverted it. The data suggest that interaction of VLO5 with PMN integrin might be related with its anti-apoptotic effect, which is dependent on PI3K and ERK2 activation and O^{-2} production. **Supported by:** FAPERJ, CNPq, IFS-Sweden

01.017

EFFECT OF OVARIECTOMY ON INTRACELLULAR SIGNALING PATHWAYS LINKED TO ACTIVATION OF MUSCARINIC RECEPTORS IN RAT HIPPOCAMPUS

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Introduction: In our laboratory, we have shown that ovariectomy up-regulates muscarinic receptors (mAChR) in rat hippocampus. This effect was reversed by estradiol (Souza et al., XXXIV Congresso SBFTE: pp.171, 2002). We now report the effect of ovariectomy on intracellular signaling pathways linked to activation of mAChR in rat hippocampus. **Methods:** The intracellular [^{3}H]-inositol phosphates content were measured in hippocampus from rats in proestrus (control) and ovariectomized (15 days) as described by Abdalla et al. (Mol. Cel. Endocr.160:2, 2000) **Results:** The ovariectomy did not change the basal level of total [^{3}H]-inositol phosphates (105.4±10.8 dpm/mg tissue). Carbachol caused a concentration-dependent rise on the accumulation of total [^{3}H]-inositol phosphates in hippocampus from ovariectomized rats when compared with control rats. The maximum response to carbachol was 2-fold higher in hippocampus from ovariectomized rats than from control rats, respectively, 59.5±12.4, n=19, and 26.0±11.8% above basal level, n= 8, $P< 0.05$. This effect was reversed by pirenzepine (M_1) or pfHHSiD (M_3 selective antagonist) in both experimental groups. On the other hand, methoctramine (M_2) or tropicamide (M_4 selective antagonist) had no effect. **Conclusion:** The results suggest that ovariectomy modulates the intracellular signaling pathways linked to activation of mAChR in rat hippocampus. **Supported by:** FAPESP

01.018

DETERMINAÇÃO DA POTÊNCIA ANTIAGREGANTE PLAQUETÁRIA E DO POTENCIAL ANTITROMBÓTICO DE NOVOS COMPOSTOS TIENILACILIDRAZÔNICOS

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Objetivo: A relevante atividade antiagregante plaquetária de compostos tienilacilidrazônicos frente a diversos agonistas fisiológicos foi anteriormente descrita (Brito *et al.*, SBFTE 2003). Neste trabalho determinou-se a potência antiagregante plaquetária de uma nova série desses derivados (LASSBio 785 LASSBio 789), frente ao colágeno e ao ácido araquidônico (AA) em plasma rico em plaquetas (PRP) citratado de coelhos, a fim de contribuirmos com o estudo da relação estrutura - atividade. Além dos estudos *in vitro*, descrevemos também resultados preliminares indicando um potencial antitrombótico para esses compostos, através da determinação do tempo de sangramento em camundongos. **Métodos e Resultados:** A potência antiagregante plaquetária foi avaliada *in vitro* em PRP citratado de coelhos, onde a agregação foi induzida por AA (200 μM) e colágeno (5 μg/ mL) e monitorada através do método turbidimétrico. O tempo de sangramento foi avaliado através de uma incisão de aproximadamente 5 mm na cauda de camundongos, previamente anestesiados com pentobarbital 25 mg/kg intra-peritoneal (i.p). Em intervalos de 15 s encosta-se papel de filtro, 2 vezes no local da lesão, até que não haja sinal de sangramento no papel. Os compostos foram administrados 1 h antes da realização da incisão, i. p. na dose de 300 μmol/kg. Os compostos apresentaram valores de CI_{50} na faixa de 0,90 ± 0,6 mM (LASSBio 785) a 21,06 ± 0,2 μM (LASSBio 787) frente ao colágeno, e de 0,018 ± 0,6 μM (LASSBio 786) a 20,88 ± 0,4 μM (LASSBio 786) frente ao AA. O tempo de sangramento foi aumentado em 29,4%* e 88,9 %*, com a administração dos compostos LASSBio 785 e 789, respectivamente (*p<0,05). **Conclusão:** Os resultados obtidos demonstram a potente atividade antiagregante plaquetária dos derivados e sugerem um perfil antitrombótico para os mesmos. Esse trabalho corrobora com resultados anteriores para essa série e permite destacar compostos, LASSBio 785 e 789, como novos candidatos a protótipos de agentes antiplaquetários e/ou antitrombóticos. **Apoio Financeiro:** PRONEX, CAPES, FAPERJ, FUJB, CNPq

01.019

DETERMINAÇÃO DO PERFIL DE SELETIVIDADE DO COMPOSTO LASSBIO 772 FRENTE A BIORECEPTORES ACOPLADOS À PROTEÍNA G EM TRAQUÉIA DE COBAIA

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Objetivo: Em comunicação anterior relatamos o perfil farmacológico de LASSBio 772, como um novo antagonista α_{1A}/α_{1D}, aquiral, com índices de seletividade frente ao subtipo α_{1B} (α_{1B}/α_{1A}: 1700 e α_{1B}/α_{1D}: 32000) compatíveis com sua utilização, no tratamento dos sintomas do trato urinário inferior (STUI) e hiperplasia benigna prostática (HBP) (Silva *et al.*, SBFTE 2003). Neste trabalho descrevemos a determinação do perfil de seletividade de LASSBio 772, derivado *N*-fenilpiperazínico obtido a partir do safrol, frente a bioreceptores acoplado à proteína G - β-adrenérgicos (β_2), colinérgicos muscarínicos (M_1) e histaminérgicos (H_1) em traquéia de cobaia. **Métodos e Resultados:** Utilizando strips de traquéia de cobaia (250 400g), avaliou-se a atividade do composto LASSBio 772 (3 nM - 30 μM) frente às contrações induzidas por carbacol (10 μM) e histamina (100 μM), assim como ao relaxamento produzido por salbutamol (10^{-10} - 3,3 x10^{-5} M). Os resultados mostraram que o derivado não foi capaz de inibir significativamente as contrações induzidas por carbacol (n = 3; % de contração = 122,1 ± 8,6) e também não apresentou efeito inibitório sobre o relaxamento produzido por salbutamol

em traquéia pré-contraída com carbacol (n= 4; % máxima de relaxamento = 96,7 ± 3,3). A avaliação de LASSBio 772 (3 nM 30 μM), frente às contrações induzidas por histamina (100 μM), evidenciou perfil inibitório significativo às contrações, apresentando CI_{50} = 309 nM. **Conclusão:** Esses resultados corroboram o perfil de seletividade antagonista α_1-adrenérgica para LASSBio 772, dissociado de efeitos em outros receptores *e.g.* colinérgicos muscarínicos e/ ou β_2 adrenérgicos. O efeito observado sobre os receptores H_1 não o torna não-seletivo, tendo em vista o índice de seletividade (I.S. H_1/a_{1A}) *ca.* 1000 vezes para o efeito antagonista no adrenoceptor α_{1A} (CI_{50} = 0,26 nM) em relação ao efeito encontrado para H_1. **Apoio Financeiro:** PRONEX, CAPES, FUJB, CNPq, UCB

01.020

OUABAIN MODULATES NUCLEAR FACTOR NF-kB IN THE CENTRAL NERVOUS SYSTEM

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Introduction: Studies have demonstrated the presence of endogenous ouabain in mammalian tissues of peripheral and central nervous system (CNS). NF-kB plays crucial roles in nervous tissue including potential roles in long-term responses to synaptic plasticity, pro- or antiapoptotic effects during developmental cell death, and neurodegenerative disorders. Our aim was to test whether ouabain modulates NF-kB in different areas of brain. **Materials and Methods:** Adult male Wistar rats received ouabain (10 nM) by indwelling cannulas in lateral cerebroventricular and hippocampus. Hypothalamus, hippocampus, pre-frontal cortex, and dorsal striatum were isolated 1 h after ouabain injection and submitted to nuclear protein extraction. Gel mobility shift assay was used to measure changes in NF-kB activity. Na,K-ATPase activity was measured by colorimetric assay. Results: Intracerebroventricular (icv) infusion of ouabain activated NF-kB in pre-frontal and hypothalamus but not in dorsal striatum. The infusion of ouabain by icv or intrahippocampal induced a decrease in NF-kB binding in hippocampus. No change in Na,K-ATPase was observed in all structures tested. **Discussion:** Our results suggest a role for ouabain, as a modulator of NF-kB in CNS. **Financial support:** FAPESP, CNPq and Bunka grant/Sumitomo Bank.

01.021

IMPORTÂNCIA DOS NÍVEIS DE GLUTATIONA NA CITOTOXICIDADE CAUSADA PELO TAXOL E PELA VINCRISTINA EM CÉLULAS LINFOBLÁSTICAS LEUCÊMICAS

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Introdução: Níveis adequados de glutationa reduzida (GSH) são cruciais para o funcionamento de importantes sistemas de defesa contra agentes pró-oxidantes, o que suprime a apoptose mediada por quimioterápicos. Assim, os objetivos desse trabalho foram: avaliar o efeito citotóxico do taxol e da vincristina associados ou não a ciclofosfamida e analisar o efeito desses compostos sobre os níveis de GSH. **Métodos e Resultados:** Células leucêmicas CEM foram incubadas por 24 horas com taxol ou com vincristina (1-10 m M; agentes despolimerizantes de microtúbulos) na presença ou na ausência de cilclofosfamida (1-10 m M; um agente alquilante, inibidor de síntese proteica). A viabilidade celular foi avaliada pelo método do MTT, e os níveis de glutationa GSH intracelular foram dosados pelo método bioquímico proposto por Tietze (1969). Nossos resultados demonstraram que o taxol e a vincristina reduziram 25% ± 3 e 30% ± 3 do número de células viáveis, respectivamente, quando comparados ao controle (100% de células viáveis). Quando associados a ciclofosfamida houve uma potencialização do efeito citotóxico de 71% ± 4 e 40% ± 2, respectivamente. Observou-se que a vincristina e a ciclofosfamida, isoladas ou associadas, não reduzem os níveis de GSH, entretanto, o taxol depletou 30 % ± 2 e quando associado a ciclofosfamida 92% ± 5 dos níveis de GSH. **Conclusão:** Nossos resultados sugerem que o taxol e a vincristina são citotóxicos para as células linfoblásticas leucêmicas CEM, e, como os níveis de GSH representam um mecanismo de resistência celular a apoptose, quando foram depletados pelo taxol houve um efeito citóxico maior quando esse foi associado a ciclofosfamida. **Apoio Financeiro:** FUNPESQUISA-2003

01.022

FKBP12 IS POSSIBLY A KEY PROTEIN INVOLVED IN RAT VAS DEFERENS FUNCTION

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Introduction: It has been demonstrated that FKBP12 is responsable for stabilization of intracellular Ca^{2+} release channels (CRC) activity of tissues like skeletal and cardiac muscle.The disruption of the complex FKBP12-CRC is being related to malignant hyperthermia and congestive heart failure.Previous work showed that temperature treatment of rat vas deferens (RVD) preparations decreases sarcoplasmic reticulum (SR) Ca^{2+} content due to an increase of Ca^{2+} leak by ryanodine receptors (RyR).The aim of the present work was to investigate the presence and the possible contribution of FKBP12 in RVD function. **Methods**: The tissue was washed, homogenized and ultracentrifuged to obtain crude homogenate or microsomes for biochemical studies.To dissociate FKBP12 from RyR, preparations were treated at 37°C for 30 min and ultracentrifuged.The hole RVD or the epididymal half were previous incubated with DMSO 0.4% or rapamycin 20μM before the addition of increased phenylephrine concentrations. **Results**: Western blotting assays showed that FKBP12 was detected in crude but not in treated homogenate (n=3).Treatment did not change neither Kd nor Bmax of [^{3}H]ryanodine in high (n=3) and low calcium conditions (n=2).As observed with temperature treatment,rapamycin diminished the SR Ca^{2+} content (n=2).Preliminary assays showed rapamycin induced contraction followed by a decrease of phenylephrine potency. **Discussion**: Data suggest that FKBP12 association with RyR contributes to the normal function of RVD. *In vivo* assays are in course to investigate the role of this immunophilin in male fertility. **Supported by:** CAPES,FAPERJ

