Setor 01. Farmacologia Celular e Molecular

01.001

NEW THIENYLACYLHYDRAZONE DERIVATIVES AS POTENT PLATELET AGGREGATION INHIBITORS

Ferreira de Brito, F. C.[1]; Kummerle, A. E.[2]; Fraga, C. A. M.[2]; Barreiro, E. J.[2]; Miranda, A. L. P.[2] - [1]UFRJ - Farmacologia Básica e Clínica; [2]UFRJ - Faculdade de Farmácia - Fármacos - LASSBio

Introduction: The cardiovascular diseases are responsible for the largest number of natural death through the entire world. Platelet adhesion and aggregation are key events in hemostasis and thrombosis. The development of new antiplatelet drugs is relevant since the available therapeutic arsenal is restricted and sometimes it is not useful. The present study has been conducted in order to investigate the antiplatelet activity of a new series of thienylacylhydrazone compounds, analogous to the lead compound LASSBio 294, and to contribute for the comprehension of its mechanism of action. **Methodology:** The antiplatelet effects have been observed through studies such as: 1) human and rabbit platelet rich plasm stimulated by AA, collagen (COL) and ADP; 2) and in washed human platelet stimulated by thrombin. The effect on cyclic nucleotides formation was evaluated in non-stimulated and stimulated (PGE_1, ODQ and SNP) human platelets quantified by EIA. **Results:** The new thienylacylhydrazone compounds have been able to interfere in platelet aggregation stimulated by these different agonists been more potent in collagen and AA induced platelet aggregation (PA). Among the compounds studied, LASSBio 785, 788 and 789 are the most potent presenting an IC_{50} for AA-PA of 0.3, 0.2 and 3.1 μM, for COL-PA of 0.9, 1.5 and 3.4 μM, respectively. They were 20-70 folds more potent than LASSBio 294 (AA-IC_{50} = 15.3 μM; COL-IC_{50} = 18.3 μM). They inhibit the release reaction by 95%* and whole blood TXB_2 production with an IC_{50} of 63.0μM, 30.4μM, 2.6μM and 257.8μM for LASSBio 294, 785, 788 and 789, respectively. Previous platelet functional studies (Brito et al, FESBE 2005) in the presence of SNP suggested a PDE_2-like effect for LASSBio 785, 788 and 789. In non-stimulated platelets the AMPc levels were not modified by the compounds but they reversed the increase observed in PGE_1-stimulated platelets as EHNA, a PDE_2 inhibitor. On the other hand, they were able to elevate the GMPc levels in non-stimulated platelets (control = 82.9±7.6; L294 = 207.7±17*; L785 = 182.4±9.4*; L788 = 177.6±14*; L789 = 189.7±8.9* fmol/well) and reversed the inhibition observed for ODQ. In both SNP (SNP = 112.0±2.2; L785 = 197.6±20*; L788 = 217.3±6.8*; EHNA = 205.2±12.4* fmol/well) and SNP+PGE_1 (SNP+PGE1 = 125.8±2.4; L785 = 216.4±16.7*; L788 = 255.7±16.6*; L789 = 297.9±15.2*) -stimulated platelets they elevated the GMPc levels with a similar behavior of PDEs inhibitors. In addition such derivatives have presented an in vivo effect increasing the bleeding time in mice and were able to inhibit the whole blood platelet aggregation by 35%-45%. **Conclusion:** We can not ascertain about an direct action over a PDE isoform, but the results suggested that the antiplatelet aggregation activity exert by the thienylacylhydrazones derivatives is through the regulation of cyclic nucleotides, mainly GMPc, and TXB2 formation. Taken together, these results shown that the structural modifications introduced in the compound LASSBio 294 led to an optimization of its pharmacological properties, indicating a potent antiplatelet activity and an antithrombotic potential for this new series of compounds. **Supported by:** PRONEX, CAPES, FAPERJ, FUJB, CNPq.

01.002

REGULAÇÃO DA PRODUÇÃO DE VEGF POR MASTÓCITOS: PAPEL DA HIPÓXIA E MAP QUINASES

Alves, A. P. G.[1]; Diaz, B. L.[1] - [1]INCA - Biologia Celular, CPQ

Introdução Mastócitos são observados em grande número em sítios angiogênicos, incluindo a periferia de tumores, principalmente os malignos. Estudos *in vivo* e *in vitro* têm mostrado que os mastócitos podem contribuir substancialmente para a formação de novos vasos através da produção de fatores pró-angiogênicos como VEGF e IL-6. Entretanto os estímulos e as vias de sinalização envolvidas nesta produção ainda não estão completamente esclarecidas. **Objetivo** Analisar os diferentes estímulos e as vias intracelulares de transdução de sinal envolvidas na produção de VEGF por mastócitos. **Métodos e Resultados** Foram utilizados mastócitos derivados de medula óssea de camundongo (BMMCs) cultivados 4-8 semanas em presença de IL-3. Os BMMCs foram ativados com PMA, SCF + IL-10 + IL-1β e/ou $CoCl_2$. O PMA e a combinação de citocinas foram capazes de induzir a produção e liberação de VEGF (162 e 26.5 pg/10^6 BMMCs, respectivamente). CoCl2, usado para mimetizar um ambiente de hipóxia presente no tecido tumoral, foi capaz de incrementar a produção de VEGF em 163% e 342%, respectivamente. A produção de VEGF induzida por PMA foi inibida em 31, 45, e 115% com a utilização de inibidores de p38, MEK 1/2, e JNK1/2/3. A modulação da produção de IL-6 e o efeito de outros estímulos, como IgE/antígeno, bradicinina, e substância P na produção de fatores angiogênicos estão sob investigação. **Conclusão** A produção de VEGF por mastócitos murinos é aumentada por hipóxia e parece depender de MAP quinases, em particular JNK. **Apoio Financeiro:** INCA/MS e PROFIX/CNPq

01.003

AUMENTO DA FAGOCITOSE MEDIADA POR RECEPTOR FC: MODULAÇÃO POR LEUCOTRIENO D4 E IMPLICAÇÕES DA VIA PI3K

Monteiro, A. P. T.[1]; Barja Fidalgo, T. C.[2]; Canetti, C.[3] - [1]UERJ - Farmacologia e Psicobiologia; [2]UERJ - Farmacologia; [3]UFRJ - Instituto de Biofísica Carlos Chagas Filho

Introdução e Objetivos: Fagocitose é um dos mecanismos fundamentais na resposta imune inata. Já é conhecido que a atividade fagocítica de macrófagos é aumentada por leucotrienos (LTs): LTB_4 e cisteinil LTs. Recentemente foi demonstrado que o aumento da atividade fagocítica promovida pelo LTB_4 envolve a amplificação da ativação da proteína tirosina quinase Syk, fato que não ocorre com o LTD_4. No presente estudo procuramos localizar em que ponto da cascata de sinalização induzida pela ativação do receptor Fc, ocorre modulação por LTD_4, e a sua implicância no processo de fagocitose. **Métodos e Resultados**: Macrófagos alveolares de rato foram plaqueados e devidamente tratados. As células foram tratadas com inibidores de fosfoinositídeo 3 quinase (PI3K; Worthmanin e LY294002) e LTD_4, e desafiadas com eritrócitos opsonizados (RBCs-IgG). Como esperado, o tratamento com LTD_4 promoveu o aumento da fagocitose (avaliada por ensaio colorimétrico). O tratamento dos macrófagos com Worthmanin ou LY294002 não foi capaz de modificar a atividade fagocítica basal, porém o mesmo tratamento foi capaz de impedir o aumento da fagocitose conferido pelo tratamento com LTD_4. A utilização dos mesmos inibidores não alterou a ligação dos macrófagos aos RBCs-IgG, sugerindo a não participação da PI3K na adesão. Demonstramos por meio de western blot que nos tempos de 40, 60 e 80 minutos após a estimulação com LTD_4 houve significativa ativação da via da PI3K (determinada por meio da ativação/fosforilação de seu substrato AKT). **Discussão**: Os resultados sugerem que o LTD_4 modula o aumento da fagocitose por receptor Fc por meio da ativação da PI3K, uma vez que os experimentos de fagocitose sugerem que o LTD_4 não consegue promover aumento na fagocitose enquanto a PI3K encontra-se inibida. **Apoio Financeiro:** Capes, CNPq, SR-2 UERJ e Faperj.

01.004

LIPOSSOMAS DE FOSFATIDILSERINA INIBEM A PRODUÇÃO DE NO E A EXPLOSÃO RESPIRATÓRIA EM MACRÓFAGOS MURINOS

Charao, C. T.[1]; Ramos, G. C.[1]; Assreuy, J.[1] - [1]UFSC - Farmacologia

Introdução: Células apoptóticas expressam fosfatidilserina (PS) no lado externo da membrana, desencadeando sinal para serem fagocitadas por macrófagos. Macrófagos ativados com lipopolissacarídeo (LPS) produzem NO. Estas células quando fagocitam, geram uma explosão respiratória caracterizada pelo aumento do consumo de oxigênio e a produção de anion superóxido. Como ambas as espécies químicas tem papel relevante na resposta inflamatória e na apoptose, neste trabalho avaliamos o efeito de PS sobre a produção de NO e a explosão respiratória. **Metodologia**: Macrófagos peritoneais murinos ($4X10^6$/mL) foram incubados com lipossomas de PS (20-400 μM), ativados com LPS (100 ng/mL) e IFN-g por 24 h e nitrito foi medido pelo método de Griess. A explosão respiratória após fagocitose de zymosan foi medida por quimiluminescência de luminol. **Resultados**: Incubação com PS resultou em menor produção de nitrito (para PS 300 μM: 6 ± 2,32 μM, 48,23 ± 3,75 μM e 12,66 ± 4,68 μM, controle, LPS, PS + LPS, respectivamente). O efeito da PS foi tempo-dependente, sendo o período de 24 h de pré-incubação o mais efetivo. PS diminuiu a explosão respiratória causada por zymosan (84,25 ± 48,40 cpm, 108763,70 ± 28967,06 cpm e 49,43 ± 16,43 cpm, controle, zymosan, PS + zymosan, respectivamente). **Discussão**: A fagocitose de PS e possivelmente, de células apoptóticas, por macrófagos pode ter importante função na modulação da resposta inflamatória. A possível existência de uma relação causal entre o efeito inibitório da PS na produção de NO e na explosão respiratória está sendo investigado no momento. **Supported by:** CNPq, CAPES, PRONEX e FUNCITEC.

01.005

INTERACTIONS BETWEEN GALANTAMINE AND KYNURENIC ACID (KYNA) ON α7 NICOTINIC RECEPTORS

Lopes, C.[1]; Pereira, E. de F. R.[2]; Schwarcs, R.[2]; Albuquerque, E. X.[3] - [1]University of Maryland / UMESP - Pharmacol. Exp. Ther. / FACBIO; [2]University of Maryland - Pharmacol. Exp. Ther.; [3]UMESP - FACBIO

Introduction: Galantamine, a drug approved to treat Alzheimer's disease (AD), acts as an allosteric potentiating ligand (APL) on nAChRs. KYNA, a kynurenine metabolite, is an endogenous inhibitor of a7 nAChRs in the brain. Here, we examined possible interactions between KYNA and galantamine on α7 nAChRs. **Methods:** Type IA, α7 nAChR-mediated currents or inhibitory postsynaptic currents (IPSCs) triggered by activation of presynaptically located a7 nAChRs were evoked by U-tube application of choline to rat hippocampal or striatal neurons in culture and recorded using the patch-clamp technique. **Results:** Galantamine (1-10 μM) increased the amplitudes of choline (1 mM)-evoked type IA currents. The voltage independence of the effect is typical of the nicotinic APL action of galantamine. KYNA blocked α7 nAChRs voltage independently with an IC_{50} of 13.9 ± 8.3 μM. Applied before and/or together with KYNA, galantamine (1 mM) shifted to the right the concentration-response relationship for KYNA-induced a7 nAChR inhibition; the IC_{50} of KYNA increased to 271 ± 131 μM. Galantamine increased, whereas KYNA reduced the net charge of choline-triggered IPSCs. The effect of KYNA on choline-evoked IPSCs was smaller in the presence than in the absence of galantamine. **Discussion:** The finding that galantamine competitively antagonizes the actions of KYNA on α7 nAChRs suggests that galantamine can aid in the treatment of neurophatology in which brain KYNA levels are elevated and α7 nAChR activity is impaired, including schizophrenia. **Supported by:** NIH grants NS25296 and NS 41671; HD16596.

01.006

IMMUNOLOCALIZATION OF TOLL-LIKE RECEPTOR 4 IN THE RAT EPIDIDYMIS.