01.023

RELAÇÃO ESTRUTURA-ATIVIDADE DE DERIVADOS DO [2-(4-BENZAMIDO) ETIL] BENZILDIMETILAMÔNIO NA TRANSMISSÃO NEUROMUSCULAR

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Introdução: A relação entre a estrutura química e o bloqueioneuromuscular produzido por uma série de brometos de [2-(4-benzamido)etil] benzildimetilamônio foi avaliada com os análogos *para*-substituídos: 2-(4-bromo) (Br-Br), 2-(4-butil) (Butil-Br) e 2-(4-hexil) (hexil-Br) em preparações neuromusculares de camundongos. **Material e Métodos**: Registros de contração do diafragma isolado de camundongo induzida por estimulação elétrica nervosa foram utilizados para determinar as ações dos derivados Br-Br (10^{-4} a $7{,}5x10^{-4}$M), Butil-Br (10^{-5} a 10^{-4} M) e hexil-Br (10^{-5} a 10^{-4} M) na transmissão neuromuscular. A interação dos derivados com o receptor nicotínico muscular foi analisada em curvas de competição com [^{125}I]-α-bungarotoxina ([^{125}I]-BUTX: 1 nM, 30 min, 25°C) em membranas extraídas de diafragma inervado de camundongo. **Resultados**: Após 30 min de incubação os compostos bloquearam as contrações do diafragma proporcionalmente às concentrações com as seguintes CI_{50}: Br-Br = $5{,}0x10^{-4}$ M; butil-Br = $2{,}4x10^{-5}$ M; hexil-Br = $1{,}2x10^{-5}$ M. A incubação de concentrações crescentes dos derivados em amostras de membrana muscular reduziu a ligação específica da [^{125}I]-BUTX com CI_{50} de: Br-Br= $5{,}78x10^{-6}$ M [LC: $2{,}31x10^{-6}$ 1,45 x 10^{-5} M]; butil-Br= $20{,}9x10^{-5}$ M [LC: $5{,}18x10^{-5}$ $84{,}2x10^{-5}$ M]; hexil-Br = 28 x 10^{-5} M [LC: $4{,}8x10^{-5}$ $169x10^{-5}$ M). A interação com o sítio do agonista dos três análogos foi 10^{4} a 10^{6} vezes menor que a da BUTXfria (CI_{50} = $3{,}85x10^{-10}$ M [LC: $2{,}4x10^{-10}$ $6{,}14x10^{-10}$ M]). **Conclusão**: A potência relativa dos derivados no bloqueio da transmissão neuromuscular foi: hexil-Br = butil-Br>Br-Br, enquanto que ada interação com o sítio ligante da BUTX foi: Br-Br>butil-Br = hexil-Br. Estes dados indicam que o bloqueio neuromuscular produzido pelos análogos não parece estar diretamente relacionado à interação com o sítio da ACh no receptor nicotínico. **Apoio Financeiro:** FAPESP, CNPq, CAPES

01.024

α_1-ADRENOCEPTORS (α_1-AR) IN THE RAT TAIL ARTERY

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Introduction: Adrenoceptor agonists contract vascular tissues through the activation of three α_1-AR subtypes (α_{1A}, α_{1B} and α_{1D}). The rat tail artery (RTA) has been used as a model for the study of contractions mediated by α_{1A}-ARs. However, the sole participation of α_{1A}-AR has been challenged by the detection of a significant component mediated by α_{1B}-ARs (Jähnichen, *EJP*, 488:157, 2004). **Objective:** To investigate the role of additional subtypes in the contractions of the RTA to adrenergic agonists. **Methods:** RTAs were excised from male Wistar rats (250-350g) cleaned and mounted in organ baths for digital recording of isometric contractions in presence of cocaine (10μM) corticosterone (10μM) propranolol (0.1μM) and yohimbine (0.1μM). **Results:** Noradrenaline could not be used as agonist to contract the RTA due to the strong activation of α_2-ARs. In the other hand, the contractions of the RTA to phenylephrine were antagonized with high affinity by prazosin (pK_B≅9.6) and 5-methyl-urapidil (≅8.5), consistent with α_{1A}-ARs. The antagonism presented by BMY-7378 against phenylephrine was characterized by a biphasic Schild plot due to the effectiveness of low concentrations of this antagonist (3-30nM). This result suggests that in addition to α_{1A}-ARs, the α_{1D}-subtype is also functional in the RTA. When the selective α_{1A}-agonist oxymetazoline was used to contract the RTA, low concentrations of BMY-7378 were ineffective and the resulting Schild plot showed slope not different from 1.0 (pK_B≅6.5). **Conclusion:** Multiple α_1-ARs subtypes are functional in the RTA and care should be taken when choosing the adrenoceptor agonist in this artery. **Supported by:** FAPESP

01.025

EFFECTS OF NORADRENALINE MEDIATED BY SINGLE POPULATIONS OF α_1-ADRENOCEPTORS (α_1-AR)

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Introduction: Radioligand binding and mRNA studies have shown that smooth muscles often co-express α_1-ARs subtypes. Despite this, some rat tissues have been used as "models" for contractions to phenylephrine or methoxamine mediated by single populations of α_{1A}-(vas deferens, RVD), α_{1B}- (spleen, RS) and α_{1D}-ARs (aorta, RA). **Objective:** To develop a protocol using noradrenaline as the agonist, since this ligand shows maximal efficacy at all α_1-AR subtypes. **Methods:** Tissues were excised from male Wistar rats (250 to 350g) cleaned and mounted in organ baths for recording of isometric contractions in presence of cocaine (6μM) corticosterone (10μM) propranolol (0.1μM) and yohimbine (0.1μM). **Results:** The contractions of the RVD and RA to noradrenaline were readily identified as mediated by α_{1A}- and α_{1D}-ARs, respectively, according to the affinities found for prazosin (pK_B≅9.4 in both organs), 5-methyl-urapidil (≅8.8 and ≅7.8) and BMY-7378 (≅7.0 and ≅8.5). The contractions of the RS to noradrenaline were antagonized with low potency by prazosin suggesting activation of α_2 in addition to α_1-ARs. Concentrations of yohimbine up to 10μM did not eliminate the α_2-ARs. However, the use of idazoxan (3μM) increased the potency of prazosin (pK_B≅8.9) indicating successful elimination. The antagonists BMY 7378 (≅6.4) and 5-methyl-urapidil (≅6.3) presented low affinities against noradrenaline in the RS in accordance with the activation of α_{1B}-ARs. **Conclusion:** The described protocol allows the use of noradrenaline in these tissues for the comparative study of the selectivity and efficacy of new ligands for α_1-ARs. **Supported by:** FAPESP and CAPES

01.026

IDENTIFICATION OF A FUNCTIONAL DITHIOTREITOL-SENSITIVE ANGIOTENSIN II (AII) RECEPTOR IN THE SOUTH AMERICAN RATTLESNAKE

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Introduction: A pharmacologically distinct AT_1 / AT_2 receptor among the reptiles was first described in *B.jararaca* (*Viperidae*) snake. In some rodents, avian, fish and amphibian species a functional non AT_1/AT_2 receptor was also identified. In mammalian AII is known to interact with specific cell surface AT_1 and AT_2 receptors, to produce multiple biological functions. The aim of this study is to investigate the AII receptor in another member of *Viperidae* family belong to a different snake genus, *Crotalus*. **Method and Results:** Isometric tension, induced by [Asp^1, Ile^5]AII and [Asn^1, Val^5]AII in aortic ring from *Crotalus durissus terrificus* (rattlesnake) was evaluated. The pD_2 and E_{max} were calculated from cumulative angiotensin concentration-effect curves. Both AII analogs produce a similar maximum vasoconstrictor effect, being [Asn^1, Val^5]AII (pD_2 6.26, n=7) less potent than [Asp^1, Ile^5]AII (pD_2 7.85, n=5). Pre-treatement of the aorta with dithiotreitol (3 mM, n=3) completely abolished the AII response (10^{-10} - 10^{-6} M). **Discussion:** Our data have shown a vasoconstrictor effect induced by AII in the *C.d.terrificus* aorta. The low potency of [Asn^1, Val^5]AII is in agreement with our previous data in *B.jararaca*, but differ from those obtained in other vertebrates where both AII analogs were equipotent. Although the dithiotreitol effect suggest the presence of an AT_1 receptor in the rattlesnake, selective AT_1 and AT_2 receptor antagonists should be used to clarify the type of AT receptor in this snake. **Supported by:** CAE and TGN are FUNDAP and CNPq/PIBIC fellowships, respectively.

01.027

EXPRESSION OF CARDIAC Na^+/K^+-ATPase α SUBUNIT ISOFORMS IN CHRONIC L-NAME-TREATED WISTAR-KYOTO (WKY) RATS

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Introduction: Three Na^+/K^+-ATPase α subunit isoforms are present in rat heart but the α_3 isoform is usually undetectable in adult stage. In models of hypertension/cardiac hypertrophy, rat heart exhibits a selective decrease in α_2 expression. However, we have shown that in stroke-prone spontaneously hypertensive rats (SPSHR) the density of cardiac α_2 is not altered but that of α_3 is very low compared to normotensives (WKY rats), which have an unexpectedly high cardiac α_3 level. We now study the expression of ventricular Na^+/K^+-ATPase α isoforms in WKY rats made hypertensive by chronic L-NAME treatment. **Methods and Results:** L-NAME-treated WKY rats (4-5 weeks, 50 mg/L in drinking water) developed significantly higher systolic blood pressure (253±8 *vs* 155±11 mmHg in control WKY), with mild but significant cardiac hypertrophy (less than 20%). Densitometric analysis of Western blots using specific antibodies revealed that in crude ventricular membrane preparations α_1 Na^+/K^+-ATPase was not modified by L-NAME (0.90±0.10 *vs* 1±0.10 in control WKY). In contrast, α_2 density was half of the control (0.47±0.07 *vs* 1±0.17 in control WKY, p=0.026) and α_3 was poorly detectable in most L-NAME samples. **Discussion:** According to our results, the global cardiac $\alpha_{2/3}$ density is reduced but the $\alpha_{2/3}$ profile seems to vary with the type of hypertensive model. Possibly the degree of hypertension and hypertrophic response are essential for the homeostatic balance of Na^+/K^+-ATPase isoform expression in the rat heart. **Supported by:** FRSM, CNPq, FAPERJ

01.028

Ca^{2+}-ATPases IN HYPERTROPHIC HEARTS TRIGGERED BY CARDIAC SELECTIVE OVEREXPRESSION OF MUTANT SELF-ACTIVATED α_{1B} ADRENOCEPTORS

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Introduction: Distinct models of cardiac hypertrophy have shown an adaptive decline of $SERCA_2$ expression and Ca^{2+} uptake ability and increase of PMCA activity, but the single contribution of overload and hypertrophy is not understood. In this study we used a model of hypertrophy without overload due to cardiac overexpression of mutant α_{1B} adrenoceptor in order to examine the heart expression and activity of Ca^{2+}-ATPases. **Methods and Results:** In order to check phenotypic differences in transgenics, heart wt/body wt ratio (20-30% greater in transgenic (AP) than control (BP), $p<0.05$) and [^{3}H]prazosin binding assays (B_{max} 70% greater in AP than BP, $p<0.05$) were measured. Crude heart membrane preparations were submitted to Ca^{2+}-ATPase activity experiments and Western blots using anti-$SERCA_2$ specific antibody. Thapsigargin-sensitive (SERCA) and -resistant (PMCA) activities were significantly lower (47% compared to BP, $p<0.05$) and higher (38% compared to BP, $p<0.05$), respectively. Specific bands for $SERCA_2$ were detected in both groups. **Discussion:** Though the phenotypic characteristics are consistent with a mild/intermediate cardiac hypertrophy and α_{1B} adrenoceptor overexpression, expression of $SERCA_2$ does not appear to be modified. Thus, lower SERCA activity might be caused by altered pump regulation. Densitometric analysis and PMCA immunoblot (to compare with PMCA activity) are under way. **Supported by:** FAPESP, FAPERJ, CAPES, CNPq

01.029

RECEPTORES PERIFÉRICOS PARA BENZODIAZEPÍNICOS NO TUMOR ASCÍTICO DE EHRLICH (TAE) E PROLIFERAÇÃO DE CÉLULAS TUMORAIS

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Introdução: Os receptores periféricos para benzodiazepínicos (PBR) têm sido relacionados à proliferação, agressividade e potencial metastático de células tumorais. Este estudo avaliou a presença destes receptores no TAE e os efeitos de ligantes deste receptor (diazepam e PK11195) sobre a proliferação destas células. **Métodos:** *Imunoistoquímica*: Células fixadas, processadas em agarose e em parafina foram utilizadas. Usou-se anticorpo anti-PBR, kit LSAB DAKO, DAB e coloração por HE. *Citometria*: Células fixadas foram incubadas com anti-PBR e anti-IgG FITC. A leitura foi realizada em Citômetro de Fluxo e a análise no Cell Quest. *MTT*: $5x10^4$ céls/poço foram incubadas em estufa a 37° C, 5% de CO_2 por 48 hs com etanol 0,01% (C1), etanol 0,001% (C2), diazepam 10 μM (T1), 1 μM (T2), 100 nM (T3), 10 nM (T4), PK 11195 100 nM (T5), 10 nM (T6), 1 nM (T7).A proliferação foi analisada por meio da técnica de MTT e a leitura foi realizada em ELISA (resultados em D.O). **Resultados:** A presença de receptores para PBR em células do TAE foi demonstrada por imunoistoquímica (n=7) e por citometria (n=9, 85,53 ± 12,60 %) entretanto, não foi possível observar efeitos dos ligantes de PBR sobre proliferação tumoral *in vitro* C1(1,28±0,17), T1(1,23±0,16), T2(1,04±0,0,16), C2(1,33±0,18), T3(1,38±0,11), T4(1,36±0,20), T5(1,46±0,07), T6(1,35±0,09), T7(1,28±0,21), n=4, para p<0,05. **Conclusão:** Este trabalho descreve, por vez primeira, a presença de PBR em células do TAE. Sugere-se que nas doses utilizadas os ligantes não tenham produzido efeitos sobre a proliferação destas células. **Apoio Financeiro:** FAPESP (02/04975-1 e 99/04228-7)

01.030

STUDIES ON THE INTERACTION OF NEOSTIGMINE AND VECURONIUM AT THE RAT NEUROMUSCULAR JUNCTION.

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Introduction: The cellular mechanisms underlying the effects of neostigmine, applied alone or in the presence of vecuronium, were studied *in vitro*. **Methods:** The sciatic nerve-*extensor digitorum longus* muscle of the rat was used. Endplate potentials (epps) were elicited in trains (50 Hz for 5s.) by means of repetitive electrical pulses applied to the sciatic nerve via a bipolar platinum electrode. The epps were recorded intracellularly and analysed in: amplitude of first and plateau epps in the train; quantal content (QC) of first and plateau epps in the train;quantal size (QS);half-decay time ($T_{1/2}$). ANOVA was used to check for significant ($p< 0.05$) differences between data means. **Results:** Neostigmine alone:$1.6x10^{-8}$M (NEO1:10 cells) and $4.8x10^{-8}$M (NEO2:16 cells) compared to 33 control (CO) cells,increased $T_{1/2}$ significantly in both cases (CO:1.35; NEO1:1.9 and NEO2: 4.0 msec).First and plateau epps in the train and the QC of plateau epps were significantly increased only in NEO2.In vecuronium alone:$8x10^{-7}$M (VEC: 22 cells)compared to 13 CO cells, first epps in the train (CO:14.5 and VEC:5.8 mV);plateau epps in the train (CO:9.5 and VEC: 3.1 mV);QC of first epps in the train (CO:250;VEC:68) and of plateau epps (CO:153;VEC:55) were all significantly decreased.In NEO1(22 cells) and NEO2(13 cells),in the presence of VEC,$T_{1/2}$ was increased significantly compared to 13 CO cells,while only in NEO2 first epps in the train (12.4 mV) and the CQ of first epps in the train (282) and of plateau epps in the train (113) were significantly increased compared to VEC alone. **Discussion:** Both pre- and post-synaptic mechanisms underlie the recovery, by NEO, of the block by VEC. **Supported by:** FAPESP,CNPq,CAPES.

01.031

MECHANISMS OF CLUSTERING AND ELONGATION RESPECTIVELY INDUCED BY PERTUSSIS (PT) AND CHOLERA (CT) TOXINS IN CHO CELLS

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Introduction: The frequently adverse side-effects induced by DPT vaccines are related to the presence of non-inactivated PT residues. **Methods & Results:** The CHO cells widely used as a model cell line for the study of the cellular effects of PT and CT were used to standardize an *in vitro* test able to quantitatively assessing the presence of these residues.The cAMP (234.3±50.0 pmol/mL; n=3) mediated the CT (115 pM)-induced elongation in CHO cells being the same effect induced by db-cAMP (704.0±94.0 μM; n=4) and 11 μM of forskolin. We evidenced the sinergistic effect of PT (3.7 pM) to the CT (115 pM)-increased cAMP levels (516.5±69.1 pmol/mL; n=3) while the isolated PT was unable to promote the effect (21.7±1.9 pmol/mL; n=3), indicating the involvement of other 2nd messenger mediating the CHO clustering, which probably is the cGMP, due to the potentiation effect induced by the IBMX (1mM). There was a significative correlation (r= 0.93) betweeen the level of the non-inactivated PT residues detected *in vivo* in mice and *in vitro* in CHO cells, as well as, should be pointed out that PT concentrations (1.8 to 28.8 pM) able to induce clustering, did not show cytotoxic effects based on the cell growth, cloning efficiency, mitotic index and on the DNA damage by the comet test. The Oligomer B (15.2 pM), a molecular fraction of PT, is the main responsible by the clustering effect in association with the intracellular proteins directly connected to the cytoskeleton. **Discussion:** We definitively demonstrated that the elongation and clustering are epiphenomena mediated by different 2nd messengers, as well as, the validity of the *in vitro* CHO cell test not only as a complementary to *in vivo* tests, but also as a possible substitute of them in the Quality Control of DPT vaccines. **Supported by:** CNPq

01.032

ORIGINAL SYNTHETIC DERIVATIVES OF WEDELOLACTONE INHIBIT NA+,K+-ATPASE ACTIVITY AND [3H]-FLUNITRAZEPAN BINDING: STRUCTURE ACTIVITY RELATIONSHIP

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Introduction: We previously reported that wedelolactone, a naturally occurring coumestan, inhibits rat kidney Na^+,K^+-ATPase and [^{3}H]-flunitrazepan binding to rat brain. The aim of this work was to perform a structure-activity relationship (SAR) study towards these two targets, using five original synthetic wedelolactone derivatives. In addition the activity of these synthetic coumestans in another P-type ATPase was also evaluated. **Methods:** The ATPase activity was measured using the colorimetric method of Fisk and Subbarow with Na^+,K^+-ATPase enriched preparations of rat kidney and brain. The (Ca^{2+}-Mg^{2+})ATPase activity was determined in microsomes from rat *Gastrocnemius*. The effect of coumestans on [^{3}H]-flunitrazepan binding was performed using rat brain crude synaptosomes. **Results:** The five derivatives were 3-6 times more potent to inhibit Na^+,K^+-ATPase than (Ca^{2+}-Mg^{2+})-ATPase activity. The ratio of IC50 (for flunitrazepam competition vs Na^+,K^+-ATPase inhibition) varies between molecules, being 0.5, 4 and >20 for PCALC27, wedelolactone and PCALC31 respectively. This indicates that methylation of the hydroxil group in position 8 ring D of wedelolactone results in loss of affinity for benzodiazepinic receptor but not for Na^+,K^+-ATPase, showing that slightly structural changes can increase the selectivity for each receptor. **Discussion:** Our results indicate that different structural requirements are important for the effects on Na^+,K^+-ATPase and flunitrazepam binding, suggesting that an extended SAR study should aid to optimize the pharmacological characteristics of these molecules, in order to obtain more selective compounds towards each of the two molecular targets examined. **Supported:** CAPES, FAPERJ

01.033

GENERATION OF A TRANSGENIC MOUSE TO STUDY BRADYKININ B1 RECEPTOR EXPRESSION USING LacZ GENE

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Introduction: Kinin B1 receptors are usually absent in most tissues, but are markedly induced by pro-inflammatory agents or after injury. Because of the low density of B1 receptors and the difficulty of detection the present study intended to develop a new transgenic mouse with LacZ gene coding sequence (b-galactosidase), under the control of B1 receptor gene promoter. **Methods:** Promoter region of B1 receptor was obtained through Long Range PCR using mouse genomic DNA. The generated product (8.9 kb) was cloned together with the LacZ gene (2.9 kb) into a pGEM-T vector. Functionality of this B1lacZ-pGEM-T vector was tested in mouse vascular smooth muscle cells, treated or not with LPS (10 µg/ml, 4 h) and stained with X-Gal solution. B1LacZ transgene was microinjected into the pro-nucleus of fertilized eggs of FVBN mice. **Results:** From the 6 founder animals obtained, 3 lines were selected based on RT-PCR. Transgene RNA was detected in several tissues: stomach, ileum, brain, spinal cord, lung and cerebellum. Expression was increased by LPS treatment (0.5µg/kg,ip,5h). X-Gal staining of stomach slices showed 24h after LPS treatment a higher amount of blue cells when compared with controls, always in the basal region of the gastric mucosa. In spinal cord slices, of LPS treated mice only a few blue cells in the grey matter part were detected. Immunohistochemistry with specific antibodies confirmed these results since staining of stomach slices presented localization of b-galactosidase in the same place observed with X-Gal staining. **Conclusion:** B1LacZ mice are a valuable new tool for kinin B1 receptor studies, especially concerning localization and pattern of expression. This new transgenic animal model can be used to clarify some questions about the participation of this receptor in different pathologies. **Supported** CNPq

01.034

INVESTIGAÇÃO DA PARTICIPAÇÃO DOS CANAIS DE Ca^{2+} VOLTAGEM-DEPENDENTES E CANAIS DE K^+ NA HIPERREATIVIDADE VASCULAR INDUZIDA POR *Schistosoma mansoni*

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Introdução e objetivos: A presença do parasita intravascular *S. mansoni* macho aumenta a reatividade vascular aos agentes serotonina (5-HT) e noradrenalina (NOR), em parte relacionada à disfunção endotelial (Silva *et al*, Parasitol. Res. 2003, 89(1):16). Assim, o objetivo deste trabalho foi investigar a participação dos canais de Ca^{2+} voltagem-dependentes (VOCC) tipo L e T, bem como de canais K_{ATP} e K_{Ca}, na hiperreatividade vascular induzida pela presença de *S. mansoni* adulto macho. **Métodos**: Segmentos de veia porta e aorta de camundongos sadios e infectados com *S. mansoni* macho foram fixados em cuba contendo solução fisiológica, e conectados a transdutor de tensão. Foram realizadas curvas à 5-HT e NOR (10^{-9} 10^{-5} M) antes e após incubação com glibenclamida (10 µM), caribdotoxina (50 nM), nifedipina (10 nM) e mibefradil (0,1 µM). **Resultados**: O bloqueio dos canais de K_{ATP} com glibenclamida aumentou a eficácia (E_{max}) da 5-HT e NOR em 30% na veia porta e aorta dos animais sadios ($p<0.05$), porém não no grupo infectado. Por outro lado, o mibefradil, antagonista de VOCC tipo T, diminuiu o E_{max} da 5-HT em ambos os grupos na mesma proporção. Contudo, o antagonista dos VOCC tipo L (nifedipina) reduziu em maior proporção (40%) o E_{max} da 5-HT e NOR no grupo infectado. **Conclusões**: Os resultados sugerem que o *S. mansoni* reduz a influência dos canais K_{ATP} na regulação do tônus vascular acompanhada pela maior contribuição dos VOCC tipo L no influxo de Ca^{2+} aumentando assim a resposta contrátil aos vasoconstrictores 5-HT e NOR. **Apoio Financeiro:** Faperj, CAPES

01.035

NUCLEAR FACTOR-κB IN RAT EPIDIDYMIS AFTER LPS TREATMENT

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Aim: Nuclear factor-κB (NF-κB) is a transcription factor involved in the cellular response to inflammation, injury, and bacterial/viral infection. Inducers of inflammation (ex. lipopolysaccharide, LPS) activate NF-κB in a variety of cells, which regulates a number of genes involved in the inflammatory response. Our aim was to study LPS effects on the activation of NF-κB in the male reproductive tract, using rat epididymis as an experimental model. **Methods:** Adult male Wistar rats (90-days-old) were injected with LPS (1mg/kg, i.v.) or saline (control) and sacrificed after 0.5, 1, 2, 3, 6, 9, 15 and 24 h after treatment. Caput and cauda epididymis were isolated and processed for nuclear protein extraction. Gel shift assays were performed to measure changes in NF-κB activity. **Results**: Constitutive NF-κB activity was detected in control caput and cauda epididymis. Two major DNA/protein complexes were displaced by an excess of unlabeled NF-κB, but not by a non-specific oligonucleotide, demonstrating the specificity of NF-κB/DNA interaction. Time course studies indicated a significant increase in NF-κB basal activity in both caput and cauda epididymis after 2 h of LPS treatment. NF-κB activity was similar to basal levels after 3 h, reaching another peak of activation between 9-15 h of LPS treatment, depending on the epididymal region analyzed. **Conclusion**: Epididymis is responsive to LPS treatment. This experimental model will be important for current studies in our laboratory of innate antimicrobial protein expression in the male reproductive tract. **Supported by:** CNPq, Fogarty International Center.

01.036

EFFECT OF THE [N-(4-PHENYL)-PHENACYL-L-HYOSCYAMINE] (PHENTONIUM -PHEN) ON ACETYLCHOLINESTERASE ACTIVITY IN PC12 CELLS.