Queiroz, D. B. C.[1]; Rodrigues, A.[1]; Honda, L.[1]; Avellar, M. C. W.[1] - [1]UNIFESP - EPM - Farmacologia

Introduction: Toll-like receptor 4 (TLR4) is a receptor for lipopolysaccharide (LPS), a highly pro-inflammatory component of the Gram-negative bacteria. Data from our laboratory indicated that rat epididymis (caput and cauda regions) responds to an *in vivo* and *in vitro* treatment with LPS from *E. coli* with changes in the activation of the transcription factor NF-kB. We have also observed that transcripts for TLR4 and CD14 are present in this tissue. To clarify the mechanism involved in the effects of LPS in the epididymis, the aim of this study was to characterize the expression and cellular localization of TLR4 along this tissue. **Methods:** Caput (CP) and cauda (CD) epididymis cryosections from 90-day old Wistar rats were used for immunohistochemistry with an antibody against rat TLR4. Antibody specificity was previously validated by Western blot using CP total protein extract. Negative controls were performed in the presence of specific blocking peptide. **Results:** Specific TLR4 immunostaining was detected in epithelial and interstitial cells from CP and CD. In epithelial cells from CP, TLR4 staining was localized in perinuclear and supranuclear regions, while in CD it was only perinuclear. CP presented a higher number of positive epithelial cells when compared to CD. In all tubules from CP and CD, TLR4-positive and -negative epithelial cells were observed, indicating that only part of these epididymal cells are involved in the local LPS response. **Discussion:** The present study indicates that the epididymis presents the elements involved in the recognition and activation of the intracellular signaling of LPS. The results contribute to the better understanding of the local mechanism of innate immunity in the male reproductive tract. **Supported by:** FAPESP, CAPES, CNPq, TW Fogarty

01.007

ATL-1 INHIBITS EXPRESSION AND ACTIVITY OF METALLOPROTEINASES IN A HUMAN MELANOMA CELL LINE

Zamith-Miranda, D.[1]; Villela, C. G.[1]; Fierro, I. M.[2] - [1]UERJ - Farmacologia e Psicobiologia; [2]UERJ - Farmacologia

Introduction: Lipoxins (LX) are arachidonic acid-derived metabolites generated through cell-cell interactions. We previously showed that ATL-1 (a synthetic LX analog) inhibited the actin cytoskeleton reorganization and proliferation of endothelial cells (Cezar-de-Mello PFT & Fierro IM, 2006). This anti-angiogenic effect of ATL-1 reflects its indirect action upon a tumor. In this study we sought to investigate a direct effect of this analog in a human melanoma cell line, focusing on the expression and activity of metalloproteinases, which are a crucial step in the angiogenic and metastatic processes. **Methodology and Results:** MV3 human melanoma cells were cultured using DMEM with 10% FBS. We first characterized the presence of the LX receptor (ALXR) on these cells, using RT-PCR assay and specific primers for both control cells (1% FBS) and IL-1β stimulated cells. For metalloproteinase assays, cells cultured on 1% FBS were pre-treated with ATL-1 (100 nM) for 30 minutes before the stimulation with IL-1β (10ng/mL). The expression of MMP-2 was measured by western blot assay, and its activity through zymography assay. The treatment with the analog inhibited both the expression and activity of MMP-2. **Conclusions:** Our data suggest a direct action of LX on tumor cells, while it was capable to inhibit the expression and activity of MMP-2, a crucial step for melanoma cell invasion. These findings infer a therapeutic potential of LX's. **Supported by:** FAPERJ, CNPq, SR-2/UERJ

01.008

PAPEL DAS ENDO-OLIGOPEPTIDASES NO METABOLISMO DE PEPTÍDEOS INTRACELULARES.

Berti, D. A.[1]; Klitzke, C. F.[2]; Ferro, E. S.[3] - [1]Instituto de Ciências Biomédicas - Biologia Celular e do Desenvolvimento; [2]Instituto Butantan - CAT/CEPID; [3]USP - ICB

Introdução: Proteínas mal formadas são inviáveis à homeostase celular e são degradadas por um sistema proteolítico intracelular, produzindo uma grande quantidade de peptídeos. Um estudo realizado por Heimann *et al.* (Heimann, A.S., Physiol. Genomics 20, 173, 2005) sugere que peptídeos intracelulares e uma diminuição da atividade de endo-oligopeptidases podem estar relacionados a uma melhora na resistência à insulina induzida por dieta rica em gordura. Estes dados nos levaram a formulação da hipótese de que peptídeos intracelulares poderiam participar na regulação de cascatas de sinalização, como a cascata de sinalização da insulina, através de sítios de modificação pós-traducional (Ferro E. S., J. Neurochem 91, 769, 2004). **Métodos:** Peptídeos intracelulares foram isolados utilizando oligopeptidases inativas (Rioli, V., J. Biol. Chem., 278, 8547 - 2003) e analisados por espectrometria de massa através da técnica LC-MS/MS. **Resultados:** Verificamos que dos 39 peptídeos potenciais substratos das oligopeptidases seqüenciados até o momento, 77% possuem motivos de modificação pós-traducional, sendo a grande maioria sítios de fosforilação para PKA (9%), PKC (13%) e CKII (4%). **Discussão:** Estes achados sugerem que endo-oligopeptidases são enzimas importantes na manutenção da homeostase intracelular, pois seus substratos têm potencial para interferir na sinalização celular, como por exemplo, na cascata de sinalização da insulina. **Agradecimento:** Laboratório Nacional de Luz Sincrotron. **Apoio Financeiro:** FAPESP e CNPq

01.009

MELATONIN INHIBITS THE PRODUCTION OF NITRIC OXIDE IN BRADYKININ-STIMULATED ENDOTHELIAL CELLS

Tamura, E. K.[1]; Silva, C. L. M.[2]; Markus, R. P.[1] - [1]IB - USP - Fisiologia; [2]UFRJ - Farmacologia Básica e Clínica

Introduction: In the CNS the constitutive production of NO is inhibited by melatonin. Moreover, this hormone also modulates vascular tonus in an endothelium-dependent way. Therefore, in this work we investigate if melatonin (MT) modulates the endothelial production *in vitro*. **Methods:** Rat endothelial cells in culture were loaded for 50 min with the probes DAF-FM (5 uM) or FLUO-3 AM (5 uM) for determination of NO or Ca^{2+}, respectively, by confocal microscopy. **Results:** Bradykinin (BK; 1-100 nM) increased the intracellular level of Ca^{2+} $[Ca^{2+}]i$ and NO (pD_2 = 7.86 ± 0.06 and 8.14 ± 0.05, n = 3, respectively). The maximum effect of BK (E_{max}) related to the production of NO was reduced by MT (1 nM) from 108.40 ± 1.01% to 9.04 ± 4.36% (n = 3, $p < 0.05$). However the E_{max} of BK related to the increase of $[Ca^{2+}]i$ was not modified. The agonist 5-MCA-NAT (MT_3 receptor, 1 nM) did not modify NO production, and the nonselective antagonist of MT_1 and MT_2 receptors, luzindol (10 uM), did not prevent the effect of MT. A possible inhibition of calmodulin by MT was also excluded since MT (1 nM) did not mimic the effect of calmidazolium (10 uM). **Discussion:** The effect of MT upon the endothelial production of NO induced by BK is unrelated to the activation of MT receptors suggesting a putative intracellular action. However, at the moment we can rule out an alteration of $[Ca^{2+}]i$ as well as an inhibition of calmodulin. The effect of MT occurs in a range of concentration compatible to the one found in the nocturnal surge suggesting a probable physiological relevance. **Supported by:** CAPES, CNPq and FAPESP

01.010

CHARACTERIZATION OF A NEW SYNTHETIC INHIBITOR OF Na,K-ATPase.

Poças, E. S.[1]; Pimenta, P. H.[1]; Berendonk Leitão, F.[1]; Touza, N.[1]; da Silva, A. J.[2]; Costa, P. R. R.[2]; Noel, F.[1] - [1]UFRJ - Farmacologia Básica e Clínica; [2]UFRJ - NPPN

The aim of the present work was to characterize the interaction between Na,K-ATPase and PCALC36, an original synthetic coumestan. Rat brain and kidney fractions enriched in Na,K-ATPase were utilized to measure inhibition of both enzymatic activity and [^{3}H]ouabain binding. Inhibition curves revealed that unlike ouabain, a thousand times more potent to inhibit brain than kidney isoforms, PCALC36 had a similar affinity for both isoforms (IC_{50}= 4.33 $\pm$ 0.90 and 11.04 $\pm$ 0.86 mM, respectively), and its effect was not antagonized by K^+. PCALC36 did not change the K_D of ouabain but decreased its maximal binding (B_{max}) in a concentration-dependent manner. This effect was not reverted after extensive washing. However, the addition of 5 mM dithiothreitol, but not ascorbic acid, completely blocked the inhibitory effect of PCALC36 suggesting that it forms a stable complex with Na,K-ATPase (in a conformation different from E2P), probably by reacting with sulphydryl groups. A structure-activity relationship study with ten coumestans showed that an hydroxyl in position 2 of the A-ring and a catechol group in D-ring are important for the inhibitory potency. Binding assays using human isoforms expressed in yeast cells showed that PCALC36 has a higher affinity for α2 and α3 (IC_{50}= 10^{-5} M) than for α1 isoform (IC_{50}= 10^{-4} M). We conclude that PCALC36, a non-steroidal molecule, has a mechanism of inhibition different from the cardiac glycosides and could thus serve as a structural paradigm for developing new inotropic drugs. **Supported by:** CAPES, FAPERJ, CNPq, Pronex

01.011

GLUCOCORTICOID RECEPTOR IN RAT EPIDIDYMIS: DISTRIBUTION AND REGULATION BY ADRENALECTOMY.

Silva, E. J. R.[1]; Queiroz, D. B. C.[1]; Rodrigues, A.[1]; Avellar, M. C. W.[1] - [1]UNIFESP - EPM - Farmacologia

Introduction: Glucocorticoid regulates several physiological functions in vertebrates including reproduction. Curiously little is known about this hormone on the epididymis, an important organ for sperm maturation, transport and storage. Here we present a systematic study to immunolocalize glucocorticoid receptor (GR) (as well as androgen receptor, AR) in the rat epididymis and to evaluate the impact of adrenalectomy (ADX) on their expression and cellular distribution. **Methods:** Wistar rats (90 days old) were sham-operated (control) or submitted to bilateral ADX (1, 2, 7 and 15 days). Caput (CP) and cauda (CD) epididymis were used in Western blot (total protein extracts) and immunohistochemistry (cryosections) with GR and AR antibodies (negative controls with specific blocking peptides). Plasma corticosterone (C) levels were monitored by RIA. **Results/Discussion**: Western blot indicated the expected MW for GR (~85 kDa) and AR (~120 kDa) in control CP and CD. Densitometric analysis revealed a significant increase in GR, but not AR protein levels, with ADX 7 and 15 days in CP when compared to control. In the CD both AR and GR increased with ADX 7 and 15 days. Specific GR and AR immunostaining in control CP and CD was detected in different cell compartments of epithelial, smooth muscle and interstitial cells (nuclear, perinuclear and cytoplasmic localization). Significant changes in the dynamic of nuclear and cytoplasmic GR and AR immunostaining were observed with ADX progression as a consequence of the significant reduction in plasma C levels with ADX. Our results show for the first time the distribution of GR and the role of endogenous glucocorticoids on the modulation of GR and AR in the epididymis, suggesting the participation of this hormone in the regulation of epididymal function. **Supported by:** CAPES, FAPESP, CNPq.

01.012

INFLUÊNCIA DE HORMÔNIOS SEXUAIS NA ATIVIDADE COLINESTERÁSICA DO PLASMA DE RATOS MACHOS E FÊMEAS

Alves Amaral, G.[1]; Andrade-Lopes, A. L.[1]; Chiavegatti, T.[1]; Pires-Oliveira, M.[1]; Bueno, M. A.[1]; Godinho, R. O.[1] - [1]UNIFESP - EPM - Farmacologia

Objetivo: As colinesterases (ChE) são classificadas de acordo com a seletividade de hidrólise dos ésteres da colina. A butirilcolinesterase (BuChE), produzida principalmente no fígado, é secretada no plasma onde atua eliminando toxinas, hidrolisando a ACh que escapa da acetilcolinesterase (AChE) e possivelmente regulando lipoproteínas plasmáticas. Considerando que a doença de Alzheimer (AD) tem maior incidência em mulheres no climatério e que a inibição específica da BuChE cerebral reduz o acúmulo do peptídeo β amilóide em ratos, o objetivo deste trabalho foi avaliar a influência de hormônios sexuais na atividade colinesterásica do plasma de ratos. **Métodos e Resultados:** A atividade da ChE do plasma de ratos Wistar adultos (n=4-8) controles (N) ou submetidos à gonadectomia (GDX) por 2-30 dias foi avaliada por método colorimétrico utilizando os substratos acetiltiocolina e butiriltiocolina. Nos machos, a GDX por 7, 15 e 30 dias elevou a BuChE em 11%, 39% e 64%, respectivamente, em relação ao N (2,8±0,2 U/mL). Nas fêmeas, a GDX por 30 dias reduziu em 49% a BuChE em relação ao pró-estro (10,2±0,9 U/mL). Em ambos os sexos, a AChE não variou com a GDX. **Discussão:** Nossos resultados mostram que as diferenças de concentração plasmática de BuChE observadas entre os gêneros são determinadas pelos hormônios sexuais e minimizadas pela gonadectomia, sugerindo que estas variações podem ter relevância na terapia medicamentosa com anticolinesterásicos. **Apoio Financeiro:** CNPq e FAPESP

01.013

EFFECT OF IVERMECTIN ON DIFFERENT ATPase ACTIVITIES FROM RAT VAS DEFERENS.