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Introduction: Therapeutic intervention in Alzheimer's patients seeks the enhancement of acetylcholine (ACh) concentration in the CNS, achieved either after administration of anticholinesterase inhibitors or by increasing ACh release. At the end-plate PHEN was shown to increase the spontaneous ACh release (Souccar *et al.*, *Gen. Pharmac.* 25:1397, 1994). The present study reports the effect of PHEN on acetylcholinesterase (AChE) activity in the neuro-endocrine PC12 cell line. **Material and Methods**: PC12 cells were grown in DMEM containing 10% horse serum, 5% fetal calf serum, penicillin/streptomycin (10000 units/10 mg/mL), 37° C, 5 % CO_2. The specific AChE activity was spectrophotometrically determined in cell homogenates (Ellman G et al., Biochem.Pharmacol. 7:88, 1961) in the presence of PHEN (10^{-8} 10^{-5}M), Iso-OMPA (10 µM), a specific inhibitor of butyrilcholinesterase or neostigmine (NEO 10^{-10} 10^{-8}M), a non-selective cholinesterase inhibitor. All drugs were incubated for 30 min prior to ASCh addition. AChE activity was expressed as nmoles of ASCh hydrolised /min/µg of protein. **Results**: The total cholinesterase activity in PC12 cells was 51.9 ± 4.3 nmoles ASCh hydrolised/min/µg of protein (n=6). After incubation of Iso-OMPA (10 µM) AChE activity was not significantly changed. In the presence of NEO (10^{-9}, 3 x 10^{-9} and 10^{-8} M, n=3), the AChE activity was decreased to 43.1 ± 3.9, 31.4 ± 3.4 and 15.3 ± 0.8 nmoles of ASCh hydrolised /min/µg of protein, respectively (IC50 = 4.25 nM). Up to the highest tested concentration, PHEN (10^{-5}M, n=3) did not significantly affect the enzymatic activity (45.9 ± 6,7 nmoles of ASCh hydrolised /min/µg of protein). **Discussion**: The results indicated that only acetylcholinesterase is expressed in the PC12 cells. PHEN did not inhibit this neuronal AChE activity reinforcing previous data reported in skeletal muscle (Fann et al., Br.J.Pharmacol. 100:441, 1990) which exclude the enzyme inhibition as cause of the increase in ACh release induced by the drug. **Supported by:** Fapesp, CNPq

01.037

EFEITO DO FENTONIO [N-(4-fenil)fenacil l-hiosciamina] NA CAPTAÇÃO DE [^{3}H]-5-HT EM SINAPTOSSOMAS DE HIPOCAMPO DE CAMUNDONGO Lima-Landman, M. T. R.[1]; Rocha, F.[1]; de Lima, T. C. M.[2]; Souccar, C.[1]; Lapa, A. J.[1] - [1]UNIFESP/EPM - Farmacologia; [2]UFSC - Farmacologia

Introdução: O fentonio (FENT) mostrou atividade do tipo antidepressiva nos modelos de natação forçada e suspensão pela cauda em camundongos. O efeito foi inibido por antagonistas nicotínicos (mecamilamina e metilicaconitina) ou por PCPA, um depletor de serotonina. O objetivo deste trabalho foi estudar o efeito do FENT na captação neuronal de [^{3}H]-5-HT. **Material e Métodos**: Camundongos machos adultos foram decapitados sob anestesia etérea, os hipocampos dissecados e homogeneizados em sacarose (0,32 M) 5% p/v. O homogenato foi centrifugado a 1.000 x g/10 min e a 40.000 x g /15 min, obtendo-se a fração sinaptossomal que foi ressuspensa em sacarose (0,27M). Amostras de sinaptossomas, em tampão Krebs a 37 °C, foram incubadas, em duplicata, com FENT (10^{-8} - 10^{-4}M) ou imipramina (IMI 75 µM e 60 nM), um bloqueador da captação de monoaminas, 10 min antes da adição de [^{3}H]-5-HT (84 Ci/mmol 4 nM). A incorporação foi interrompida após 6 min com Krebs gelado e filtração a vácuo, medindo-se a radiatividade remanescente no filtro em cintilador β. O efeito do FENT na captação de [^{3}H]-5-HT foi expresso em % da captação total da amostra. **Resultados**: Na presença de FENT, ou de IMI, a captação de [^{3}H]-5-HT foi bloqueada de forma concentração-dependente com CI_{50} de 8,4 µ M (LCI 3,5 µM - LCS 21 µM, n=5) e de 62,5 nM (LCI 36,9 LCS 106,2 nM, n=4), respectivamente. Na presença da CI_{50} da IMI (60 nM), a incubação de FENT (10^{-8} - 10^{-4}M) não produziu efeito somatório e na presença de FENT 10^{-6}M o efeito da IMI (10^{-9} 3 x10^{-4}M) não foi aditivo. **Conclusão**: A atividade do tipo

antidepressiva do FENT observada *in vivo* pode ser explicada pela inibição neuronal da captação de 5-HT, provavelmente em um sítio diferente do da IMI. **Apoio :** Fapesp, CNPq

01.038

EFFECT OF CHLORIDE CHANNEL BLOCKERS ON THE PROLIFERATION OF GLIOMA CELLS

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Introduction: Gliomas are glia derived brain tumors that accounts for 60% of all primary intracranial neoplasms. The hallmark of these tumors is the rapid proliferation with invasion of adjacent structures and poor responsiveness to chemotherapy. Chloride channels have been identified on both *in vitro* slices and cell lines of human gliomas. The present work concerns with the effect of two chloride channel blockers, NPPB and Zn^{2+}, on the proliferation of the human multiform glioblastoma cell line U373. **Methods:** U373 cells were grown during a period of 72h on DMEM supplemented with 10% FBS where NPPB 100µM or Zn^{2+} 100µM were added. The growing rate was quantified using MTT assay. For determination of cell death, experiments were performed using propidium iodide Flow Cytometry (FACS). **Results:** NPPB 100µM decreased by 36 ± 11,78 % cell proliferation, whereas Zn^{2+} 100µM had no effect (mean ± s.d, n=5). FACS analysis showed no cell death on both NPPB and Zn^{2+} treatment. **Discussion:** NPPB and Zn^{2+} are blockers of volume activated Cl^- channel and CLC 1 channel. These channels have been detected on many glioma cell lines, including U373. The effect of NPPB on cell growth shows the involvement of Cl^- channels on glioma proliferation. Further studies are required to clarify the exact role of Cl^- channels on the biology of gliomas.

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01.039

EFEITO COMPARATIVO DO AZUMOLENE E DANTROLENE SÓDICO NA REVERSÃO E PREVENÇÃO DA CONTRATURA DE MÚSCULO ESQUELÉTICO INDUZIDA POR CAFEÍNA.

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Introdução: O dantrolene sódico (DS) é uma hidantoína que inibe a liberação de Ca^{2+} interagindo com o receptor da rianodina (RyR), indicado no tratamento da hipertermia maligna (HM). A HM está associada a disfunção da regulação intracelular de Ca^{2+} conseqüente a mutação do RyR do músculo esquelético. O azumolene (Az) é alvo de investigação, pela sua ação semelhante no RyR e ser 30 vezes mais hidrossolúvel. Este trabalho investiga comparativamente a potência do Az e do DS em reverter e prevenir a contratura de músculo esquelético induzida pela cafeína. **Métodos:** Músculos solear de camundongos suíços, machos (20-30g) eram dissecados, posicionados em cubas para registro de abalos induzidos eletricamente. A cuba era preenchida com solução de Ringer equilibrada com gás carbogênio, a 37°C e pH 7,4. O primeiro protocolo experimental consistiu em expor os músculos ao Az ou DS (10 µM) durante a contratura induzida pela cafeína (8mM). Num segundo protocolo, os músculos eram previamente equilibrados com Az ou DS antes da exposição à cafeína. **Resultados:** O Az (n=7) provocou relaxamento dos músculos após contratura com cafeína de 62.7± 5.1% e o DS (n=6), de 74.6±3.6%, não apresentando diferença estatística significativa entre as substâncias. Já no protocolo profilático, o Az (n=5) e DS (n=8) preveniram a contratura cafeínica em 39.3±7.4% e 73.8±5.3%, respectivamente ($P<0.05$). **Conclusões** O Az e o DS são equipotentes em reverter a contratura induzida pela cafeína de músculo esquelético, porém, o DS é mais potente em prevenir essa contratura. **Apoio Financeiro:** Cristália, CNPq

01.040

PARTICIPAÇÃO DO CÁLCIO MITOCONDRIAL NA CONTRAÇÃO DO MÚSCULO LISO.

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Introdução: O Ca^{2+} é um íon que atua como mensageiro em processos celulares. Demonstrou-se que a mitocôndria atua como estoque, modulando as concentrações do Ca^{2+}, que pode ser captado ou liberado durante a sinalização. **Objetivos:** Verificar a participação do Ca^{2+} mitocondrial na contração do músculo liso pelo estudo da contração de fundo gástrico na ausência e presença de inibidores mitocondriais. **Metodologia***:* Prepararam-se fundos gástricos de ratos adultos para registro de contração isotônica. Estimulou-se o tecido com concentrações únicas de carbacol (CCh-10^{-5}M) ou de KCl (80mM), na ausência e presença dos inibidores mitocondriais FCCP e antimicina A, que são drogas que colapsam o potencial de membrana mitocondrial. Os parâmetros calculados foram efeito inibitório máximo (Imax), e concentração que causou 50% de inibição (IC_{50}). **Resultados**: CCh e KCl causam contração bifásica, com componente fásico inicial (F) seguido de componente tônico (T). FCCP inibiu os componentes F e T tanto por CCh (Imax - F:69,2±2,0% e T:75,2±1,65%; IC_{50} - F:9,89±0,77uM e T:9,12±0,97uM) como por KCl (Imax - F:78,2±5,3% e T:76,7±3,8 %; IC_{50} - F:6,46±0,36uM e T:6,46±0,96uM). Observou-se o mesmo com a antimicina A, para o CCh (Imax - F:96,8±1,24% e T:97,8±0,98%; IC_{50} - F:8,4±1,2ug/ml e T:3,2±0,9ug/ml) e para o KCl (Imax - F:97,0±1,3% e T:84,8±3,9%; IC_{50} - F:6,8±1,3 ug/ml e T:2,33±1,4 ug/ml). **Discussão***:* Os resultados mostram que o FCCP e a antimicina A bloquearam a contração por CCh, que depende de Ca^{2+} intracelular, e por KCl, dependente de Ca^{2+} extracelular. **Apoio Financeiro:** CNPq

01.041

PROTEIN DISULFIDE ISOMERASE (PDI): A NOVEL REGULATORY MECHANISM OF SUPEROXIDE GENERATION

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Introduction: The role of protein thiols in the control of NADPH oxidase activity is incompletely understood. Recently, we showed oxidase inhibition by thiol oxidizing reagents regardless of glutathione redox status. We postulate that thiol oxireductases such as PDI could regulate NADPH oxidase activity. **Methods and Results:** Western Blotting confirmed robust PDI expression in vascular smooth muscle cells (VSMC) and neutrophils. PDI inhibition with bacitracin (0,5-1mM), scrambled RNase (100ug/ml) or inhibitory RL90 antibody led to 25-60% inhibition ($p<.05$) of oxidase activity assessed with lucigenin (5um) chemiluminescence or EPR spin-trapping in VSMC; and total inhibition ($p<.01$) assessed by superoxide dismutase-inhibitable cytochrome c reduction in neutrophils. VSMC incubation with angiotensin II (A II) increased oxidase activity, accompanied by an increase in PDI activity, assessed by scrambled RNase renaturation assay. A II-induced NADPH oxidase activation was inhibited 71% by antisense oligonucleotide against PDI (PDI-AS oligo), which decreased PDI activity by 42%. Co-immunoprecipitation experiments indicated close spacial association between PDI and oxidase subunit p22phox. Confocal microscopy showed strong co-localization between PDI and p22phox particularly after NADPH oxidase activation. **Discussion:** PDI displays spacial and functional interaction with NADPH oxidase and strongly affects oxidase activation. PDI could be a novel regulator of NADPH oxidase. **Supported by:** FAPESP and CNPq.

01.042

THE $T^{-786}C$ GENETIC POLYMORPHISM OF ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) GENE MAY AFFECT METALLOPROTEINASE-9 (MMP-9) ACTIVITY

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Introduction: Alterations in the activity of metalloproteinases (MMPs) are of major significance in vascular remodelling. Since nitric oxide (NO) affects MMP-9 activity and gene expression, we hypothesized that the $T^{-786}C$ genetic polymorphism of eNOS gene, which reduces eNOS activity by 50%, could affect MMP-9 activity. **Methods:** We determined MMP-2 and MMP-9 activities in 34 healthy male subjects by gelatin zymography of MMP-2 and MMP-9 from plasma samples. Samples were subjected to electrophoresis on 12% SDS-PAGE co-polymerized with gelatin (0.1%). Gels were washed Triton X-100 and incubated at 37°C for 16 h in $TrisCaCl_2$ buffer, and stained with Coomassie Brilliant Blue. Enzyme activity was assayed by densitometry. Genotypes for the $T^{-786}C$ genetic polymorphism were determined by polymerase chain reaction (PCR) and restriction fragment length digestion of PCR products. **Results:** Subjects with genotype TT (n=19) tended (P=0.10) to have lower pro-MMP-9 plasma activity when compared with subjects with the genotype CC (n=15). Non-significant differences were observed when plasma active-MMP-9 activities were compared. Moreover, non-significant differences were observed in MMP-2 activities. **Discussion:** Our results show that subjects with genotype TT tended to have lower plasma MMP-9 activities compared with those with genotype CC. It is possible that the $T^{-786}C$ polymorphism affects MMP-9 activity and the development of atherosclerosis. **Financial support:** FAPESP-CAPES-CNPq

01.043

ESTRESSE OXIDATIVO EM PACIENTES COM LEUCEMIA MIELÓIDE CRÔNICA (LMC)

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Objetivo: Avaliar a geração de espécies reativas de oxigênio (ROS) e a expressão de enzimas pró e antioxidantes em células do sangue de pacientes com LMC ainda não submetidos a tratamento e em tratamento com imatinib (inibidor da BCR-ABL, proteína responsável pelas anormalidades da LMC). **Métodos e resultados**: A produção do ânion superóxido ($O_2^{\cdot-}$) foi medida por espectrofotometria da reação de redução do ferricitocromo-c inibível pela superóxido dismutase (SOD) nos grupos celulares: linfomononucleares (LM) e polimorfonucleares (PMN). Frente à estimulação com PMA (éster de forbol, ativador da PKC) por 20 minutos, a geração de $O_2^{\cdot-}$ pelas LM de pacientes sem tratamento (0,37±0,04 nmol $O_2^{\cdot-}/10^6$cél/min, $P<0,001$) foi significativamente maior que a dos saudáveis (0,16±0,03 nmol $O_2^{\cdot-}/10^6$cél/min) e a dos tratados (0,19±0,02 nmol $O_2^{\cdot-}/10^6$cél/min). Após 10 minutos de estímulo com PMA, houve geração significativamente maior pelas PMN dos pacientes com LMC sem tratamento (1,10±0,08 nmol $O_2^{\cdot-}/10^6$cél/min, $P<0,01$) em relação aos saudáveis (0,89±0,05 nmol $O_2^{\cdot-}/10^6$cél/min). A geração basal de $O_2^{\cdot-}$ pelas LM e PMN foi semelhante entre os grupos. Não foram encontradas diferenças na expressão gênica (avaliada por RT-PCR) das enzimas catalase, SOD e NADPH oxidase entre os grupos. **Conclusão**: A geração de $O_2^{\cdot-}$ via PKC está aumentada nas células LM e PMN de pacientes com LMC. A maior geração de ROS, inibida pelo imatinib, não está relacionada a alterações da expressão das enzimas catalase, SOD e NADPH oxidase. **Apoio Financeiro:** CNPq

01.044

EFEITOS DOS ANÁLOGOS DA L-ARGININA NO TRANSPORTE DE L-ARGININA EM PLAQUETAS HUMANAS

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Estudos recentes sugerem que concentrações plasmáticas elevadas dos análogos da L-arginina, assimétrico dimetilarginina (ADMA) e monometilarginina (L-NMMA), inibidores endógenos da produção de óxido nítrico (NO), são fatores de risco para doença cardiovascular. O objetivo deste estudo foi investigar os efeitos inibitórios de análogos exógenos e endógenos no transporte de L-arginina, precursor da síntese de NO, em plaquetas humanas. **Métodos:** Dezoito indivíduos saudáveis participaram deste estudo. As plaquetas foram isoladas e incubadas a 37° com L-arginina tritiada e adicionadas concentrações crescentes (5-500 μM) de ADMA, L-NMMA, N (G)-nitro-L-arginina (NOARG), aminoguanidina (AG) ou N-(G)-nitro-L-arginina metilester (L-NAME). **Resultados:** O influxo total de L-arginina em plaquetas humanas foi inibido de uma maneira dose dependente pelo L-NMMA (K_i = 42 ± 6 μM, n=5) e ADMA (K_i = 27 ± 2 μM, n=4). Em contraste, o NOARG demonstrou ser um fraco inibidor competitivo do influxo de L-arginina em plaquetas de controles (K_i = 398 ± 8 μM, n=3) assim como o L-NAME (K_i = 1917 ± 319 μM, n=3). Enquanto que a AG não mostrou ter um efeito inibitório no influxo de L-arginina em plaquetas. **Conclusão:** Nossos achados demonstraram pela primeira vez que o ADMA e L-NMMA inibem dramaticamente o transporte de L-arginina em plaquetas humanas, o que não ocorre com os análogos exógenos da L-arginina. É possível que o aumento de análogos endógenos da L-arginina presente em patologias cardiovasculares inibam a via L-arginina-NO em plaquetas predispondo a uma hiperagregabilidade plaquetária e eventos trombóticos. **Apoio Financeiro:** Wellcome Trust

01.045

PURMORPHAMINE ENHANCES OSTEOGENESIS ACTIVITY OF HUMAN OSTEOBLASTS DERIVED FROM BONE MARROW MESENCHYMAL CELLS

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Introduction: Purmorphamine induces osteogenesis in mesenchymal cells. However, there are no studies evaluating the effect of purmorphamine on human cells. This study investigated the effect of purmorphamine on osteogenesis activity of human osteoblasts differentiated from bone marrow mesenchymal cells (hBMMCs). **Methods:** hBMMCs were cultured in supplemented medium. Subconfluent cells in primary culture were enzimatycally harvested and first passage cells weresubcultured in 24-well culture plates (2 x 10^4 cells/well) in culture medium containing purmorphamine (1, 2 and 3 mM, each) or vehicle. At 7, 14, and 21 days, cell proliferation, viability, and alkaline phosphatase (ALP) activity were evaluated. Bone-like nodule formation was evaluated at 21 days. All experiments were done in quintuplicate and submitted to ANOVA. **Results:** Purmorphamine did not affect cell proliferation (p=0.935) and viability (p=0.833). ALP activity (p=0.029) and bone-like nodule formation expressed as both number of bone-like nodules (p=0.0001) and average area of bone-like nodules (p=0.0001) were increased by purmorphamine in a dose dependent way. Discussion: These results indicate that events related to osteoblast differentiation including increased both ALP activity and bone-like nodule formation are enhanced by purmorphamine. It means that this molecule could be used as an adjunct therapy to bone formation. **Acknowledgements:** FAPESP and CNPq for financial support and Dr. Peter Schultz (The Scripps Research Institute-USA) for purmorphamine supplied. **Financial support:** FAPESP and CNPq

01.046

ESTROGEN RECEPTOR A PARTICIPATION ON OSTEOBLAST DIFFERENTIATION INDUCED BY TAK-778 IN BONE MARROW MESENCHYMAL CELLS

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Introduction: TAK-778 has been shown to act via estrogen-receptor (ER)-mediated signaling to induce in vitro osteogenesis. Here we tested the hypothesis that TAK-778 acts via an ERα-dependent pathway to up-regulate osteoblast differentiation of human bone marrow mesenchymal cells (hBMMCs), using a selective anti-ERα, MPP. hBMMCs were cultured in supplemented medium. Subconfluent cells in primary culture were enzimatycally harvested and first passage cells were subcultured in 24-well culture plates (2 x 10^4 cells/well) in culture medium containing TAK-778 (10^{-5} M), MPP (10^{-6} M), TAK-778 (10^{-5} M)+MPP (10^{-6} M), and vehicle. At 7, 14, and 21 days, cell proliferation, cell viability, and alkaline phosphatase (ALP) activity were evaluated. Bone-like nodule formation was evaluated at 21 days. All experiments were done in quintuplicate and submitted to ANOVA. Cell number (p=0.81) and viability (p=0.60) were not affected by any treatment. ALP activity (p=0.042) and bone-like nodule formation (p=0.00001) were increased by TAK-778. Similar to vehicle, MPP did not have any effect on cell behavior. However, when cells were cultured in medium containing both TAK-778 and MPP, the effect of TAK-778 on osteoblast differentiation was abolished. The present results show that TAK-778 up-regulates osteoblast differentiation of hBMMCs via an ERα-dependent pathway, since its effect was abolished by MPP, an ERα antagonist. Acknowledgements: FAPESP and CNPq for financial support and Takeda Chemical Industries for TAK-778 supplied. **Financial support:** FAPESP and CNPq

01.047

EFFECTS OF AGING ON THE SEDENTARY AND EXERCISED MURINE ILEUM RESPONSIVENESS

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Introduction: Aging and exercise are related to oxidative stress, which is due to the unbalance between tissue deterioration and repair mechanisms. We investigated the effects of either aging or association of aging and aerobic exercise on isolated murine ileum responsiveness to stimulant agents. **Methods:** C57BL/6 male mice were divided in sedentary (N=6), aged 3, 6, 12 and 18 months, and exercised group. Exercise program consisted of a 60-min daily treadmill running session, at 13 m/min, 5day/week from 3 to 18 months. Tissue responsiveness was evaluated by determining Emax and EC_{50} from carbachol- and KCl concentration-isometric contraction curves. Tissue oxidative stress was indirectly assessed by [MDA]measurement; morphologic alterations were evaluated by optical microscopy analysis from HE tissue sections. **Results:** Emax (KCl: -1,88±0,04; -1,81±0,03; -1,83±0,03; -1,80±0,03; -1,82±0,02; and CCh -6,23±0,08; -6,03±0,05; -6,23±0,09; -6,21±0,05; -6,21±0,04) and $logEC_{50}$ (KCl: 2,65±0,71; 2,39±0,28; 2,51±0,37; 2,38±0,27; 1,66±0,19; and CCh: 2,92±0,81; 3,38±0,82; 4,06±1,05; 2,80±0,39; 2,25±0,37) between sedentary and exercised animal groups, at the corresponding age were similar. Aging caused a muscle layer hypertrophy and enhanced lipid peroxidation, while aging associated to exercise caused atrophy and decreased lipid peroxidation. **Conclusion:** Contractile response was not affected by ileum oxidative level, animal aging or association of aging and aerobic exercise, even though morphological alterations of the muscular layer were observed. **Supported by:** FAPESP and CNPq

01.048

THE ROLE OF NITRIC OXIDE IN ISOPROTERENOL INDUCED SALIVARY GLAND HYPERTROPHY

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Introduction: Nitric oxide (NO) contributes to control of vascular tone and exocrine secretion of rat salivary glands. NO production can be increased by selective β-adrenergic agonist isoproterenol. Drug repeated administration results in enlargement of parotid and submandibular glands. Activation of beta-adrenoceptors causes secretion of only parotid amylase through a NO/cGMP-dependent pathway. The aim of this study was to investigate the NO role in salivary gland throughout isoproterenol effect. **Methods:** Male Wistar rats received (8 days), isoproterenol (2 [n=4] or 5 [n=18] mg/kg s.c.) or saline [n=10], in association (30 minutes before), with L-NOARG (40mg/kg, i.p.) or saline. After treatment, animals were anesthetized and had the three major salivary glands removed. They were individually dissected, weighted, fixed in PFA 4%+PBS 0.1M, pH 7.4 (2 hs) and immersed in PB 0.1 M, 15% sucrose (24 hs). They were 14µm cryostat cut and stained for NADPH-d to quantify salivary ducts density. **Results:** Isoproterenol induced parotid and submandibular glands hypertrophy which was not inhibited by L-NOARG. NADPH-d ducts density decreased in parotid glands of isoproterenol treated animals (2mg/kg, 19.20 ± 2; ducts/0.5mm^2, 16.22 ± 0.39 ducts/0.5mm^2; control 32.94 ± 1.15 ducts/0.5mm^2; *t*- test, P< 0.05). **Conclusion:** The major findings of this study are: i. isoproterenol chronic treatment decrease NADPH-d positive duct density only in parotid gland; ii. NO may not influence chronic isoproterenol parotid and submandibular hypertrophy effect. **Supported by:** CNPq

01.049

CHARACTERIZATION OF ENDOTHELIN RECEPTORS IN THE AORTA OF THE SNAKE *Bothrops jararaca*

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Introduction: Endothelins (ET_1, ET_2 and ET_3) and sarafotoxins (S6a, S6b, S6c and S6d) are two families of active peptides with close structural and pharmacological activities. Their effects are mediated via ET receptors, types ET_A and ET_B. ET_A mediates contraction, has high affinity for ET_1 and S6b, and low affinity for ET_3. ET_B does not discriminate between the isopeptides. The subtype ET_{B2} mediates contraction, whereas ET_{B1} mediates dilatation. Our aim was to characterize the ET receptors in isolated aorta of the snake *Bothrops jararaca* (Bj). **Methods:** Concentration-response curves to ET_1, S6b, S6c and ET_3 were performed in the absence and presence of selective antagonists. mRNA expression, by RT-PCR, was analyzed using primers corresponding to conserved regions of human (H), rat (R) and chicken (Ch) ET_A and ET_B receptors. **Results:** The order of potency of agonists was ET_1>S6b>> ET_3. Maximal effect of S6c was not reached with 1µM. In the presence of selective ET_A (BQ_{123}), ET_B (IRL_{1038}) or non-selective (PD_{142893}) antagonists, ET_1 response was partially antagonized, whereas total antagonism was reached using 3 µM of both BQ_{123} and IRL_{1038}. ET_A receptors were detected with primers of the species H, R and Ch, whereas ET_B receptors expression was detected only with Ch primers and was less intense. **Discussion:** Our results suggest the presence of atypical ET_A and ET_B receptors in the Bj aorta. Expression of mRNA for ET_A is more abundant than ET_B. **Support:** Fapesp and Fundação Butantan.

01.050

EFFECT OF OLEIC ACID AND LINOLEIC ACID ON REACTIVE OXYGEN SPECIES PRODUCTION IN LYMPHOCYTES

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Introduction: Oleic acid (18:1) and linoleic acid (18:2) are the major unsaturated fatty acids consumed in Western diets and are presented in higher concentration in human plasma. Linoleic acid, from soybean oil, is an essential polyunsaturated fatty acid and is required for the biosynthesis of eicosanoids. Monounsaturated fatty acids (MUFA), such as oleic acid from olive oil, are non-essential and are present in Mediterranean diet. Numerous studies have described that dietary fatty acids (FA) are involved in the modulation of the immune system. One important effect of fatty acids can be stimulating the generation of reactive oxygen species (ROS), which act as a second messenger in lymphocytes. **Objective:** The effect of oleic and linoleic acid on ROS generation by Jurkat cells (T lymphocyte) and Raji cells (B lymphocyte) was investigated. **Methods:** The cells were grown in RPMI-1640 medium plus 10 % fetal calf serum (FCS) and treated with various concentrations (25, 50, 100 and 200 micromolar) of oleic acid and linoleic acid. Dyhidroethidium was used for the flow cytometric measurement of intracellular ROS production in lymphocytes. **Results:** Jurkat cells treated for 1h with linoleic acid increased ROS production at 200 micromolar (4.5-fold) and for 24 h at 50, 100 and 200 micromolar (1.2, 4 and 2.5-fold, respectively). Raji cells treated with linoleic acid (100 and 200 micromolar) also triggered ROS production after 1 h treatment (2-fold, and 8-fold, respectively) and for 24 h (4.2-fold, and 9.8-fold, respectively). **Conclusion:** Linoleic acid induced a pronounced increase while oleic acid had no effect on ROS production at plasma concentration. Thus, the consumption of olive oil may be able to prevent ROS production. **Supported by:** FAPESP

01.051

SUPERSENTIVITY FOR CARBACHOL IN THE RAT ISOLATED DETRUSOR SMOOTH MUSCLE AFTER LONG-TERM L-NAME-TREATMENT.