Muzi-Filho, H.[1]; Souza, D. R. A.[1]; Scaramello, C.[1]; Cunha, V. M. N.[1] - [1]UFRJ - Farmacologia Básica e Clínica

Introduction: Ivermectin (IVM) is a macrocyclic lactone described as an activator of RyR and an inhibitor of SERCA1 and SERCA2b isoforms. IVM is also described as an inhibitor of Mg^{2+} ATPase activity associated with efflux pumps, as is the case of P-glycoprotein. The aim of the present work was to investigate the effect of IVM on Mg^{2+}-ATPase and Ca^{2+}- ATPase present in rat vas deferens (RVD). **Methods:** Male Wistar rats (250-300g) were sacrificed and the RVD were removed. The tissue was washed, homogenized and centrifuged at 108.000xg to obtain ultracentrifuged homogenate (FKBP(+) fraction). To dissociate FKBP12-CRC complex, part of the ultracentrifuged homogenate was treated at 37°C for 30 min before further ultracentrifugation (FKBP(-) fraction). These fractions were used for the measurement of $^{45}Ca^{2+}$ uptake, Mg^{2+}ATPase and (Ca^{2+}+Mg^{2+})ATPase activity. **Results:** 10uM IVM did not alter significantly the $^{45}Ca^{2+}$ content and the total Ca^{2+}-stimulated ATPase activity of SR vesicles present in FKBP(+) and FKBP(-) fractions, with or without 5mM oxalate (n=3; $P>0.05$), but inhibited the basal ATPase activity (28.4±3.5% and 30.8±7.6%; n=4 or 3; $P<0.05$, respectively). The basal ATPase activity of FKBP(+) fraction was also inhibited by 10mM Vanadate (45.0±2.5%; n=4; $P<0.05$) and 100uM Trifluoperazine (65.9±2.3%; n=4; $P<0.05$) but not by 40 or 100uM verapamil. **Discussion:** These data show that IVM does not inhibit the Ca^{2+} pump activity of RVD. However, the NaN_3 resistant-Mg^{2+} ATPase activity is inhibited by this drug as well as other inhibitors of P-glycoprotein. **Supported by:** CAPES, FAPERJ

01.014

MEXITIL® INIBE O BRONCOESPASMO COLINÉRGICO E A HIPERREATIVIDADE DE VIAS AÉREAS EM UM MODELO MURINO DE ASMA

Nascimento, J. B.[1]; Cardozo, S. V. S.[2]; Pires, A. L. de A.[1]; Serra, M. F.[1]; Perez, S. A. C.[1]; Silva, P. M. R. e[1]; Martins, M. A.[1] - [1]FIOCRUZ - Fisiologia e Farmacodinâmica; [2]UFRJ - Faculdade de Farmácia

Introdução e Objetivo: O mexitil® é um anestésico local ativo por via oral e utilizado clinicamente como agente antiarrítimico. Neste trabalho investigou-se o efeito do tratamento oral com mexitil sobre a hiperreatividade de vias aéreas induzida por estimulação antigênica em camundongos. **Métodos:** Camundongos Balb/c foram sensibilizados subcutaneamente nos dias 1 e 14 com uma mistura de $Al(OH)_3$ e ovoalbumina (OVA) e desafiados nos dias 19, 20 e 21 com OVA (25 μg/25 μl, i.n.). Pletismografia de corpo-inteiro (*enhanced pause*, Penh) foi utilizada para monitoramento de mudança no fluxo aéreo pulmonar em resposta à estimulação com metacolina aerosolizada 24 h após a última provocação antigênica. Alterações no Penh após estimulação com metacolina foram também monitoradas em animais normais. **Resultados:** O tratamento com mexitil® (100 mg/kg, oral), 1 h antes da provocação antigênica, aboliu a hiperratividade à metacolina observada nos animais sensibilizados e desafiados. De maneira similar à teofilina, o tratamento com mexitil® (20-60 mg/kg, oral) inibiu também o aumento de Penh induzido por metacolina (6 a 25 mg/ml) em animais naives. **Conclusão:** Os resultados demonstram que o tratamento oral com mexitil® inibe a hiperreatividade de vias aéreas observada em camundongos "alérgicos", sendo também capaz de bloquear o broncoespasmo colinérgico em animais normais. **Apoio Financeiro:** CNPQ, FAPERJ, PDTIS

01.015

EFFECT OF TAK-778 IN A LARGE-SCALE GENE EXPRESSION OF HUMAN OSTEOBLASTS

Bellesini, L. S.[1]; Passos, G. A. S.[2]; Bombonato-Prado, K. F.[3]; Beloti, M. M.[4]; Junta, C. M.[5]; Marques, M. M. C.[5]; Rosa, A. L.[4] - [1]FMRP - USP - Farmacologia; [2]USP - Genética; [3]FORP - USP - Morfolofia, Estomatologia e Fisiologia; [4]FORP - USP - Cirurgia; [5]FMRP - USP - Genética

Introduction: TAK-778 induces osteogenesis *in vitro* and *in vivo.* However, there are no studies evaluating the effect of TAK-778 on gene expression of human osteoblasts. Thus, the aim of this study was to investigate the effect of TAK-778 on gene expression by using cDNA microarray technology. Methods: Cells at first passage were cultured in osteogenic medium containing TAK-778 (10^{-5}M) or vehicle. Total RNA was isolated at day 7 from all samples and cDNA generated after reverse transcription, in the presence of 33P isotope, were hybridized with *microarrays.* To investigate the gene expression a nylon cDNA microarray was prepared containing 687 clones from the IMAGE CONSORTIUM. Gene expression profiling of both treated and control cells were compared by means of hierarchical clustering algorithm. Results: TAK-778 induced the expression of some genes related with skeletal development and histogenesis such as BMP5, CDH11, ALPL, BMPRII, MSX2, SMURF1 and CTGF. In addition, TAK-778 induced the expression of ICAM-1, which promotes osteoclastogenesis. Discussion: These results indicate that TAK-778 enhances osteoblast differentiation, since the expression of some genes like ALPL and OMD were induced at day 7, while such genes were suppressed in the control group. Acknowledgements: FAPESP and CNPq for financial support and Takeda Chemical Industries for TAK-778 supplied. **Supported by:** FAPESP and CNPq

01.016

PRODUCTION OF NITRIC OXIDE INDUCED BY P2Y1 RECEPTORS IN RAT PINEAL GLAND.

Armelin, M. A.[1]; Markus, R. P.[1]; Ferreira, Z. S.[2] - [1]IB - USP - Fisiologia; [2]USP - Fisiologia

Previous results show the presence of perivascular nerve fibers immunoreactive to the neuronal form of nitric oxide synthase (NOS) in rat pineals and support that noradrenaline (NE) enhances NOS activity and increases the formation of a nitric oxide (NO)-like compound in the rat pineal gland. Purinergic cotransmission has been demonstrated in rat pineals where P2Y1 receptors potentiate NE-induced melatonin synthesis. An attempt has been made to test a fluorescent probe to measure in vitro NO levels in rat pinealocytes and to investigate the effects of the activation of P2Y1 receptors on NO production in pineals. The drugs were applied to the cells in the presence of DAR-4M (1μM) in a microplate-based assay. Stimulation of pinealocytes with the agonist AMP-PNP (0.03–1mM) increased NO formation in a concentration-dependent manner (from 8.4±2.6 to 15.2±2.6% over basal, n=3). This stimulatory effect was abolished by 41.9% ($p<0.05$) in the presence of the pre-treatment with A3P5P (selective P2Y1 antagonist, 0.3mM, n=3) or L-NAME (0.1mM, n=3). Even more, AMP-PNP also increased the content of cGMP in a concentration-dependent manner. AMP-PNP 0.3mM increased by 5 times cGMP content. We conclude that DAR-4M, which detects as little as a few nanomolar concentration of NO in cell-free conditions, is a suitable probe for monitoring NO production in rat pinealocytes. Even more the P2Y1 receptor stimulation leads to a measurable increase in NO and cGMP production in the pineal gland, suggesting the involvement of nitrergic pathway in the synchronization of neural output. **Supported by:** FAPESP, CNPq.

01.017

FUNCTIONAL ROLE OF RELAXIN IN THE ISOLATED VAS DEFERENS

Cardoso, L. C.[1]; Pimenta, M. T.[1]; Santos, M. F.[1]; Avellar, M. C. W.[1]; Porto, C. S.[1]; Lazari, M. F. M.[1] - [1]UNIFESP - EPM - Farmacologia

Aim. Relaxin (RLX) activates the G-protein coupled receptors RXFP1 and RXFP2 and induces cAMP production in several tissues. We have previously shown by RT-PCR that transcripts for RLX receptors are widely distributed in the male reproductive tract. We confirmed the expression of the proteins by immunohistochemistry in the epithelial and muscular layers of the rat vas deferens. In the present study we aimed to identify the role of these muscular RLX receptors. **Methods and results.** We compared the effect of relaxin (RLX) and forskolin (FSK) on isometric contractions and on cAMP production of the rat vas deferens. RLX (80 μM) had little effect on the contraction induced by a depolarizing solution (8% relaxation, versus 36% relaxation with 50 μM FSK, and 34% relaxation with 50 μM isobutylmethylxanthine). Similar contractions of the rat uterus were reduced by 40% with 40 μM RLX. Incubation with RLX (30 min, 80 μM) failed to reduce the contraction induced by 2.5 μM noradrenaline, whereas this contraction was reduced by 80% with FSK. RLX also failed to inhibit spontaneous contractions of the vas deferens isolated from castrated animals, whereas these were blocked by FSK. Finally, RLX (800 μM) was much less potent than FSK (50 μM) at inducing cAMP production (increase of 150% vs. 2000%). **Conclusion:** RLX is not involved in the contractile activity of the rat vas deferens. It remains to be determined whether RLX plays a role in the absorptive and secretory functions of the organ. **Supported by:** FAPESP, CAPES.

01.018

CORRELAÇÃO ENTRE ATIVIDADE DO TIPO ANTIDEPRESSIVA E INIBIÇÃO DA CAPTAÇÃO DE [^{3}H]–5HT NA PRESENÇA DE TROPOL, IPRATRÓPIO e *endo*-FENTÔNIO

Lima-Landman, M. T. R.[1]; Barros, J. S.[2]; Duarte, F. S.[3]; De Lima, T. C. M.[3]; Souccar, C.[1]; Lapa, A. J.[1] - [1]UNIFESP - EPM - Farmacologia; [2]CBA - AM - Farmacologia; [3]UFSC - Farmacologia

O exo-Fentônio [N-4-(fenil) fenacil l-hiosciamina] (exoFEN) é antimuscarínico competitivo e antinicotínico não-competitivo em receptores $\alpha 1_2\beta\,\gamma\varepsilon$, $\alpha 7$, $\alpha 4\beta 2$ e $\alpha 3\beta 4$. O exoFEN também tem ação do tipo antidepressiva no teste de suspensão pela cauda em camundongos e bloqueia a captação de monoaminas em sinaptossomas cerebrais. Este trabalho estudou a correlação desses efeitos nas ações do enantiômero *endo*-Fentônio (endoFEN) e dos antimuscarínicos [N-(4-fenil)-fenacil-tropan-3-ol] (Tropol) e [8-iso-propil-noratropina] (Ipratrópio). Imipramina (IMI 75 μM e 180 nM) e exoFEN foram os controles. Em sinaptossomas de hipocampo de rato, a captação de [^{3}H]-5-HT (102 Ci/mmol – 4 nM durante 6 min) a 37 °C, foi inibida na presença de exoFEN (CI_{50} = 0,1 μM, n=3), IMI (CI_{50} = 120 ηM, n=5) e endoFEN (CI_{50} = 0,9 μM, n=3) incubados por 10 min. Tropol (10^{-4}M) ou Ipratrópio (10^{-4}M) não alteraram a captação de [^{3}H]-5-HT. A injeção *i.c.v* de 2 μL de exoFEN (10^{-4} M), Tropol (10^{-4} M) e Ipratrópio (10^{-4} M) ou de IMI (15 mg/kg, *i.p.*) reduziu de ~30% o tempo de imobilidade no teste de suspensão pela cauda. O endoFEN (10^{-4} M) não alterou a resposta controle. A diminuição do tempo de imobilidade no teste de suspensão pela cauda sugere ação do tipo antidepressiva para os antimuscarínicos Tropol e Ipratrópio, mas este efeito *in vivo* não foi correlacionado à inibição da captação de 5-HT *in vitro*. Ao contrário, o endoFEN inibiu a captação de 5-HT, mas não modificou a reação dos animais. CEP/Unifesp – 0031/04 **Apoio Financeiro:** Fapesp, CBA-AM

01.019

SUPPRESSION OF SILICA-INDUCED LUNG FIBROSIS BY EARLY TREATMENT WITH FLUNISOLIDE AND NCX 1024 IN MICE.