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Introduction and goals: The rat detrusor smooth muscle (Rd) contains a heterogeneous population of muscarinic receptors that controlls bladder contractility. Nitric oxide (NO) is produced in the urothelium and nitrergic neurons, but its role in modulating Rd is not clear[1]. Therefore, the aim of this work was to investigate the contractile response mediated by carbachol (CCH) in Rd after long-term NO inhibition. **Methods**: Wistar male rats were treated orally with L-NAME (20 mg/rat/day) for 7, 15, 30 and 60 days. Age-matched control animals received tap water alone. Concentrationresponse curves to CCH were obtained and the pEC_{50} and maximal responses (Emax) were calculated. **Results**: L-NAME treatment induced an increase in the systolic blood pressure. The pEC_{50} values for CCH was not modified by 7 days whereas a significant increase in the potency was seen 15, 30 and 60 days after L-NAME treatment, respectively (ctl: 6.03±0.03 vs treated: 6.46±0.04, n=11-17, ctl: 6.09±0.02 vs treated: 6.82±0.06, n=10-5 and ctl: 5.88±0.04 vs treated: 6.30±0.04, n=12-9). The Emax to CCH were not affected by L-NAME treatment in all studied time. **Conclusion:** Our findings show that L-NAME treatment induces detrusor muscle hyperactivity in the rat suggesting that NO exert a modulatory role. **Reference:** (1) Alm, *J Auton Nerv Syst*, 5(1-2): 105-114, 1995.

01.052

POLYMERIC MICRO AND NANOPARTICLES APPLIED TO DRUG DELIVERY SYSTEMS: PREPARATION, CHARACTERIZATION AND IN VITRO ACTIVITY

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Introduction: Micro and nanoparticles offer advantages when compared to systems of conventional dosage due progressive liberation of the bioactive agent. This work is related to the preparation, application and characterization of a controlled release systems containing streptomycin and propolis by emulsification and solvent evaporation method. Antimycobacterial activity of the encapsulated drug acting on *Mycobacterium tuberculosis* was studied. **Methods:** STM was encapsulated in biodegradable microparticles of poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV) by water-oil-water (W/O/W) double emulsion solvent evaporation method and propolis was encapsulated in PHBV by the oil-water (O/W) emulsion. The micro and nanoparticles were characterized in terms of morphology, encapsulation efficiency, and *in vitro* release kinetics. **Result and Discussion:** The applied method was efficient in the particle formation and the entrapment of the drug (25% and 50% for propolis and STM, respectively). The molar mass of polymer also influences in the formation and the size distribution of particles as well as in the encapsulation efficiency and the release kinetics. At the optimal conditions a 60% and 80% of drug release to propolis and STM were observed, respectively. 1. Martin, M.A; Miguen, F.C.; Rieumont (2000). Tailoring of the external and internal morphology of poly-3 hydroxy butyrate microparticles. *Colloids Suf. B: Biointer.* 17, 111. 2. Ruiz, P.; Rodriguez-Cano (2002). Investigation of the *in vitro* activity os streptomycin against *Mycobacterium tuberculosis. Microbial. Drug Resitance* 8, 147. **Financial support:** CNPq, FAPESP and Network Nanobiotechnology MCT/CNPq

01.053

MICRO AND NANOPARTICLES: PREPARATION AND CHARACTERIZATION OF STREPTOMYCIN AND RIFAMPICIN ENCAPSULATION

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Introduction: Calcium alginate has been widely studied in drug controlled release specially in tuberculosis[1-3]. Another important biodegradable polymer is PLGA. These polymers were used in antigens encapsulation of several pharmaceutical drugs[4]. To improved the entrapment of hydrophilic drugs, the double emulsion (W/O/W) method was used and the streptomycin and rifampicin were used as model drugs. Streptomycin (STM) and rifampicin (RIF) are antibiotics used as second and first-line therapy, respectively[5]. **Methods:** The aqueous phase containing sodium alginate, STM and distilled water was emulsified adding into the oil phase containing ether, liquid Vaseline and Span 80 with a magnetic stirrer. Alginate gel microparticles are prepared by the additional of calcium chloride solution 10%. The microparticles were precipitated by centrifugation at 5.000 rpm and 4°C for 50 minutes and washed with ethanol. Similar procedure with rifampicin was carried out. **Results and Discussion:** Based on the scanning electronic microscopy (SEM) was possible to choose the best viscosity quality of sodium alginate for the STM encapsulation. Micro and nanoparticles made with PLGA/RIF were also produced. Use of paraffin instead of benzyl benzoate showed better results to form micro/nanoparticles. In the case of PLGA/rifampicin a smooth surface was observed. 1. Gaserod,O., Sannes,A. and Skjak-Braek,G. *Biomaterials* 20,773-783. 1999 2. Lemoine,D., Wauters,F., Bouched'homme,S. and Préat,V. *Intern.J. Pharm.* 176,9-19, 1998 3. Qurrat-ul-Ain, Sharma,S., Khuller,G.K. and Garg,S.K. *J.Antimicrob. Chemother.* 51,4,931-938. 2003 4. Chen, J. and Davis, S.S. *J. Microencap.* 19,2,191-201.2002 5. Ruiz,P., Rodriguez-Cano,F., Zerolo,F.J., Casl,M.. *Microbiol Drug Resistance* 8,147-149.2002. **Financial support:** FAPESP, CNPq and Network Nanobiotechnology MCT/CNPq

01.054

GLUTAMINA E PEPTÍDEOS RESTABELECEM O TRANSPORTE DE ÁGUA E ELETRÓLITOS, NA DIARRÉIA INDUZIDA POR TOXINA DA CÓLERA E METOTREXATO EM CAMUNDONGOS

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Toxina do *Vibrio cholerae* (TC) e metotrexato (MTX) induzem diarréia. A solução de reidratação oral (SRO) melhora a absorção de água e eletrólitos. Objetivou-se avaliar a eficácia da suplementação de Gln e peptídeos, e da SRO acrescida destes na diarréia induzida em camundongos por TC ou por MTX. Camundongos *Swiss* ♂ (35-40g, n=6/grupo), foram tratados por 3 dias com MTX (2,75mg/kg/24h *sc*) ou perfundidos com TC (1mg/ml), concomitantemente à suplementação de Gln e peptídeos (111mM). Utilizou-se os protocolos de perfusão (cálculo do transporte de água e eletrólitos) e de permeabilidade (teste de lactulose-manitol, L/M) intestinais. O grupo TC apresentou secreção de água (ml/g/min), -0,02±0,004 *vs* 0,001±0,017, Na^+ (-12,87±1,42 *vs* 8,41±0,53 mEq/g/min) e de Cl^- (-12,89±4,55 *vs* 2,32±1,16 mEq/g/min). Gln e Ala-Gln, respectivamente foram mais eficazes que a SRO-OMS na reversão da secreção de água (0,03±0,08; -0,03±0,05 *vs* 0,53±0,018), de Na^+ (6,02±2,10; 23,85±3,13 *vs* 0,23±1,76) e de Cl^- (10,1±5,14; 35,28±6,25 *vs* 1,94±0,39. Hyprol®4107 restabeleceu apenas a secreção de água (0,03±0,007 *vs* 0,02±0,004). MTX induz o aumento da razão L/M (0,74 *vs* 0,35). Gln e peptídeos restauraram a permeabilidade intestinal aumentada pelo MTX. SRO+Gln e peptídeos é mais eficaz na redução da diarréia secretória que SRO-OMS. Conclui-se que a suplementação com Gln e peptídeos restabelece a função intestinal, com potencial terapêutico na doença diarréica. **Apoio Financeiro:** CAPES, CNPq, FUNCAP

01.055

VITAMINAS A E E, GLUTAMINA E PEPTÍDEOS RESTABELECEM O TROFISMO INTESTINAL EM CAMUNDONGOS TRATADOS COM METOTREXATO

Nunes-Monteiro, S. M.[1]; Monteiro, M. C. S. A.[1]; Aguiar, C. V.[1]; Monteiro, A. F. M.[1]; Menezes, D. B.[2]; Lima, A. A. M.[1] - [1]UFC - Fisiologia e Farmacologia; [2]UFC - Patologia e Medicina Legal

Metotrexato (MTX) em roedores reproduz enteropatia com dano na função absortiva. Objetivou-se avaliar a eficácia da suplementação com vitaminas A (VITA) e E (VITE), glutamina (Gln) e peptídeos na diarréia induzida por MTX. Camundongos *Swiss* ♂ (35-40g,n=6/grupo) foram tratados com MTX (2,75mg/kg/24h *sc* por 3 dias) e suplementados com Gln, alanil-glutamina, Ala-Gln; hyprol 4107, HYP; hifoama 77, HYF (0,6g/kg/24h *sc*). Efetuou-se morfometria de vilos e criptas, estudo de mitose e apoptose intestinais. O grupo MTX mostrou atrofia de vilos (VL) no duodeno (DUO, 364,8±19,9 *vs* 548±15,97 μm) e no jejuno (JEJ, 363,3±20,65 *vs* 564±15,14 μm) e hiperplasia de criptas (CR) no DUO (251±19,24 *vs* 89±5,26 μm) e JEJ (217±14,23 *vs* 124±4,76 μm). Houve aumento no comprimento de VL no DUO com Gln, Ala-Gln e HYP (517,8±9,65, 537±14,5 e 537±20,9, respectivamente *vs* 364,8±19,9 μm) e no JEJ nos grupos Gln, Ala-Gln, HYP e HIF (521,7±9,46; 518,3±9,25; 563,7±20,9 e 542,5±114,5, respectivamente *vs* 363,3±20,65 μm).E redução na profundidade das CR no DUO e JEJ, em todos os grupos *vs* MTX. Ocorreu maior quantidade de corpos apoptóticos (ca) nas CR no DUO (MTX, 7,3±0,91 *vs* 6±0,22 ca) e no JEJ (1,8±0,47 *vs* 0,3±0,15 ca), e redução com VITA, VITE, Gln, Ala-Gln, HYP e HIF. E aumento de figuras mitóticas (fm) com Gln, Ala-Gln, HYP e HIF no DUO (1,1±0,15; 0,8±0,13; 1,0±0,16; 1,0±0,16 fm). Conclui-se que VITA, VITE, Gln e peptídeos recuperam o trofismo intestinal na diarréia. **Apoio Financeiro:** CAPES, CNPq, FUNCAP, UFC.

01.056

TOXICOLOGIA E AVALIAÇÃO PONDERAL E ALIMENTAR EM CAMUNDONGOS TRATADOS COM 5-FLUOROURACIL

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5-Fluorouracil (5-F.U) é um antineoplásico que causa mucosite oral e intestinal em humanos e roedores. Objetiva-se investigar uma dose eficaz de 5-FU para indução de diarréia com baixa mortalidade em camundongos. Utilizaram-se camundongos *Swiss* ♂ (32-36g; n=10/grupo) para estudos toxicológicos, de avaliação ponderal e do comportamento alimentar. Os 3 primeiros dias serviram de controle. No 4° dia iniciou-se a administração de 3 diferentes doses de 5-F.U (50,100,150 mg/kg/dia *sc*) por 3 dias, seguindo-se a observação dos animais nos 5 dias posteriores. * $p<0,05$ (ANOVA, Bonferroni). Observou-se mortalidade de 1/10 animais na dose de 50 mg, 2/10 na de 100mg e de 6/10 na de 150 mg. Ocorreu diarréia em 8/10 animais nas doses de 100 e 150 mg/kg/24h *sc* por 3 dias. O consumo de ração foi reduzido nas doses de 100 (2,57±0,364 *vs* 4,895±0,865g) e 150 mg/kg/dia (1,87±0,139 *vs* 4,55±0,592 g/animal/dia) durante o tratamento com 5-FU. Essa redução foi mais acentuada no período pós tratamento. O consumo de água foi reduzido apenas na dose 150 mg/kg/dia (6,813±1,07 *vs* 13,04±3,44 ml/animal/24h). Observou-se redução da massa corpórea de maneira dose-dependente no pós tratamento principalmente na dose 150 mg/kg (-5,581±0,915 g/animal/24h). Conclui-se que o tratamento com 100 mg/kg/dia *sc* durante 3 dias é mais eficaz na reprodução do modelo de diarréia experimental em camundongos *Swiss*.Tal modelo propicia estudos futuros da pato-fisiologia da doença diarréica induzida por 5-FU. **Apoio Financeiro:** CAPES, CNPq, FUNCAP,UFC

01.057

EFFECT OF STI571 AND TEMOZOLOMIDE ON INHIBITION OF GLIOMA CELL LINES PROLIFERATION

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Introduction: Malignant gliomas are the most common primary brain tumors in humans and despite available treatment they recur early. STI571 (imatinib mesylate) is a kinase inhibitor which can lead to glioblastoma growth arrest. It has been established that STI571 inhibits the kinase activity of the platelet-derived growth factor receptor (PDGFR). Hyper-expression of this receptor and its ligand has been documented in some malignant gliomas. The cellular response of glioblastoma cells to STI571 does not appear to involve an apoptotic mechanism. Temozolomide is an alkylating agent that may induce apoptosis and has characteristics that make it suitable for combination therapies. In this study we examine the effect on inhibition of PDGFR-mediated glioblastoma proliferation by combination of an active kinase inhibitor (STI571) and temozolomide. **Methods:** Cell proliferatin assay was performed by cell counting, and cytotoxicity of STI571 (3µM, 6µM, 10µM), temozolomide (0.1µM, 1µM, 10µM) and their combinations was assessed by using microculture MTT assay. **Results and Conclusion:** Results of cell counting demonstrated that STI571 and temozolomide alone inhibited the growth of C6 rat glioma cell line and U138-MG human glioma cell line in the concentrations above cited. The combinations STI3 +T1 and STI6+T3, significantly inhibited cell proliferation of C6 cells by 56.4%± 14.6 and 75.4%±12.4 respectively, with MTT assay, but this inhibition was not different from either agent alone. The combination of these drugs could be an alternative to treat brain tumors and further studies are being performed to confirm these results. **Supported by:** FAPERGS

01.058

HEPATIC ALTERATIONS IN RATS TREATED CHRONICALLY WITH N^{ω}-NITRO-L-ARGININE METHYL ESTER (L-NAME)

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Introduction: Chronic treatment of rats with N^{ω}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide biosynthesis, causes cardiac and renal damage. In this work, we examined the changes in hepatic morphology in this model. **Methods:** Male Wistar rats received L-NAME (20 mg rat^{-1} day^{-1}) in the drinking water for 2, 4 and 8 weeks, after which the livers were processed for histological analysis by light and transmission electron microscopy. **Results:** Treatment with L-NAME caused hydropic degeneration, compactation of the sinusoidal lumen and deposition of connective tissue in the portal space. There was also a significant increase (*p<0.05) in arterial wall thickness. Electron microscopy showed damage to Kupffer cells, vacuolation, and changes in nuclear and sinusoidal morphology. PAS staining and direct quantification showed an increased glycogen content [5.58±1.03 (control) vs 10.09±0.78* (L-NAME), 4.46±0.93 vs 10.54±0.89* and 5.45±1.05 vs 11.27±1.28* mg glycogen/100 mg tissue, for 2, 4 and 8 weeks, respectively; n=5/group; mean±S.D.; *p<0.05]. There were no significant changes in tissue total cholesterol, HDL, LDL and triglyceride levels (n=5/group). **Conclusion:** Chronic treatment with L-NAME caused hepatic alterations. However, ischemia, necrosis and fibrosis were less common than in cardiac and renal tissue. **Supported by:** FAPESP

01.059

CYTOCHROME P450 ACTIVITIES IN RATS TREATED CHRONICALLY WITH N$^{\omega}$-NITRO-L-ARGININE METHYL ESTER (L-NAME)

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Introduction: Nitric oxide (NO) can modulate cytochrome P450 (CYP450) activity and expression. We examined the effect of treatment with L-NAME, an inhibitor of NO synthase, on hepatic CYP1A1/2, CYP2B1/2, CYP2C11 and CYP2E1 activities. **Methods:** Male Wistar rats received L-NAME (20 mg/rat/day) in the drinking water for 2, 4 and 8 weeks and, when required, phenobarbital or B-naphthoflavone (80 mg/kg/day each, i.p., 4 d) or pirazole (200 mg/kg/day, i.p., 2 d) prior to sacrifice. Liver microsomes were prepared by differential centrifugation. The CYP450 content was determined colorimetrically. The activities of CYP2A1/2 and CYP2B1/2 were assayed fluorimetrically. CYP2C11 and CYP2E1 were determined colorimetrically. **Results:** The microsomal P450 content in control livers was 6.7±1.8, 10.1±2 and 10±1.9 nmol/mg of protein (2, 4 and 8 wks, respectively; mean±S.D., n=5) and increased significantly ($p<0.05$) with the three inducers. L-NAME did not alter the P450 content in any group. The activities of CYP1A1/2 (6418±1058, 5331±1528, 4578±745 AUF/min/mg), CYP2B1/2 (669±114, 1295±368, 793±115 AUF/min/mg), CYP2C11 (0.166±0.04, 0.130±0.047, 0.140±0.03 A_{410} nm/min/mg) and CYP2E1 (1.19±0.1, 1.14±0.3, 1.28±0.6 nmol/min/mg) (n=5 each) after 2, 4 and 8 weeks, respectively, were unaltered by L-NAME, nor did L-NAME affect the level of enzyme induction. **Conclusion:** Chronic treatment with L-NAME did not affect basal or induced CYP450 activities. This lack of effect may reflect the ability of the liver to adapt to the long-term absence of NO. **Supported by:** FAPESP

01.060

ATIVIDADE ANTI-TUMORAL *IN VITRO* E *IN VIVO* DE ORGANO METÁLICO DE ESTANHO PLANEJADO RACIONALMENTE

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Introdução: Estudos de compostos de coordenação ou organometálicos com metais de transição têm tido um papel de destaque na química medicinal como agentes antineoplásicos. Neste trabalho avaliamos a letalidade e a atividade anti-tumoral *in vitro* e *in vivo* de um organometálico de estanho (PCS 10). **Metodologia e resultados:** Foi determinada, pelo método do MTT ou contagem do número de células viáveis, uma IC_{50} de $3{,}02x10^{-7}$ moles/L para $B_{16}F_1$, de $3{,}72x10^{-7}$ moles/L para $B_{16}F_{10}$ (melanomas murinos), de $4{,}59x10^{-7}$ moles/L para células de tumor de Ehrlich e > 10^{-6} moles/L para MCF-7, MDA MB 231 (carcinomas mamários humanos) e duas linhagens celulares normais. Em função dos efeitos citotóxicos seletivos, demos continuidade à avaliação anti-tumoral *in vivo*. Em camundongos Swiss (*n* entre 5 e 8), foram administradas, i.p., doses diárias de 10^2 e 10^1 mg/kg. por 21 dias sendo, observada uma queda de 21,9% da sobrevida na menor dose e mortalidade de 100% na maior. Camundongos Swiss portadores de tumor de Ehrlich na pata ($2{,}5x10^5$ cél, s.c. - *n* entre 5 e 7) foram submetidos ao tratamento, i.p., com PCS-10 (10^{-1} mg/Kg). Foi observada uma pequena, mas significativa ($p< 0{,}005$) atividade anti-tumoral, com redução da espessura da pata. **Discussão:** Estes resultados indicam que a PCS 10 é uma substância com promissora aplicação como antineoplásico, uma vez que em células normais não apresenta efeito e em tumorais, *in vitro* e *in vivo,* mostra-se ativa em concentrações consideradas relevantes. **Apoio Financeiro:** FAPEMIG e CAPES

01.061

EXPRESSION OF Na^+/K^+-ATPase α SUBUNIT ISOFORMS AND ACTIVATED MAP KINASES IN A MODEL OF HYPERTROPHY BY CARDIAC SELECTIVE OVEREXPRESSION OF MUTANT α_{1B} ADRENOCEPTORS

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Introduction: Several models of cardiac overload have shown a selective decrease in Na^+/K^+-ATPase α_2 isoform expression. However, it is still unclear whether this reduction constitutes an immediate response to overload or a consequence of hypertrophic adaptation itself. Here we used overload-free hypertrophic hearts from transgenic mice overexpressing a mutant α_{1B} adrenoceptor in order to examine the expression of Na^+/K^+-ATPase α isoforms and MAP kinase pathways which may be involved. **Methods and Results:** Heart wt/body wt ratio (20-30% greater in transgenic (AP) than control (BP), $p<0.05$) and [^{3}H]prazosin binding assays (B_{max} 70% greater in AP than BP, $p<0.05$) were used for phenotypic evaluation. Crude heart membrane preparations (15 or 30µg ptn) were resolved on a 7.5 or 10% SDS-PAGE and Western Blots performed with Na^+/K^+-ATPase $\alpha_{1,\ 2\ or\ 3}$ isoform-specific antibodies or phospho-p38 kinase antibodies. Although α_3 blot is lacking, preliminary results showed the presence of $\alpha_{1\ and\ 2}$ isoforms and phospho-p38 kinase specific bands in both groups. **Discussion:** Phenotype is consistent to a mild-to-intermediate cardiac hypertrophy. Nonetheless, the preliminary blotting results do not seem to indicate different expression of Na^+/K^+-ATPase isoforms and activated p38 kinase. Densitometric analysis and phospho-Erk1/2 immunoblots are under way. **Supported by:** FAPESP, FAPERJ, CAPES, CNPq

01.062

PROTEÍNA ANTI-APOPTÓTICA Bcl-x_L INIBE A LIBERAÇÃO DE Ca^{2+} MITOCONDRIAL OCASIONADA PELA PROTEINA PRO-APOPTÓTICA BAX

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Introdução: A morte celular pode envolver alterações mitocondriais. Na apoptose, Bax, uma proteína da família da Bcl-2, transloca do citosol para as mitocôndrias e diminui o potencial de membrana mitocondrial que pode causar liberação de Ca^{2+} mitocondrial (Ca^{2+}_m). A superexpressão da proteína Bcl-x_L protege as células dos efeitos deletérios produzidos pela Bax. **Objetivos:** Neste trabalho investigamos o papel da Bcl-x_L no transporte de Ca^{2+}_m assim como seu efeito na respiração mitocondrial, na presença e ausência da Bax. **Métodos:** Astrócitos em cultura primária foram suspendidos em tampão intracelular e permeabilizados com digitonina (7μ g/ml). A liberação de Ca^{2+}_m foi investigada por fluorimetria após adição de Mag-Fura-2 sal (5μM) na presença de Tapsigargina (Tap,2μ M). O consumo de oxigênio da Bax e Bcl-x_L foi avaliado por meio de oxígrafo. **Resultados:** Nossos estudos mostraram que a Bcl-x_L libera Ca^{2+}_m de maneira dose-dependente. Este efeito foi bloqueado pela pre-incubação com FCCP (10μ M), porém, não foi inibido pela Tap. A Bax também induziu uma liberação de Ca^{2+}_m (64.5%) que foi inibida pela Bcl-x_L (4.5%). A proteína Bcl-x_L não inibiu a respiração mitocondrial (5.8%) como observada com a Bax (46.7%). **Discussão:** Os dados sugerem que os efeitos protetores da Bcl-x_L não estão relacionados à cadeia respiratória, mas a uma interação com a Bax que inibe a liberação de Ca^{2+}_m. A Bcl-x_L pode prevenir alterações nos estoques de Ca^{2+}_m e proteger as células de estímulos que modificam a homeostase de Ca^{2+} e conduzem à morte celular. **Apoio Financeiro:** FAPESP, CNPq

01.063

PHARMACOLOGICAL INVESTIGATION OF NEW A_1-ADRENERGIC RECEPTOR ANTAGONISTS

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Introduction: The α_1-adrenergic receptor (AR) is divided into three subtypes (α_{1A}, α_{1B}, α_{1D}). The pharmacological blockade of α_{1B} has a role on the hypertension, while the inhibition of $\alpha_{1A/D}$ is useful in the treatment of human benign prostate hypertrophy. The objective of this work was to evaluate pharmacologically the synthetic N-phenylpiperazine compounds named LASSBio 772 (1) and LASSBio 772B (2). **Methods:** For functional studies we used rat aorta as mentioned before (Silva *et al*, 2002, Br J Pharmacol.135:293). Cumulative concentration-response curves for norepinephrine (NE) were performed in the absence or presence of compounds 1 or 2 (0.1, 0.3, 1.0 nM) for calculation of the antagonist affinity (pK_b). Alternatively, we used binding studies in which we evaluated the potency of the compounds in displacing the specific binding of [^{3}H]-prazosin. Results: In rat aorta, compounds 1 and 2 displaced to the right the NE curves compatible to a competitive antagonism at α_{1D} receptor, with an affinity higher than the non-selective antagonist prazosin (10.86, 10.7 and 9.77, respectively). In binding studies using rat (α_{1B}) or rabbit (α_{1A}) liver membranes compound 1 inhibited the [^{3}H]-prazosin binding (IC_{50}= 0.4 nM and 0.45 μM, respectively). In the same protocol prazosin had an IC_{50} of 0.78 and 0.25 nM, respectively. **Conclusions:** Compounds 1 and 2 are both AR antagonists more selective for α_{1D}AR as compared to the non-selective antagonist prazosin. Such profile is verysuitablefor the development of newuroselective drugs. **Supported by:** CNPq-PIBIC

01.064

EFFECT OF DIAZEPAM ON THE PROLIFERATION AND 5' NUCLEOTIDASE ACTIVITY IN RAT GLIOMA CELL LINE

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Introduction: Benzodiazepines are widely used drugs in anxiolytic, sedative and muscle relaxant therapy. Diazepam in micromolar concentrations can inhibit DNA synthesis in different human cell lines. Lately it has been suggested an involvement of purines in benzodiazepines actions. Benzodiazepines can inhibit adenosine reuptake in rat sinaptossomes and diazepam appears to be a competitive modulator of the efficacy of adenosine at A2 receptor in glioma cells. This study has the aim to evaluate the effects of diazepam on proliferation and on the ecto-5'-nucleotidase activity in C6 rat glioma cell line. **Methods:** The C6 glioma cells, obtained from American Type Culture Collection, were plated and treated with diazepam (0.5, 1, 5, 20, 30 μM). Proliferation was measured by cell counting and cell viability was assessed by MTT assay. The 5'-nucleotidase activity was measured by inorganic phosphate liberation, based on the malaquite green method, and protein was quantified by Comassie Blue assay. The treatment with diazepam for cell proliferation assays and enzymatic activities were 72 and 12 hours, respectively. **Results and Conclusion:** Our results show an inhibition on the proliferation of C6 glioma cells with diazepam 0.5 μM (60%±0.8, n=3) a 30μM (85%±0.5, n=3). There was no alteration on the ecto-5'-nucleotidase activity in all diazepam concentrations tested. The effect of higher concentrations of diazepam on 5'-nucleotidase activity is being tested since preliminary data indicates a reduction on the activity with diazepam 100 μM. **Supported by:** FAPERGS, CNPq.