Lima, J. G. M.[1]; Ferreira, T. P. T.[1]; Farias-Filho, F. A.[1]; Arantes, A. C. S. de[1]; Ciambarella, B. T.[1]; Cordeiro, R. S. B.[1]; Lagente, V.[2]; Martins, M. A.[1]; Wallace, J.[3]; Silva, P. M. R. e[1] - [1]FIOCRUZ - Fisiologia e Farmacodinâmica; [2]Université de Rennes 1 - Faculté des Sciences Pharmaceutiques et Biologiques ; [3]University of Calgary - Proteases and Inflammation Network (PAIN)

Introduction: We previously reported that flunisolide (FLU) and the nitrosteroid NCX-1024 exert a curative effect on silica-induced lung fibrosis. Since up to now there is no clinical evidence proving that lung fibrosis occurs after an acute inflammatory response, we investigated the effect of an early treatment with FLU and NCX-1024 on late fibrosis in silicotic mice. **Methods:** Anaesthetized Swiss-Webster mice were nasal instillated with silica particles (10 mg) and treated intranasally with FLU and NCX-1024 (0.022 µmol/kg), from days 0 to 7. The analyses were made on day 28 and included leukocyte counts in the bronchoalveolar (BAL) fluid as well as lung morphology performed by histological techniques. **Results:** We found an increase in the number leucocytes in BAL, mainly neutrophils and mononuclear cells, and a marked collagen deposition and granuloma formation in the lung tissue. Intranasal administration of FLU and NCX 1024, from 0-7 days, led to a reduction in the number of total leukocytes in the BAL fluid from $6.8 \pm 1.2 \times 10^5$ cells to $2.1 \pm 0.4 \times 10^5$ cells and $2.6 \pm 0.3 \times 10^5$ cells (mean ± SEM; n=7), respectively). Attenuation of granuloma formation and collagen deposition was also attested by means of haematoxilin & eosin and Picrus sirius staining, respectively. **Conclusion**: Our findings show that the early administration of FLU as well as NCX 1024 suppressed silica-induced chronic lung fibrosis, and offer evidence that there is a direct relationship between inflammatory parameters and the evolution of the disease. **Supported by:** CNPq, PAPES4/FIOCRUZ.

01.020

PROFILE OF mRNA NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS IN BRAIN AND SKELETAL MUSCLE OF ADULT MICE.

Ghedini, P. C.[1]; Avellar, M. C. W.[1]; Honda, L.[1]; Lima-Landman, M. T. R.[1]; Lapa, A. J.[1]; Souccar, C.[1] - [1]UNIFESP - EPM - Farmacologia

Introduction: Nicotinic acetylcholine receptors (nAChRs) are classified in muscular ($\alpha1,\beta1,\delta$ and ε or γ subunits) and neuronal ($\alpha2$- $\alpha10$ and $\beta2$-$\beta4$ subunits) subtypes. Neuronal nAChRs were also reported in non-neuronal cells, and muscular $\alpha1$ subunit was described in chick ciliary ganglia. In this work the profile of different mRNA nAChRs subunits ($\alpha1,\varepsilon,\gamma,\alpha7,\alpha4$ and $\beta2$) was examined in skeletal muscle and different brain regions of adult male mice. **Methods:** RNAs extracted from diaphragm muscle, cortex, hippocampus and cerebellum were screened using RT-PCR with primers designed to amplify specific regions coding for the different nAChRs subunits. DNA products were confirmed by direct nucleotide sequencing performed with an automated sequencer. Muscular (diaphragm) and neuronal (cortex) nAChR binding sites were determined by radioligand binding techniques. **Results:** Maximal binding of [^{125}I]-α-bungarorotoxin and [^{3}H]-cytisine in membrane preparations were (in fmol/mg protein): 52.2± 3.4 (muscle nAChR), 48.6 ± 3.6 and 98.2 ± 3.0 ($\alpha7$ and $\alpha4\beta2$ subtypes, respectively); (K_d = 0.5 to 1 nM). Transcripts encoding $\alpha1$, ε, $\alpha7$, $\alpha4$ and $\beta2$ nAChRs subunits were detected in all samples tested. No specific γ subunit DNA product was observed in the brain regions, but low expression of this transcript was detected in the muscle. **Discussion:** Expression of $\alpha7$, $\alpha4$ and $\beta2$ subunits transcripts in adult rodent muscles were previously reported and were related to non-contractile Ca^{2+} mobilization and prejunctional modulation of ACh release. Expression of $\alpha1$ and ε transcripts in neuronal tissue, however, were not so far described and their functional role remains to be determined. CAPES, CNPq, FAPESP

01.021

HEME, A PROINFLAMMATORY MOLECULE, DELAYS HUMAN NEUTROPHIL APOPTOSIS: INVOLVEMENT OF CLASSICAL AND NOVEL PKC ISOFORMS

Barcellos-de-Souza, P.[1]; Serezani, C. H.[2]; Barja Fidalgo, T. C.[1]; Arruda, M. A.[1] - [1]UERJ - Farmacologia; [2]USP - Imunologia

Introduction: Our group has previously shown that free heme is a proinflammatory molecule able to delay human neutrophil spontaneous apoptosis, indicating that heme may play a major role during acute and chronic inflammation related to hemolytic episodes. Although our observations that PKC activity plays an essential role on most of heme-evoked proinflammatory responses, as well as on the up-regulation of the pro-survival pathway PI3K/Akt, the use of pan-PKC inhibitors were unable to abolish heme antiapoptotic effect. This scenario points to a differential role of distinct PKC isoenzymes on the regulation of heme-induced delay of neutrophil spontaneous apoptosis. Methods and Results: Inhibition of the classical PKC-α isoform with the selective inhibitor RO32-0432 (10 nM) significantly reverted the delay of neutrophil apoptosis evoked by heme (21% ± 1.5 versus 46% ± 1.2, n = 5). However, Rotlerin (6 μM), a selective inhibitor of the novel PKC-δ, as previously described, inhibited neutrophil spontaneous apoptosis, an effect that was not affected by heme treatment. The involvement of PKC-α and PKC-δ isoforms on pro-survival signaling cascades as well as on mitochondrial transmembrane potential and Bcl-2 members activity in heme-stimulated neutrophils are under investigation. Conclusion: Our results indicate a complex, hierarquical role of PKC isoforms on neutrophil stimulation by heme, a phenomenon which may corroborate to the understanding of the signaling events that govern neutrophil activation and survival. **Supported by:** CAPES, CNPq, SR-2/UERJ

01.022

INHIBITION OF PLATELET ADHESION BY STAPHYLOCOCCAL ENTEROTOXIN B (SEB) INVOLVES HYDROGEN PEROXIDE FORMATION

Prada Morganti, R.[1]; Marcondes, S.[1]; Antunes, E.[1] - [1]UNICAMP - Farmacologia

Introduction: SEB inhibits human platelet adhesion (PA) to fibrinogen (FB), but the mechanisms involved are still unclear. In this study we investigated the participation of reactive oxygen species (ROS) in the inhibition of PA by SEB. **Methods:** Human washed PA was evaluated using FB-coated 96-well microtiter plates. Platelets were pretreated with SEB (0.0001–30 μg/ml) and/or SOD (100 U/ml), PEG-SOD (30 U/ml) and PEG-Catalase (300-1000 U/ml) for 5 to 120 min, after which they were transfered to FB-coated plates and maintained for 15 min. Adhered platelets were quantified through the measurement of acid phosphatase activity. Levels of cAMP and cGMP as well as cell viability were also evaluated. **Results:** SEB (0.0001-30 μg/ml) dose and time-dependently inhibited the spontaneous PA (for 0.1 μg/ml: 31±6 and 42±8% inhibition at 5 an 60 min, respectively; for 30 μg/ml: 33±5 and 59±11% inhibition at 5 an 60 min, respectively). Pre-incubation of platelets with either SOD (100 U/ml) or PEG-SOD (30 U/ml) did not change the inhibitory effects of SEB. However, PEG-Catalase (1000 U/ml) reduced the inhibitory responses of SEB (by approximately 60%). No changes in the cAMP and cGMP levels were found in SEB-treated platelets. Platelet viability was not changed after incubation with SEB, according to the MTT assay. **Conclusions:** Hydrogen Peroxide plays a role in the inhibition of PA by SEB. **Supported by:** FAPESP

01.023

REGULAÇÃO DA ATIVIDADE DA ENZIMA CONVERSORA DE ANGIOTENSINA I PELO RECEPTOR B_2 DE CININAS

Sabatini, R. A.[1]; Fernandes, L.[1]; Bersanetti, P. A.[1]; Mori, M. A.[1]; Navarro, A. I.[1]; Santos, E. L.[1]; Carmona, A. K.[1]; De Andrade, M. C. C.[2]; Casarini, D. E.[2]; Chagas, J. R.[3]; Paiva, A. C. de M.[1]; Pesquero, J. B.[1] - [1]UNIFESP - EPM - Biofísica; [2]UNIFESP - EPM - Medicina; [3]UNIFESP - EPM - Psicobiologia

Introdução: Dados recentes da literatura têm sugerido uma heterodimerização entre a enzima conversora de angiotensina (ECA) e o receptor B_2 das cininas, entretanto essa interação sempre foi avaliada pela alteração de parâmetros farmacológicos do receptor. Dessa forma, o objetivo deste trabalho foi verificar esta possível interação avaliando a influência da expressão do receptor B_2 de cininas na atividade da ECA**. Métodos:** A atividade da ECA foi determinada por ensaios fluorimétricos com o substrato Abz-FRK (Dnp)-P em células CHO transfectadas com a forma somática da ECA e o receptor B_2 e em culturas primárias de células endoteliais de animais nocautes para o receptor B_1 de cininas. A expressão da enzima foi avaliada por *Western blotting*. **Resultados:** A presença do receptor B_2 promoveu um aumento na atividade da ECA quando comparada com o controle nas CHO transfectadas (0,67 ± 0,04 U/ µg de proteína vs 0,47 ± 0,01 U/µg de proteína , respectivamente, $p<0,05$), sendo esse efeito bloqueado na presença do antagonista específico de receptores B_2, icatibant 1 nM (28%, $p<0,05$ e 33 % nas células endoteliais, $p<0,05$). Esses resultados mostram pela primeira vez que a expressão dos receptores B_2 das cininas modula positivamente a atividade da ECA e sugerem a existência de uma interação funcional entre estas duas moléculas. Além disto, nossos resultados mostram também que antagonistas do receptor B_2, ligando-se ao receptor, são também capazes de causar inibição da atividade da ECA. **Apoio Financeiro:** CAPES, FAPESP

01.024

EFFECTS OF BAY 41-2272 ON THE HUMAN NADPH OXIDASE SYSTEM FROM THP-1 CELLS

De Oliveira-Jr, E. B.[1]; Thomazzi, S. M.[2]; Rehder, J.[3]; Antunes, E.[1]; Condino-Neto, A.[4] - [1]UNICAMP - Farmacologia; [2]UFSE - Fisiologia; [3]UNICAMP - Pediatria; [4]ICB - USP - Imunologia

Introduction: The NADPH oxidase system has a central role in bacterial killing and host defense. The 5-cyclopropyl-2-[1-(2-fluoro-benzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]-pyrimidin-4-ylamine (BAY 41-2272) was described as a non-NO-based sGC activator. The original compound YC-1 inhibits human neutrophil functions through a cAMP/PKA-dependent pathway. Recent study demonstrated that BAY 41-2272 increased in human eosinophils both cGMP and cAMP levels. **Methods**: This study was designed to investigate the effects of the BAY 41-2272 on superoxide release, gp91-*phox* gene expression, and cGMP and cAMP levels in the THP-1 cells. **Results:** BAY 41-2272 produced a increase on superoxide release (1.5±0.1, 2.5±0.5, 2.8±0.2, 3.1±0.3 and 2.6±0.4 nmolO_2^-/10^6 cells/h for control, and 0.3, 1, 3 and 10μM of BAY 41-2272; $P<0.05$; $n=5$), and increased the expression of gp91-*phox* (1.0±0.0, 1.7±0.1 and 1.6±0.1 relative expression for control, and 3 and 10μM of BAY 41-2272; $P<0.01$; $n=3$). In the presence of zaprinast or IBMX, BAY 41-2272 produced a larger increase in the gp91-*phox* expression ($P<0.05$; $n=3$). BAY 41-2272 significantly increased the cGMP and cAMP levels ($n=4$). **Discussion:** Our findings show that BAY 41-2272 caused a significant increase on the superoxide release and gp91-*phox* expression by THP-1 cells. The elevations of cGMP and cAMP levels have also showed that they may be involved in these intracellular mechanisms. **Supported by:** FAPESP and CNPq

01.025

EFFECTS OF 17B-ESTRADIOL IN THE RAT SERTOLI CELLS

Lucas, T. F. G.[1]; Lazari, M. F. M.[2]; Porto, C. S.[1] - [1]UNIFESP - EPM - Farmacologia Celular; [2]UNIFESP - EPM - Farmacologia

Aim: Sertoli cells (Sc) are important for the establishment and maintenance of spermatogenesis. Previous studies from our laboratory showed that estrogen receptors (ERa and ERb) are expressed in Sc and that ERs translocate to the cell membrane after estrogen treatment, suggesting that the action of estrogen on Sc may involve non-genomic mechanisms (Lucas et al., Fesbe, 2005). In other systems E2 triggers a variety of second messenger signaling, including activation of the MAP Kinases (Thomas et al., 2005 Endocrinol 146:624). The aim of this study was to determine the functional role of estrogen on Sc proliferation and ERK1/2 activation. **Methods and Results**: Primary culture of Sc was obtained from the testis of 15-day old rats. The treatment of cultured Sc with 10^{-10}M 17b-estradiol (E2) for different periods (2 min to 4 h) at 35°C induced a time-dependent increase in the incorporation of ^{3}H-thimidine, with a maximum increase of 70% above basal after 10 min of incubation. An increase in cell proliferation was also observed after 24 h of incubation with E2. All these effects were blocked by pre-treatment of the cells for 30 minutes with 10^{-9} M of the estrogen receptor antagonist ICI 182,780. Western blot with anti-p44/42 and anti-phospho-p44/42 antibodies showed that E2 induced a maximum 8-fold increase in the phosphorylation state of ERK1/2 after 10 min incubation. **Conclusion**: Our data indicate that E2 induces Sc proliferation by a nongenomic mechanism that probably involves ERK1/2 activation. **Supported by:** FAPESP, CNPq

01.026

PHARMACOLOGICAL REACTIVITY OF PROSTATIC AND EPIDIDYMAL PORTIONS OF VAS DEFERENS OF NEWBORN RATS AFTER TREATMENT OF MOTHERS WITH FLUOXETINE DURING PREGNANCY AND BREAST-FEEDING.