01.065

EFFECT OF AEROBIC EXERCISE PROGRAM AND HYDROGEN PEROXIDE (H_2O_2) ON THE MURINE INTESTINAL RESPONSIVENESS

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Introduction: Oxidative stress caused by exercise depends on its intensity and is associated to the balance between tissue oxidants and antioxidant defense.We have previously demonstrated that H_2O_2 impairs the signal transduction in intestinal muscle. We investigated the effects of the association of H_2O_2 and *aerobic exercise program (AEP)* on the responsiveness of the ileum. **Methods:** C57BL/6 mice, male, aged three months, were submitted to AEP.Ileum reactivity was determined by E_{max} and -logCE_{50} values obtained from KCl or CCh concentration-isometric contractile curves (n=10) in both *sedentary (SD)* and *exercised mice (EX)* before and after add H_2O_2 in bath solucion. **Results:** Animal adaptation was assessed by the increase of 33% in the maximum velocity, and a 12% increase of the *heart wet weight/ body weight (HBR)* in the EX over SD groups.The values of E_{max} for KCl curves were 1.01±0.05, 0.08± 0.15, 1.05±0.04 and 0.19±0.05g, and -logCE_{50} were 2.0±0.1, 1.3± 0.2, 2.1±0.2 and 1.6±0.2 in SD, SD+$H_2O_2$0,3 mM, EX and EX+$H_2O_2$0,3, respectively;and for CCh these values were 1.29±0.07, 0, 1.3±0.1, 0 and 6.9±0.4, 0, 6.3±0.1 and 0.Preliminary results show that the lower the [H_2O_2],the lower its effect on the contraction, suggesting a protective effect of exercise against H_2O_2-dependent oxidative stress. **Conclusions:** It is concluded that AEP did not protect the ileum responsiveness from the oxidative stress induced by high concentration of H_2O_2. Further studies should be done with lower H_2O_2 concentrations to verify if there is any protective effect of aerobic exercise on the ileum responsiveness. **Supported by:** FAPESP and CAPES

01.066

HUMAN PROTEOLYSIS-INDUCING FACTOR (hPIF) ACTIVATES PROTEASOME AND CASPASE PROTEOLYTIC PATHWAY DURING SKELETAL MUSCLE WEIGHT LOSS IN MICE

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We identified a novel human gene which encoding a protein partially homologue at N-terminal amino acid sequence to a 24 kDa glycoprotein secreted by MAC-16 tumor, responsible for induction of skeletal muscle proteolysis and named proteolysis-inducing factor (PIF). **Objectives**: Development of methods for detecting and analysis of biological effects of hPIF and its signaling pathway in cancer cachexia. **Methods**: Two cell lines transfected with pcDNA-hPIF vector: (1) melanoma cell B16-F10 (B16-PIF) and (2) skeletal myoblast C2C12 (C2-PIF) were evaluated for biological effects of hPIF on body weight, survival time, lung metastasis, proliferation, differentiation and cell death during growth in mice (1) and cell culture (2). Biochemical and morphological assays were performed to evaluate the proteolytic activity and expression of caspases, ubiquitin-proteasome E3 ligases and apoptosis in cells and tissues from mice. **Results:** The B16-hPIF bearing animals had an increased body weight loss (15.9 ± 1.5% p<0,001, N=20), reduced survival time (<21 days) and developed 90% more lung metastasis (p<0,001, N=20). In the low calorie fed mice injected with rhPIF, the body weight did not change, but the weights of soleus and gastrocnemius muscles decreased significantly in relation to controls (18.2 ± 5.2% and 12 ± 4.1%, respectively; p<0.005, N=12). In skeletal muscle samples from B16-hPIF tumor and rhPIF-injected mice, we detected a significantly up-regulation of MURF1 and MAFbx/Atrogin-1 E3-ubiquitin-ligases (2.76 and 3.97-fold; respectively, p<0.05). The increased expression and activity of ubiquitin-proteasome, caspases-3, -8 and -9 correlated with the morphological features of apoptosis and DNA fragmentation observed in the skeletal muscle tissues from mice and cells from C2-PIF cultures. **Conclusion:** The induction of skeletal protein breakdown by hPIF is mainly due to an increase in the expression and activity of the ubiquitin-proteasome system components and caspases pathways. **Supported by:** Fapesp, CNPq and CAPES

01.067

$P2Y_1$ RECEPTOR SIGNAL TRANSDUCTION IN THE RAT PINEAL GLAND THE ROLE OF PHOSPHOLIPASE C (PLC)

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Introduction: Stimulation of $P2Y_1$ receptors, present in denervated rat pineal glands, increases the extracellular acidification rate by a mechanism dependent on a transient increase in intracellular calcium concentration (Ferreira et al., Pharmacology 69:33, 2003). This effect is mediated by inhibition of a Na^+/H^+ exchanger, since it is blocked by amiloride (Bomfim et al, Annals 35th Congress Pharmacology 72, 2003). **Aim:** Determine the role of PLC in the $P2Y_1$ receptor signaling pathway that mediates proton extrusion. **Methods and Results:** $P2Y_1$ receptor-mediated acidification was detected by microphysiometry, a silicon-based biosensor system which continuously monitors the extracellular pH surrounding cells in culture, and reports receptor activation by measuring increases in extracellular acidification rate. Pinealocytes were dissociated by trypsinization, and the variation in extracellular acidification rate induced by ADP (300 μM) was determined in the absence or presence of increasing concentrations of U73122 (3 μM 300 μM), an inhibitor of PLC. Blockage of PLC with U73122 reduced the ADP-induced response in a dose dependentmanner (log IC_{50} = -4.66 ± 0.05; n = 5). **Conclusion:** Therefore, stimulation of pineal gland $P2Y_1$ receptors activates PLC, increases intracellular calcium and promotes activation of Na^+/H^+ exchanger, a plasma membrane protein present in cell membrane, which increases the extracellular acidification rate, by electroneutral reversible exchange of Na^+ against H^+. **Supported by:** FAPESP, CNPq

01.068

ALCOHOL INGESTION DURING PREGNANCY AND NURSERY: PHARMACOLOGICAL EFFECTS ON MOTHER AND FEMALE LITTERS

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Introduction and goals: There are evidences that the use of alcohol during pregnancy affects the development of sympathetic nervous system (Zimmerberg, B., *Alcohol* 12:71,1995, Juárez, J. *Alcohol* 21:181,2000). Our objective was to check if treatment of rats with alcohol during pregnancy and nursery affects the smooth muscle autonomic innervation of mothers and female litters. **Methods:** Wistar rats, 4 months old, were treated from the 1st day of pregnancy until the 10th day post-partum with 20, 25, or 30% oral alcohol ad libitum. Controls received drinking water. Functional experiments were made in stomach fundus by testing barium chloride and 5HT contractions, and relaxation (under a tonus by carbachol), by the adrenergic agonists Phenilephrine and noradrenaline (NA), on mothers and 40-day old female litters. **Results:** In litters, the following mean weights were lower than in controls: body (66,4±7,3 and 111,4± 4,5g), fundus (68,2±6,5 and 93,7±9,4mg), ovary (30,4±3,7 and 58,6±6,0mg) and heart (282,1±28,7 and 440,0± 20,1mg), respectively. Only the values of apparent affinity (pD_2) for NA were significantly lower from controls, for litters (6.8± 0.14 and 7.2± 0.12). In addition, when using NA after cocaine, corticosterone and propranolol to block uptake I and II and β-adrenoreceptors, pD_2 values were also lower (6.2± 0.25 (alcohol). and 7.0± 0.16 (controls)). **Conclusion:** The treatment with alcohol, caused a decrease of morphologic parameters, added to alterations of the affinity of NA in stomach smooth muscle of female litters. **Supported by:** Fapesp, CNPq and Capes.

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MATERNAL ALCOHOL CONSUMPTION DURING PREGNANCY AND NURSERY AFFECTS CONTRACTILE RESPONSE IN RAT VAS DEFERENS OF DESCENDENTS

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Introduction and objectives: Considering previous evidences (Zimmerberg, B., *Alcohol* 12:71,1995), our objective was to check if treatment of parents with alcohol during pregnancy and nursery produces changes of adrenergic and cholinergic systems of rat litters. **Methods:** Wistar rats, 4 months old, were treated from the 1st day of pregnancy until the 10th day post-partum with 20, 25, or 30% oral alcohol ad libitum. Controls received drinking water. Functional experiments were made by testing the contraction induced by agonists in vas deferens of 40 days old litters. **Results:** The following weights were lower in treated male litters than in controls: body (74,7± 8,12 and 130,3± 5,31g), vas deferens (13,7±1,68 and 23,9± 1,96mg), heart (381,4,±32,6 and 678,7±37,0mg), testicle (436,7±66,8 and 812,48±50,7mg), respectively. The contractile maximal response (g) of the following agonists were also lower: noradrenaline (0,88±0,15 and 1,45± 0,09), phenilefrine (0,84±0,15 and 1,46±0,12), (0,73±0,09 and 1,14± 0,10), $BaCl_2$ (1,0±0,21 and 2,0±0,13) without changes of apparent affinity (pD_2). The relative responsiveness (ρ) for noradrenaline was significantly increased (0,91±0,04 and 0,73±0,04). **Conclusion:** Alcohol use during pregnancy and nursery affects body and organ weights in young descendent males, as well as the contractile response of the vas deferens. Although the mode of action of alcohol is still under study, it is assumed that the functional changes are related, at least in part, to alterations of receptor systems. **Supported by:** Fapesp, CNPq and Capes

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CARACTERIZAÇÃO FARMACOLÓGICA DAS RESPOSTAS CONTRÁTEIS A ANGIOTENSINA II E A ENDOTELINA-1 EM FUNDO DE ESTÔMAGO DE CAMUNDONGOS NOCAUTE B_1 E B_2 DE CININAS

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Cininas estão envolvidas na regulação de homeostase cardiovascular, inflamação e nocicepção. Dois tipos de receptores de cininas B_1 e B_2, são ambos constitutivamente expressos no estomago de camundongo. Em animais deficientes em receptor B_1, verificou-se que a potencia da bradicinina (BK) na resposta contrátil não estava alterada, mas o efeito máximo estava inibido. Foi constatado também que os níveis de óxido nítrico (NO) estava elevado no fundus do estomago isolado desse animal nocaute. Baseado no conhecimento que pode ocorrer interação entre os receptores B_2 de cininas e AT_1 da angiotensina II (AII), interação entre os efeitos da BK e endotelina-1 (ET-1) e que o efeito contrátil induzido pela ET-1 pode ser inibido pelo NO, o nosso objetivo foi caracterizar farmacologicamente as ações da AII e da ET-1 no fundus do estomago isolado de animais normal e nocaute B_1 ou B_2. Registros de contração isométrica foram obtidos para a determinação da potencia e também do índice taquifilático aplicando-se repetidas doses do mesmo agonista em intervalos curtos. Os valores de pD2 (-log da concentração do agonista que induz 50% do efeito máximo, E_{max}.) foram: 8,4 ± 0,1, 8,3 ± 0,1 e 8,2 ± 0,2 para o caso da AII e 7,5 ± 0,2, 7,6 ± 03 e 7,6 ± 0,5 para a ET-1, respectivamente no animal controle (WT), nocaute B_1 (KOB_1) e nocaute B_2 (KOB_2). Os valores de E_{max}. dos agonistas determinados em relação ao do carbacol considerado 100%, foram: 31±2%, 31± 3% e 36 ± 5% para a AII e 59%, 55% e 62% para a ET-1, respectivamente no WT, KOB_1 e KOB_2. Quanto à taquifilaxia à 100 nM AII, a terceira resposta em relação à primeira foi: 50% (WT) 31% (KOB_1) e 32% (KOB_2). Com relação à ET-1 (100 nM) a terceira

resposta foi totalmente bloqueada, em todos os casos, selvagem e nocautes. **Discussão e Conclusão**: os resultados indicam que a potência e a eficácia foram semelhantes para ambos os agonistas nos animais nocaute e normal. A observação que a taquifilaxia à AII no fundus de estomago foi mais atenuada nos animais nocaute, sugere que ela decorre de falta do receptor B_1 ou B_2 e não da presença de NO que se encontra aumentado apenas no nocaute B_1. Entretanto a possível interação cruzada entre a BK e a ET-1 não foi verificada, visto que tanto a potencia como a intensa taquifilaxia foram semelhantes nos animais normal e nocautes. Conclui-se que pode haver um papel importante da interação entre os receptores de cininas e AT_1 para o fenômeno de taquifilaxia, mas não para a ação contrátil. Sugere-se também que o nível de NO não tem influência sobre os efeitos mediados pela ET-1, os quais foram semelhantes no animal normal e nos nocautes. **Apoio Financeiro:** FAPESP e CNPq.