Pereira, J. D.[1]; Caricati-Neto, A. C.[1]; Jurkiewicz, A.[1]; Jurkiewicz, N. H.[1] - [1]UNIFESP - EPM - Farmacologia

Background and aims: The contractile response of the vas deferens (VD) of 30-day old Wistar rats whose mothers were treated during pregnancy and breast-feeding with fluoxetine, a serotonin uptake blocker, was studied. **Material and methods**: Wistar rats (n=10/group) were treated with fluoxetine hydrochloride 10mg/kg/day/i.p. (F) during pregnancy and breast-feeding, while the control group (C) was treated with the vehicle (saline + DMSO, 1mL/kg). The epididymal (EP) and prostatic (PP) portions of the VD of the corresponding 1 month old newborn rats were mounted *in vitro*. Pharmacological parameters (E_{max}, pD_2, alpha, rho) were measured from concentration-response curves for adrenergic agonists noradrenaline (NA), phenylephrine (PHE), dopamine (DA) and clonidine (CLO) as well as for serotonin (5HT) and barium chloride ($BaCl_2$). **Results**: Differences were not found for pD_2 values for $BaCl_2$, NA and PHE in both portions of the VD. However, much lower pD_2 values were obtained for DA (F=3.72 ± 0.46, C=5.08 ± 0.19) in PP, and for CLO (F=4.45 ± 0.43, C=6.35 ± 0.13) in EP. In addition, the effects of 5HT were almost totally blocked in PP. b>Conclusions: The entire blockade of 5HT effect in PP, and the decrease of pD_2 values for CLO in EP may be related to the fact that both agents are indirect agonists, releasing endogenous substances. In relation to the decreased affinity (pD_2) for DA, but not for NA or PHE, it may indicate that DA is interacting with another receptor, besides the alpha-adrenergic. In these cases, fluoxetine mechanism of action remains unknown. **Supported by:** Fapesp, CNPq, Capes

01.027

DISCREPANCIES BETWEEN THE FUNCTIONAL CHARACTERIZATION AND THE IDENTIFICATION OF RNA ENCODING ALPHA$_1$-ADRENOCEPTOR (AR) SUBTYPES IN THE RAT TESTICULAR CAPSULE (RTC).

Jurkiewicz, N. H.[1]; Caricati-Neto, A. C.[1]; Verde, L. F.[1]; Avellar, M. C. W.[1]; Reuter, A. R.[1]; Jurkiewicz, A.[1] - [1]UNIFESP - EPM - Farmacologia

Introduction and Methods: Experiments were made in order to compare the characterization of alpha1-AR subtypes in RTC by means of apparent affinity (pA_2) of competitive antagonists on noradrenaline (NA)-induced contractions with reverse transcription polymerase chain reaction (RT-PCR) assays to check for corresponding expression of alpha$_1$ -AR subtypes. **Results**: NA-induced contractions were competitively blocked by the selective alpha$_{1A}$-AR antagonists WB 4101 (pA_2=8.88), phentolamine (pA_2=8.39) and by the alpha$_{1B}$-AR antagonist spiperone (pA_2=8.57), indicating the presence of functional alpha$_{1A}$- and alpha$_{1B}$-AR. In addition, contractions were not blocked by the selective alpha$_{1D}$-AR antagonist BMY 7378 (up to 10^{-6}M), suggesting a minor role, if any, for alpha$_{1D}$-AR. On RT-PCR assays, the presence of mRNA encoding alpha$_{1A}$- and alpha$_{1B}$-AR was also shown. Unexpectedly, alpha$_{1D}$-transcripts were also detected in these assays. **Conclusion**: These results show that contractions for NA in RTC are mediate by both alpha$_{1A}$- and alpha$_{1B}$-AR, but not by alpha$_{1D}$-AR. The fact that alpha$_{1D}$-AR could not be detected in contraction experiments, in spite of the presence of the corresponding mRNA, can indicate another function for this subtype, unrelated to contraction of RTC. **Supported by:** Fapesp, CNPq, Capes

01.028

FUNCTIONAL CHARACTERIZATION AND EXPRESSION OF ENDOTHELIN RECEPTORS IN RAT CAROTID ARTERY: MECHANISMS UNDERLYING ET_B-INDUCED RELAXATION.

Tirapelli, C. R.[1]; Casolari, D. A.[2]; Yogi, A.[2]; Montezano, A. C. I.[3]; Tostes, R. C. A.[2]; Legros, E.[4]; D´orleans-Juste, P.[4]; Oliveira, A. M. de[5] - [1]EERP - USP - Farmacologia; [2]USP - Farmacologia; [3]ICB - USP - Farmacologia; [4]Universite de Sherbrooke - Pharmacologie; [5]USP - FCFRP

Introduction: We aimed to functionally characterize endothelin (ET) receptors in the rat carotid artery. Methods and Results: mRNA and protein expressions of both ET_A and ET_B receptors were detected in carotid segments. Immunohistochemical assays showed that ET_B receptors are expressed in the endothelium and smooth muscle cells, while ET_A receptors are expressed only in the smooth muscle. Vascular reactivity experiments, using standard muscle bath procedures, showed that ET-1 induces contraction in endothelium-intact and -denuded carotid rings. Endothelial removal enhanced ET-1-induced contraction. BQ123 and BQ788, selective antagonists for ET_A and ET_B receptors, respectively, produced rightward displacements of the ET-1 concentration-response curves. IRL1620, a selective agonist for ET_B receptors, induced a constraction that was abolished by BQ788. ET-1 and IRL1620 relaxed carotid rings with intact endothelium. The relaxation was reduced by BQ788 and completely abolished after endothelium removal. Preincubation of intact rings with L-NAME, ODQ, indomethacin, tetraethylammonium (TEA) or 4-aminopyridine reduced IRL1620-induced relaxation. Discussion: The rat carotid posses ET_A and ET_B vasoconstrictor receptors located on the smooth muscle and endothelial ET_B receptors that mediated vasorelaxation via NO-cGMP pathway, vasodilator cyclooxygenase product(s) and the activation of voltage-dependent K^+ channels.

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01.029

CROSS-TALK BETWEEN THE SARCOPLASMIC RETICULUM AND THE MITOCHONDRIAL CALCIUM HANDLING SYSTEMS MAY PLAY AN IMPORTANT ROLE IN THE REGULATION OF CONTRACTION IN ANOCOCCYGEUS SMOOTH MUSCLE

Restini, C. A.[1]; Moreira, J. E.[2]; Bendhack, L. M.[1] - [1]FCFRP - USP - Física e Química; [2]FMRP - Biologia Celular e Molecular

Introduction Activation of mitochondrial Ca^{2+} influx can modulate IP_3 receptors and cytosolic Ca^{2+} signaling leading to amplification of Ca^{2+} signal. Therefore, we investigated the contribution of mitochondrial Ca^{2+} in the phenylephrine (PHE)-induced contraction in the rat anococcygeus muscle. **Methods** Contractile response induced by PHE and the effect of mitochondrial inhibitors were recorded in Ca^{2+}-free and normal Ca^{2+} solution, in absence and in presence of caffeine and the IP_3-receptor antagonist, 2-APB. The morphology of the cells was analyzed by electronic microscopy (EM). **Results** In normal Ca^{2+}, carbonyl cyanide p-(trifluoro-methoxy)phenyl-hydrazone (FCCP, 5 μM) and oligomycin (OLIGO,1 μg/mL) produced contraction similar to that elicited by 0.1 μM PHE. Contraction induced by PHE was reduced by FCCP+OLIGO from 100% to 8.9 ± 1.2% (n=5). In Ca^{2+}-free FCCP+OLIGO did not induce contraction. Following Ca^{2+} repletion, response to FCCP+OLIGO was reduced from 100% to 42.9 ± 7.9% (n=5). This response was abolished by 100 μM 2-APB. Contractile response induced by 20 mM caffeine was reduced from 100% to 24,4 ± 6.9% (n=6) after incubation with FCCP+OLIGO. Images from ME showed that a profuse net of sarcoplasmic reticulum (SR) encloses mitochondria. **Conclusion** A cross-talk between SR and mitochondria may play an important role in the PHE-induced contraction in presence of extracellular Ca^{2+} as well Ca^{2+} released from SR in rat anococcygeus muscle cells. Financial support: FAPESP, CNPq. **Supported by:** FAPESP, CNPq

01.030

REGULATION OF PROTEIN SYNTHESIS AND SECRETION BY CARBACHOL IN RAT EFFERENT DUCTULES AND EPIDIDYMIS.

Siu, E. R.[1]; Avellar, M. C. W.[1]; Porto, C. S.[1] - [1]UNIFESP - EPM - Farmacologia

Aim: The presence of M_1, M_2 and M_3 muscarinic acetylcholine receptor (mAChR) subtypes was shown in the rat efferent ductules and epididymis (Siu et al., 2006, Cell Tissue Res 323:157-166). M_3 subtype is involved in the cauda epididymal tubule contraction, but the role of the other mAChRs remains unknown. Considering the involvement of the cholinergic system in secretory processes in several tissues and the presence of mAChRs over the epithelium of efferent ductules and epididymis, the aim of the present study was to determine the effects of carbachol on protein synthesis and secretion in these tissues. **Methods and Results**: Efferent ductules and epididymis from 50 day-old rats were incubated with [^{35}S]-Methionine in the presence and absence of carbachol (10^{-4}M), 4 h, 30^{o}C. After incubation, total incorporation of [^{35}S]-Methionine was estimated in proteins released to the medium (secreted proteins) and tissue proteins. Carbachol induced a significant increase in [^{35}S]-Methionine incorporation in proteins secreted by efferent ductules and in secreted and tissue proteins of caput of the epididymis, when compared to their respective basal values (absence of carbachol). These effects were abolished by atropine. In the cauda of the epididymis, carbachol did not have any effects on total incorporation of [^{35}S]-Methionine. **Conclusion**: The present study showed that the activation of mAChRs increased the [^{35}S]-Methionine incorporation in synthesized and/or secreted proteins by efferent ductules and caput of the epididymis. More studies are necessary to understand the role of each mAChR subtype in the efferent ductules and epididymis and, consequently, in the acquiring mechanisms and/or maintenance of male (in)fertility. **Supported by:** FAPESP e CAPES

01.031

PEPTÍDEOS GERADOS INTRACELULARMENTE SÃO CAPAZES DE MODULAR A SINALIZAÇÃO DE GPCRs

Cunha, F. M.[1]; Ferreira, Z. S.[2]; Markus, R. P.[3]; Ferro, E. S.[4] - [1]UNIFESP - EPM - Bioq./Dep de Bio Cel e Desenv; [2]USP - Fisiologia; [3]IB - USP - Fisiologia; [4]USP - ICB

Introdução Durante o metabolismo protéico há geração de um grande número de peptídeos intracelulares. O presente trabalho investiga os efeitos destes peptídeos em cascatas de sinalização de receptores acoplados a proteínas G (GPCRs). **Métodos** A ativação de vias de sinalização foi avaliada por ensaios de gene-repórter e microfisiometria. Para a internalização celular, os peptídeos escolhidos foram sintetizados fundidos à seqüência TAT do HIV. **Resultados** O pré-tratamento das células H293, por exemplo, com o peptídeo 5A (LTLRTKL, 100 μM), que possui um sítio para a PKC, inibiu 60% da atividade da luciferase induzida pela estimulação com angiotensina II e forskolina, em relação ao controle. Por outro lado, o mesmo peptídeo foi ineficaz em inibir a ativação da luciferase induzida pela estimulação com isoproterenol e forskolina. De forma diferente, o peptídeo 3A (DITADDEPLT, 1 μM) com sítio para CKII, apresentou um efeito dual dependente da concentração. Nos ensaios de microfisiometria, o tratamento com os peptídeos 5A e 3A (500 μM) inibiu em 54% e 46%, respectivamente, o aumento da taxa de acidificação extracelular (indicador da ativação de vias de sinalização), induzida pela estimulação com angiotensina II, enquanto que o inibidor padrão da PKC, chelerythrine (5 μM) reduziu a resposta em 60%. **Discussão** Os resultados do presente trabalho sugerem que peptídeos contendo sítios de fosforilação, gerados endogenamente durante o metabolismo protéico, são capazes de modular cascatas de sinalização ativadas por GPCRs. **Apoio Financeiro:** CNPq e FAPESP

01.032

EFEITO DE MUTAÇÕES SÍTIO-DIRIGIDAS NAS HÉLICES I, V E VI DO RECEPTOR AT_1 NA MATURAÇÃO, DIMERIZAÇÃO E INTERAÇÃO COM O AGONISTA.

Pignatari, G. C.[1]; Rozenfeld, R.[2]; Cunha, F. M.[3]; Oliveira, L.[1]; Ferro, E. S.[4]; Devi, L. A.[2]; Paiva, A. C. de M.[1] - [1]UNIFESP - EPM - Biofísica; [2]Mount Sinai School of Medicine - Pharmacology and Biological Chemistry; [3]UNIFESP - EPM - Biologia Celular; [4]USP - ICB

Introdução: O receptor AT_1 possui resíduos aromáticos e alifáticos altamente conservados nas hélices I, V e VI. Este trabalho teve como objetivo estudar o papel de alguns desses resíduos na maturação, tráfego, formação do complexo agonista/receptor e dimerização. **Métodos:** Um vetor contendo o AT1-EGFP foi construído e utilizado para a obtenção de mutantes para alanina (Ala) e ácido glutâmico (Glu)**.** Células HEK foram transfectadas com essas construções e utilizadas nos experimentos de microscopia de fluorescência, ensaios de ligação, BRET e ensaios funcionais, utilizando gene repórter. **Resultados:** Através da microscopia observou-se que o receptor selvagem, os mutantes de Ala e os de Glu da hélice V encontram-se na membrana. As substituições feitas na hélice VI para Glu afetaram o tráfego causando retenção no retículo endoplasmático. A afinidade do AT_1 pela AngII nos ensaios de ligação para os mutantes de Ala não foi afetada, enquanto nas mutações para Glu aparecem diminuídas. A homodimerização ocorreu na ausência de AngII e a maioria das mutações não afetaram este processo. Ensaios com o gene repórter confirmam a funcionalidade de alguns desses mutantes. **Discussão:** Sugerimos que o AT_1 seja um homodímero e que resíduos específicos nas hélices V e VI sejam responsáveis pela configuração do sítio de ligação da AngII e correto dobramento do receptor. **Apoio Financeiro:** FAPESP e NIH- NCI.

01.033

CHARACTERIZATION OF BENZODIAZEPINE RECEPTORS IN ADULT *SCHISTOSOMA MANSONI* AND THEIR RELATION TO THE CONTRACTILE EFFECT OF CLONAZEPAM

Thibaut, J. P. B.[1]; Mendonça-Silva, D. L.[1]; Noel, F.[1] - [1]UFRJ - Farmacologia Básica e Clínica

We recently described a GABAergic signalling pathway in adult *Schistosoma mansoni* that could participate in the control of its motonervous system. Present objective was to verify if the previously reported contraction of whole worm produced by clonazepam should be related to activation of putative allosteric benzodiazepine (BZP) modulatory sites present on worm $GABA_A$ receptors. Competition binding assays were performed in a mitochondrial fraction obtained after centrifugation of adult worm homogenate, using 0.2 nM [^{3}H]-flunitrazepam as radioligand. The sequence of potency was: flunitrazepam (IC_{50}=40 nM)>zolpidem>diazepam>>clonazepam (IC_{50}=5.000 nM). Body area measurements of adult male worms freshly recovered from mice portal veins indicated that 10 mM clonazepam contracted the worms in a time-dependent manner (30% reduction after 15 min, $p<0.0001$, n = 15) whereas flunitrazepam, zolpidem and diazepam were without effect. When applied directly on muscle fibers, freshly dissociated from adult worms by a mechanical and enzymatic process, 10 mM clonazepam contracted 52% of the fibres. The other BZP were without effect. We conclude that BZP binding sites present in *S. mansoni* are different from the central BZP receptors present in mammals and that they are probably not related to the worm contraction produced by clonazepam. **Supported by:** CNPq; FAPERJ

01.034

EFFECT OF CYCLOSPORIN A ON *Schistosoma mansoni:* THE MAIN SCHISTOSOME SPECIE IN BRAZIL

Gonçalves, J. P.[1]; Cunha, V. M. N.[1] - [1]UFRJ - Farmacologia Básica e Clínica

Introduction: Cyclosporin A(CsA) is an immunosuppressant agent that selectively inhibits SERCA1 Ca^{2+} pump isoform in mammals. In addition to its immunomodulatory properties, CsA is a potent anti-schistosomal agent. However, the molecular basis of this action is still unclear. The aim of the present work was to investigate if CsA could directly affect the body length and the spontaneous movements of the adult male worms and if these effects are related to an alteration of Ca^{2+} pump activity. **Methods:**Mice were killed 45 days after infection and the adult male worms were collected from portal vein and washed. Five worms were placed in different plastic dishes and the effects of CsA were measured according to Silva & Noel (1995).The Ca^{2+}-ATPase activity present in P_4 fraction was determined according to Cunha et al.(1996). **Results:**50 or 100µM CsA did not modify the motility of the worms ($n=12, P>0.05$), but 100µM of this drug promoted an increase of the length of the worms in the absence of serotonine (5-HT) ($n=12, P<0.05$). However, in the presence of 5-HT, this effect of CsA was not different from the control ($n=12, P>0.05$). P_4 showed the highest and the lowest specific Tg-sensitive and -resistant activity (11.38±3.22 and 0.57±0.15µmolPi.mg^{-1}.h^{-1}). CsA (100µM) significantly stimulated Tg resistant- activity (-0.28±0.49*vs* 1.92±1.22µmolPi.mg^{-1}.h^{-1};$n=4$, $P<0.05$). **Discussion:** Our data suggest that CsA produces relaxation of the worm musculature that may be due to the stimulation of Ca^{2+} pumps. The addition of 5-HT in the nutritive medium seems to antagonize the relaxation effect of CsA. **Supported by:** CAPES; FAPERJ; UFRJ

01.035

COMPETITIVE ANTAGONISM OF NATIVE AND RECOMBINANT α_{1B}-ADRENOCEPTORS (α_{1B}-AR) BY L772, A N-PHENYLPIPERAZINE DERIVATIVE

Akinaga, J.[1]; Mueller, A.[1]; Romeiro, L. A. S.[2]; Pupo, A. S.[3] - [1]UNESP - Botucatu - Farmacologia; [2]Universidade Católica de Brasilia - Núcleo de Química Bioorgânica e Medicinal; [3]UNESP - Farmacologia

Introduction: α_1-ARs are involved in several important actions of norepinephrine and epinephrine. In fact, drugs interacting with α_1-ARs are useful for treatment of several diseases. The development of new compounds showing selectivity for specific receptor subtypes might result in new and improved therapeutics. **Objective:** To investigate the antagonism of native and recombinant α_{1B}-ARs by L772, a new N-phenylpiperazine derivative, and to compare its properties with those of 5-methylurapidil (5-MU), a known α_1-AR antagonist. **Methods:** The contractions of the rat spleen to norepinephrine were used as models of responses mediated by α_{1B}-ARs and the inhibition of the specific binding of [^{3}H]-prazosin to membranes from HEK-293 cells stably expressing human α_{1B}-ARs was used to study the interactions of theses compounds with recombinant receptors. **Results:** In the rat spleen, L772 and 5-MU competitively antagonized the contractions to norepinephrine, and L772 was ≅30 times more potent than 5-MU (n=5). As expected, 5-MU showed low affinity for the human α_{1B}-ARs in the radioligand binding assays ($pK_i \cong 6.4$, n=3). Surprisingly, the affinity of L772 for human α_{1B}-ARs was much higher ($pK_i \cong 9.5$, n=3). **Discussion:** L772 showed high affinity for human recombinant α_{1B}-ARs. It is important to determine to investigate the selectivity of L772 for affinities for α_{1A}- and α_{1D}-ARs since there are only few drugs that interact with subnanomolar affinity with α_{1B}-ARs. **Supported by:** FAPESP, CAPES

01.036

EFFECTS OF TESTOSTERONE MANIPULATION ON THE EXPRESSION OF MUSCARINIC RECEPTORS IN THE RAT SEMINAL VESICLE

Hamamura, M.[1]; Yasuhara, F.[1]; Avellar, M. C. W.[1]; Porto, C. S.[1] - [1]UNIFESP - EPM - Farmacologia

Aim: Androgens have potent effects on many aspects of neuronal regulation of reproductive organs during development and adulthood (Keast, 2000, J Auton Nerv Sys 79:67). In the seminal vesicle we have previously detected the presence of five muscarinic acetylcholine receptor (mAChR) mRNA subtypes, with M_3 mAChR involved in the seminal vesicle contraction (Hamamura et al., 2006, Mol Cell Endoc 247:192). The aim of the present study was to investigate whether changes in the testosterone level with sexual development or surgical castration have effects on the expression of mAChRs in this tissue. **Methods and Results**: Ribonuclease protection assays, using specific probes for m1-m5 mAChR transcripts, were carried out with seminal vesicle total RNA from 30, 90 and 120-day old rats, castrated (at 75-day old rats castrated and sacrificed 15 days after surgery), castrated and treated with testosterone (rats castrated and treated for further 7 days with testosterone 0.5 mg/100 g, s.c., daily). The presence of five protected fragments was confirmed in the seminal vesicle of the different experimental groups tested. Densitometric analysis indicated that m1 transcript levels decreased with sexual development and increased with orchidectomy, this latter effect reverted by testosterone replacement. The expression of m3 transcript, however, was similar among all experimental groups. **Conclusion**: These data suggest that sexual development and/or testosterone may play a role in the regulation of mAChR expression in the rat seminal vesicle. Further studies will be necessary to evaluate the androgen dependence of m2, m4 and m5 transcript expression. **Supported by:** FAPESP, CAPES and CNPq

01.037

EFEITO DE FRAÇÕES PROTÉICAS DO VENENO DE *BOTHROPS LANCEOLATUS* (VBL) NA ADESÃO DE PLAQUETAS HUMANAS AO FIBRINOGÊNIO.

Biasotto, M. C. C. C.[1]; Lôbo de Araújo, A.[1]; Antunes, E.[1]; Marcondes, S.[1]; Hyslop, S.[1] - [1]UNICAMP - Farmacologia

Introdução: Venenos de serpentes contém vários componentes que afetam a hemostasia, através da ativação ou inibição plaquetária ou por fatores da coagulação.Neste estudo investigamos a ação de frações do (VBL) na adesão de plaquetas humanas ao fibrinogênio. **Método:** Adesão de plaquetas humanas lavadas foi avaliada usando placas de 96- well recobertas com fibrinogênio. Plaquetas foram mantidas na placa com frações do (VBL) por 15 e 30 min. Plaquetas aderidas foram incubadas com substrato para fosfatase ácida por 1h. A placa foi lida a 405nm. **Resultados:** Frações do (VBL) inibiram a adesão de plaquetas ao fibrinogênio. A FI (0.1; 0.3; 1.0mg prot/well) inibiu a adesão de modo concentração-dependente em 15min de incubação, As frações FII e FIIc (1.0mg prot/well) inibiram a adesão de modo tempo-dependente, FIIc e FIII (1.0 mg prot/well) foram as que mais inibiram a adesão (48%) porém tal inibição foi significativamente menor quando FIIc e FIII (1.0 mg prot/well) foram fervidas (100°C) por 30min. **Conclusão:** As frações FIIc e FIII (1.0 mg prot/well) são compostas por enzimas (metaloproteinase), provavelmente por isso inibiram a adesão de plaquetas ao fibrinogênio, já que enzimas desta família atuam na função plaquetária. Deve-se fazer testes complementares para confirmarmos esses resultados. **Apoio Financeiro:** CNPq

01.038

CLONING AND EXPRESSION OF ANTIMICROBIAL SPAG11 (SPERM ASSOCIATED ANTIGEN 11) GENE IN TISSUES FROM FETAL AND ADULT *Bos Taurus*.

Honda, L.[1]; French, F.[2]; Hall, S.[2]; Avellar, M. C. W.[1] - [1]UNIFESP - EPM - Farmacologia; [2]UNC-Chapel Hill - Reproductive Biology

Introduction. SPAG11 gene codes for antimicrobrial proteins and is peculiar among the β-defensin genes due to its complex genomic structure and mRNA splicing pattern. SPAG11 is a single gene derived from 2 ancestrally independent β-defensin genes that can also function independently in primates or entirely independent genes in rodents. Here the deduction of the Bos taurus SPAG11 gene and the expression of its alternative splicing mRNAs were evaluated in fetal and adult bovines. **Methods.** Full length cloning of *Bos taurus* SPAG11 cDNAs was achieved by using the caput epididymis (CP) poly(A^+) RNA in PCR screening of a directional cDNA library and 5´-3´-RACE method followed by subcloning and sequencing of the resulting PCR products. RT-PCR was used with total RNA extracted from: 1) testis (T), CP, corpus (CO) and cauda (CA) epididymis and vas deferens (VD) from adult bovines; 2) T, intestine, kidney, ovary and liver from fetal (90-150 day of pregnancy) and adult bovines. **Results/Discussion.** Based on the human SPAG11, bovine SPAG11 gene was deduced as 8 exons, with 2 promoters, coding for 6 alternative splicing mRNAs (SPAG11C, D and E and the bovine-specific SPAG11U, V and W). In the reproductive tract SPAG11E and U mRNAs were abundant and broadly detected in all adult tissues. SPAG11V and W were only amplified in T. Although not detected in VD, SPAG11D was observed in T and CP, with low abundance in CO and CA. SPAG11C mRNA was detected in all tissues, except T. When tissues from fetal and adult bovines were compared the results indicated that the mRNA levels were dependent on the SPAG11 transcript and tissue analyzed, showing for the first time the possible differential involvement of SPAG11 gene products in embryonic and postnatal physiological events. **Supported by:** FAPESP, CNPq, Fogarty International Center

01.039

INTERAÇÃO DO FENTÔNIO [N-(4-fenil) fenacil-l-hiosciamina] COM RECEPTORES NICOTÍNICOS (nAChR) GANGLIONARES SUBTIPO α3β4.

Munhoz, E.[1]; Souccar, C.[1]; Lapa, A. J.[1]; Lima-Landman, M. T. R.[1] - [1]UNIFESP - EPM - Farmacologia

No músculo esquelético, o Fentônio (FENT) aumenta a liberação quantal espontânea de acetilcolina e bloqueia competitivamente o nAChR subtipo $\alpha 1_2\beta\gamma\varepsilon$, sem afetar as propriedades elétricas da membrana (Souccar et al, 1994,1998). Neste trabalho avaliou-se a interação do FENT com nAChRs subtipo α3β4 expressos nas terminações simpáticas do ducto deferente de rato. A porção prostática do ducto deferente de ratos Wistar (200-300g) em líquido nutritivo carbogenado (LNV), a 30^o C, contraiu com a incubação de DMPP (3μM – 1,0 mM, n=4) com CE_{50} = 84,1 μM. Na presença de metilicaconitina (10 - 300 nM, n=5) a resposta ao DMPP não foi alterada, mas, após incubação de hexametônio (n=3) ou mecamilamina (n=6), a contração foi bloqueada com CI_{50} de 1 μM e 10 nM, respectivamente. Estes dados indicaram a participação de nAChR α3β4 na resposta ao DMPP. A atropina, (100 nM), não alterou a contração induzida por DMPP, mas a inibição com Prazosin (30 μM, n=5) ou com Suramin (30 μM, n=4) indicou ativação de α1-adrenoceptores e P2X-purinoceptores pós-sinápticos, respectivamente. O Fent bloqueou a contração induzida por DMPP (CI_{50}: 1 μM) sem afetar a contração induzida por noradrenalina (1 μM – 1 mM) ou por ATP (1 μM – 1 mM). Os dados sugerem que o Fent é um antagonista não-competitivo do nAChR α3β4 pré-sináptico com potência 100 vezes menor que a da mecamilamina, porém equipotente ao hexametônio, atuando, provavelmente, em sítios alostéricos do colinoceptor. CEP - UNIFESP 1605/04 **Apoio Financeiro:** FAPESP, CNPq, CAPES

01.040

STIMULATION OF ALFA-1 ADRENOCEPTOR ACTIVATES PKC AND MAP KINASE (ERK) IN SNAKE VENOM GLAND.

Zablith, M. B.[1]; Luna, M. S. A.[1]; Kerchove, C. M. de[1]; Yamanouye, N.[1] - [1]Instituto Butantan - Farmacologia

Introduction and aim: Alfa-1 adrenoceptor has an atypical pharmacological profile and undergoes long-term desensitization just after stimulation (Kerchove et al, JEB 207:411, 2004). This receptor activates phospholipase C signaling pathway, promoting an increase in inositol phosphate formation and mobilization of calcium from thapsigargin-sensitive stores. In this study we further evaluated the participation of PKC and MAP kinase (ERK) in alfa-1 signaling pathway in *B. jararaca* venom gland. **Methods:** Dispersed quiescent secretory cells were stimulated by phenylephrine (PHE). PKC activity was measured by phosphorylation of a specific substrate peptide ([Ser^{25}PKC (19-31)]) through the use of ATPγ^{32}P. The activation of ERK was measured by Western Blotting using primary antibody against phosphorylated ERK. **Results and Discussion:** Phenylephrine 1.10^{-4}M and 3.10^{-4}M promoted an increase in PKC activity of 10.2±1.4% (n=3) and 18.2±1.2% (n=4) over basal, respectively. PKC inhibitors H89 (90μM) and Staurosporine (100nM) inhibited completely the PHE response. Incubation with PHE (3.10^{-4}M) for 5 and 10 min also increased the activity of ERK (83.5±49% and 144.9±72.9%, respectively). Staurosporine (100nM) partially inhibited 5 min PHE response (31±10.8%, n=5. $P<0.05$) but has no effect in 10 min response (136.8± 50.6%, n=6). These results showed that alfa-1 adrenoceptor in venom gland triggers typical alfa-1 signaling pathway. Besides, PKC activates ERK only in the earliest step of the cascade. **Supported by:** FAPESP, CNPq, Fund. Butantan

01.041

CHARACTERIZATION OF [^{35}S]GTPγS BINDING TO RAT EPIDIDYMAL MEMBRANES

Patrao, M. T. C. C.[1]; Andrade-Lopes, A. L.[1]; Godinho, R. O.[1]; Avellar, M. C. W.[1] - [1]UNIFESP - EPM - Farmacologia

Aim: In the present study, we established a protocol to analyze the functional coupling of epididymal receptors to heterotrimeric G-proteins by measuring the binding of non-hydrolysable GTP analogue [^{35}S]GTPγS to membrane preparation from adult (120 day-old) Wistar rat epididymis. **Methods:** Epididymal membranes (5–50 μg) were incubated with [^{35}S] GTPγS (0,1 nM or 1 nM) in the presence or absence of 10 to 100 μM GDP in a 50 mM Tris buffer containing 1mM EGTA, 5mM $MgCl_2$, 100mM NaCl (pH 7.4), 1mM PMSF and 100μg/mL bacitracin. Nonspecific [^{35}S] GTPγS binding was obtained in the presence of 25μM GTPγS. Samples were incubated for 0.5-2h at 30^{o}C. Phenylephrine (PHE) or oxotremorine-M (OXO-M) were used as agonists for α_1 and muscarinic receptor, respectively. **Results and Discussion:** The optimal conditions for the assays were: 5 μg of membrane, 1h incubation at 30^{o}C, 1nM [^{35}S]GTPγS and 50μM GDP. Incubation of membranes (5 μg) with GDP (50μM) shifted the G-proteins to inactive state, reducing by 71% the [^{35}S]GTPγS binding to G-proteins. The [^{35}S]GTPγS binding increased linearly with membrane concentration (r^2= 0,99) in the presence of 50 μM GDP, whereas it fitted to an hyperbolic curve (r^2= 0,98) in the absence of GDP. Incubation of membranes (5 μg) with PHE (10^{-5} M) or OXO-M (10^{-5} M) increased by 1,22 and 1,23 fold the basal [^{35}S]GTPγS binding, respectively, indicating that the [^{35}S]GTPγS binding assay is a sensitive method to analyze the conformational changes of Gα subunit following agonist occupation of G-protein coupled receptors in rat epididymis. **Supported by:** Fapesp, CNPq, Capes.

01.042

MODELOS MOLECULARES DE ATIVIDADE DE RECEPTORES CANABINÓIDES E VANILÓIDES: NOVAS VIAS DE TRANSDUÇÃO DE SINAIS

Mesquita, C. M.[1]; Julius, D.[2]; Guimaraes, M. Z. P.[1] - [1]UFRJ - Farmacologia Básica e Clínica; [2]UCSF - Cellular and Molecular Pharmacology

Introdução: O receptor canabinóide CB1 encontra-se em abundância no sistema nervoso, enquanto o CB2 está presente em células de linhagem imune. Entretanto, algumas das ações neurais de substâncias canabinóides, não são eliminadas através do nocaute do gene CB1. Essa e outras evidências parecem indicar a existência de um outro receptor, já cunhado de CB3. **Métodos e resultados**: Iniciamos a busca pelo terceiro receptor canabinóide, pela expressão de cDNAs em células de mamíferos e imagem de acúmulo de cálcio intracelular. Surpreendentemente, ao isolarmos um único cDNA que conferia respostas a canabinóides, esse codificava a enzima fosfolipase C delta 4 (PLCd4). Verificamos em células transfectadas com PLCd4 que a farmacologia de resposta a canabinóides é bastante semelhante ao descrito para o CB3, isto é: ativação por WIN55212-2, anandamida, 2 araquidonoilglicerol e capsaicina e bloqueio por SR141716A. Entretanto, através de hibridização *in situ*, verificou-se que a PLCd4 não está presente no hipocampo, local supostamente rico em CB3, mas sim em gânglios da raiz dorsal e no cerebelo. **Discussão**: O método de *screening* revelou, de forma imparcial, que a PLCd4 pode ser ativada por canabinóides. Entretanto, não é possível afirmar que esta enzima seja o CB3, mas não se pode excluir a hipótese de que algumas atividades de canabinóides não-CB1/CB2/TRPV1 possa ser atribuída a PLCd4. Por isso estamos investigando seu possível papel como adjunto transducional de receptores do tipo TRP, em modelos de células transfectadas com registro de cálcio e eletrofisiologia de ovócitos de *Xenopus*. **Apoio Financeiro:** CNPq e PEW Latin American Program in Biomedical Sciences.

01.043

ISOPRENALINE INCREASES CYTOSOLIC Ca^{+2} BY EXTRACELLULAR INFLUX AND Ca^{+2} RELEASE FROM THE ENDOPLASMIC RETICULUM IN RAT AORTA ENDOTHELIAL CELLS.

Neto, M. A.[1]; Bendhack, L. M.[2] - [1]FCFRP - USP - Física e Química; [2]USP - FCFRP

The aim of this study was to investigate the effect of b-adrenoceptors (b-AR) agonist isoprenaline (ISO) on $[Ca^{+2}]_c$ and to investigate if $[Ca^{+2}]_c$ is increased by extracellular Ca^{+2} infux and/or release from endoplasmic reticulum (ER) via IP_3 receptors (IP_3R) and/or ryanodine receptors (RyR) in aorta endothelial cells. **Methods:** Endothelial aortic cells were isolated and loaded with 5 mmol/L Fura-2 AM in order to measure $[Ca^{2+}]c$ by fluorescence of Fura-2/AM (ratio 340/380 nm). Data are expressed as the difference between basal and stimulated $[Ca^{+2}]_c$. **Results:** In 1.6mM Ca^{+2} solution ISO increased $[Ca^{2+}]c$ in 0.131±0.026 (n=3; $p<0.01$), that was reduced by verapamil (10 mM) to 0.048±0.003 (n=3; $p<0.001$). In Ca^{+2} free solution ISO increased $[Ca^{+2}]c$ in 0.039±0.01 (n=3; $p<0.05$). The IP_3R antagonist 2-APB reduced the effect of ISO to 0.0165±0.0023 ($p<0.001$) and the RyR antagonist tetracaine reduced the effect of ISO to -0.042±0.005 ($p<0.001$). The combination of 2-APB and tetracaine completely blocked the effect of ISO. **Discussion:** ISO activating b-AR coupled to Gs protein activates the adenylyl cyclase enzyme inducing cAMP production and PKA activation that can phosphorilate the Ca^{+2} channel, IP_3R and RyR, causing $[Ca^{+2}]c$ increase that associated with the NO production, could be a reason to higher potency of ISO in aorta ring with intact endothelium. **Conclusion:** ISO increases $[Ca^{+2}]c$ by Ca^{+2} influx and by Ca^{+2} release from ER through IP_3R and RyR in rat aorta endothelial cells. **Supported by:** FAPESP and CNPq.

01.044

VENOM EXTRACTION ACTIVATES AP-1 IN THE VENOM GLAND OF SNAKE.

Luna, M. S. A.[1]; Ferreira, Z. S.[2]; Yamanouye, N.[1] - [1]Instituto Butantan - Farmacologia; [2]USP - Fisiologia

Introduction: Viperidae snake venom gland has a central lumen where all venom produced is stored. Secretory cells are stimulated for new cycle of venom synthesis after emptying the lumen either by manual extraction or biting. Both alpha and beta-adrenoceptor play a role in triggering venom production cycle by inducing the synthesis of proteins of the gland. Besides, we have shown that venom extraction increases the activation of NFkB in quiescent secretory cells. The aim of this study is to further verify whether venom extraction could activate other potencially important transcription factors such as AP1. **Methods:** Nuclear extract were obtained from male and female snake venom glands in quiescent stage and 30, 60 and 120 min after venom extraction. The activation AP1 was analyzed by electrophoretic mobility shift assay, using ^{32}P-AP1 oligonucleotide. **Results:** We detected AP1-DNA complex in quiescent cells. After venom extraction, an increase of NFkB-DNA complex was observed. The highest increase in female and male venom gland occurs after 60 min (111.53 ± 27.27%, n=3) and 120 min (101.30 ± 61.25%, n=3) of venom extraction, respectively. **Discussion:** The data showed that AP1 is activated in quiescent venom gland and its activity increases after venom extraction, suggesting that stimulation of both alpha and beta-adrenoceptors could activate transcription factors that probably induce the synthesis of proteins important for venom production and secretion process. Besides, the activation of AP1 presents a sexual dimorphism in venom gland. **Supported by:** FAPESP, CNPq, Fund. Butantan.

01.045

KNOCKDOWN OF PKR EXPRESSION BY RNA INTERFERENCE IN B16 MELANOMA CELLS

Delgado Andre, N.[1]; De Lucca, F. L.[1] - [1]FM - USP - Biochemistry and Immunology

Introduction: The RNA-dependent protein kinase (PKR) is a serine-threonine kinase activated by intermolecular autophosphorylation upon binding RNA. Accumulating evidence during recent years has implicated PKR in cell growth, differentiation and apoptosis. It has also been suggested that PKR acts as a tumor suppressor which is still controversial. RNA interference (RNAi) has become a powerful tool to investigate the function of mammalian genes by degrading a specific mRNA target. The mediators of sequence-specific mRNA degradation are double-stranded small interfering RNAs (siRNAs). In the present study, we examined the possibility of RNAi to inhibit the expression of PKR gene in B16-F10 melanoma cells. **Methods**: We have used a short hairpin RNA (shRNA) expressing plasmid (psiSTRIKE U6 hairpin cloning systems, Promega) controlled by Pol III U6 promoter. The B16-F10 cells were transfected (5, 24 and 48 hours) with the PKR-specific shRNA and lipofectamine 2000, and PKR gene silencing was monitored by RT-PCR and Western blot analysis. Non-related shRNA was used as control. **Results**: We found that the maximum reduction of PKR mRNA (98%) and PKR protein (95%) levels occurs 48 hours after transfection of B16-F10 melanoma cells with PKR-specific shRNA.**Discussion**: This study indicated that PKR-specific shRNA is effective in PKR gene silencing in B16-F10 melanoma cells. This finding is relevant because these tumor cells have been used as a model of experimental metastasis since these cells are able to colonize lungs of animals after intravenous injection. Thus, the injection of the B16-F10 cells transfected with PKR-specific shRNA may contribute to determine whether PKR has a tumor suppressor function in this model of experimental metastasis. **Supported by:** CAPES; FAPESP

01.046

EFFECT OF AZUMOLENE ON INTRACELLULAR Ca^{2+} HOMEOSTASIS IN RAT HEART MICROSSOMAL PREPARATION

Bezerra, P. M.[1]; Scaramello, C.[1]; Zapata-Sudo, G.[1]; Sudo, R. T.[1]; Cunha, V. M. N.[1] - [1]UFRJ - Farmacologia Básica e Clínica

Introduction: Azumolene is a structural analogue of dantrolene with higher water solubility. Several studies show that both drugs are equipotent in prevent and abolish caffeine / halothane induced contracture, but their sites of action are still unclear.The aim of the present work was to investigate the effects of azumolene on intracellular Ca^{2+} homeostasis in rat heart microssomes. **Methods:**The tissue was washed, homogenized and differential centrifuged to obtain heart microsomes (Ms). Ca^{2+} uptake and (Ca^{2+}+Mg^{2+}) ATPase activity were measured at the same experimental conditions, in the absence/presence of 10 mM dantrolene or azumolene, 3 mM thapsigargin and using 1 or 10mM free Ca^{2+} concentration. **Results:**The total Ca^{2+} uptake using 1 and 10 mM free Ca^{2+} concentration in the presence of dantrolene (6.9±1.4 and 11.4±2.7nmolCa/mg) or azumolene (9.8±1.3 and 11.7±2.9nmolCa/mg) were not statistically different from total Ca^{2+} uptake in control Ms (absence of the substances; 7.8 ±1.9 and 11.9±3.3nmolCa/mg). SERCA ATPase activity, measured in the absence of the drugs (372±117mmolPi/mg; with 10 mM free Ca^{2+}) is not statistically different from that measured in the presence of dantrolene (1153±376mmolPi/mg) or azumolene (254±81mmolPi/mg). **Conclusions:**SERCA dependent Ca^{2+} accumulation and SERCA ATPase activity are not altered by dantrolene nor azumolene in rat heart microssomes. These data are consistent with previous functional studies suggesting that azulmolene alike dantrolene does not promote cardiovascular side effects during treatment of malignant hyperthermia syndrome. **Supported by:** FAPERJ, FUJB, FINEP, CRISTALIA

01.047

ISOLAMENTO E CARACTERIZAÇÃO PARCIAL DE NOVA PROTEÍNA DO VENENO DE *BOTHROPS LANCEOLATUS* (FER-DE-LANCE)

Dotto, P. L.[1]; Souza, G. H. M. F.[2]; Eberlin, M. N.[3]; Hyslop, S.[1]; Lôbo de Araújo, A.[1] - [1]UNICAMP - Farmacologia; [2]UNICAMP - Farmacologia Bioquímica; [3]UNICAMP - Instituto de Química

Venenos de serpentes do gênero *Bothrops* contêm proteínas que são responsáveis por efeitos locais como dano tecidual, incluindo edema, dor, hemorragia e necrose; e também distúrbios sistêmicos como coagulopatia, hemorragia sistêmica e falência renal. Portanto, o isolamento e caracterização de proteínas de venenos de serpentes do gênero *Bothrops* auxilia na elucidação do envenenamento por estas serpentes. Uma nova proteína do veneno de *Bothrops lanceolatus* foi isolada por combinação de cromatografias de gel filtração em Sephadex G-100, troca iônica em Hi Trap Q e afinidade em Hi Trap Chelating. Esta nova proteína possui peso molecular de aproximadamente 17 kDa, de acordo com SDS-PAGE. Foram testadas as atividades hemorrágica e fosfolipásica, sendo que a proteína em questão não apresentou nenhuma das atividades mencionadas. A digestão da nova proteína isolada do veneno de *B. lanceolatus* e a análise da massa e da seqüência dos peptídeos precursores selecionados serão feitos através do espectrômetro de massas Q-tof (Waters, Manchester, UK). Os espectros dos peptídeos obtidos serão analisados a fim de se obter as seqüências, que serão posteriormente submetidas ao BLAST para se verificar o score com possíveis proteínas homólogas. **Apoio Financeiro:** CAPES, CNPq e FAPESP.

01.048

THAPSIGARGIN, A IRREVERSIBLE INHIBITOR OF Ca^{2+} PUMP, AFFECTS THE MOTILITY AND BODY LENGTH OF *Schistosoma mansoni*: COMPARISON WITH IVERMECTIN

Azevedo, R. P.[1]; Cunha, V. M. N.[1] - [1]UFRJ - Farmacologia Básica e Clínica

Introduction: ivermectin (IVM) is an anthelmintic drug that inhibits SERCA1 and SERCA2b while thapsigargin (TG) is a sesquiterpenic lactone that inhibits all Ca^{2+} pump isoforms of SERCA type. In a previous study we showed that IVM that is not effective against adult *S. mansoni*, does not modify the motility nor the body length of the worms. The aim of the present work was to investigate if a known inhibitor of SERCA pumps (TG) could directly affect the body length and the spontaneous movements of the adult male worms. **Methods:** Mice were killed 45 days after infection and the adult male worms were collected from portal vein and washed. Five worms were placed in different plastic dishes and the effect of 0.5 or 5µM TG was measured according to Silva & Noel (1995). The (Ca^{2+}-Mg^{2+})ATPase activity was determined in the microsomal fraction (P_4) according to Cunha et al. (1996). **Results:** In the presence of 10µM serotonine (5-HT), TG (0.5 and 5µM) significantly increased the motility and decreased the length of the worms, and these effects were not reversed by wash in relation to their controls (0.01 and 0.1% DMSO, respectively (n=12; $P< 0.05$). While 3µM TG inhibited 95% (n=4; $P<0.05$), 30µM IVM inhibited 27.7±2.41% (n=4;$P<0.05$) of the specific (Ca^{2+}-Mg^{2+})ATPase activity present in P_4 fraction. **Conclusions:** Our data indicate that the increment of motility is related to the decrease of body length and both effects are due to the inhibition of Ca^{2+} pumps from *S. mansoni*. Although, IVM also inhibits the Ca^{2+} pumps, this action is not related to any modification in body worms. **Supported by:** FAPERJ;UFRJ

01.049

IN VITRO AND *IN SITU* ANALYSIS OF PROBENECID-SENSITIVE CYCLIC AMP EFFLUX AT RAT SKELETAL MUSCLE

Chiavegatti, T.[1]; Andrade-Lopes, A. L.[1]; Costa Junior, V. L. da[1]; Godinho, R. O.[1] - [1]UNIFESP - EPM - Farmacologia

Objective: We have recently shown the existence of an extracellular cAMP signaling cascade mediated by adenosine (ADO) in cultured rat skeletal muscle (cAMP–ADO pathway; Chiavegatti, FESBE 2005). The aim of the present work was to determine if the cAMP–ADO pathway also occurs in isolated rat skeletal muscle. **Methods and Results:** The cAMP-ADO pathway was analyzed at *extensor digitorum longus* (EDL) of male rats pre-incubated (30 min) with Tyrode containing 50µM uridine, 0.1µM iodotubericidine, 10µM EHNA and 0.1mM IBMX ± 100µM probenecid (PROB) and treated for 30 min with 10µM forskolin (FSK) or isoproterenol (ISO). The intra (cAMP***i***) and extracellular cAMP (cAMP***e***) were quantified by radioassay. Both ISO and FSK increased the basal cAMP***i*** (31.6±16.3 pmol/g, n=8) by 3.6 and 12.6 fold and cAMP***e*** (3.0±1.9 pmol/g) by 10 and 36 fold. PROB reduced by 58% the cAMP***e*** induced by ISO and FSK, respectively, without interfering with cAMP synthesis. In another set of experiments, rat cultured skeletal muscles (n=4) were sequentially treated with 1mM IBMX and 3µM ISO for 30 and 15min ± 1-100nM propanolol or 100µM PROB. Propranolol reduced in a dose-dependent manner both the cAMP***i*** and cAMP***e*** whereas PROB reduced exclusively cAMP***e*** by 63%. **Discussion:** Our results show the existence of a cAMP efflux in skeletal muscle mediated by a PROB sensitive transporter and dependent on cAMP***i*** levels. This efflux may be relevant to the autocrine and paracrine extracellular signaling of cAMP through the activation of skeletal muscle receptors coupled to G proteins. **Supported by:** FAPESP, CNPq

01.050

INTERACTIONS OF DOPAMINE (DA) WITH α_1-ADRENOCEPTORS (α_1-ARs)

Lima, V.[1]; Pupo, A. S.[1] - [1]UNESP - Farmacologia

Introduction It is known that dopamine (DA) interacts with α_1-ARs. However, it is unknown whether DA shows selectivity for any of the α_1-ARs subtypes. This study further investigates the interactions of DA with α_1-ARs by determining its actions in rat tissues used as models of responses mediated by each of the α_1-ARs subtypes. **Methods** The contractions of the rat epididymal vas deferens (RVD), spleen (RS) and aorta (RA) were used as models of actions mediated by α_{1A}-, α_{1B}- and α_{1D}-ARs, respectively (Lima, *EJP*, **508**:183, 2005). **Results** In the RVD and RA, DA behaved as a full agonist (RVD, pD_2=4.9±0.1 and a=1.25±0.07; RA, pD_2=5.6±0.1 and a=1.33±0.05) in relation to norepinephrine (NE). In these tissues, prazosin (non-selective α_1-antagonist) and BMY7378 (α_{1D}-selective antagonist) antagonized the contractions induced by DA with the same affinities as it antagonized the contractions induced by NE. These results suggest that the contractions induced by these two agonists are not mediated by different receptors. This is further supported by protection experiments in which DA protected the α_1-ARs from alkylation by phenoxybenzamine (POB 10 nM/15min). Interestingly, DA was unable to contract the RS, but competitively antagonized the contractions induced by NE (pA_2=3.7±0.04). **Discussion** DA is a full agonist of the α_{1A}-ARs of the RVD and α_{1D}-ARs of the RA, and although it interacts with the α_{1B}-ARs of the RS, DA has no agonist activity at these receptors. It will be important to check if the absence of agonism in the RS is related to the poor coupling of this receptor in this tissue or if it is a specific behavior of DA at α_{1B}-ARs. **Supported by:** Fapesp

01.051

TISSUE DISTRIBUTION AND SUBCELLULAR LOCALIZATION OF FBXO25 UBIQUITIN LIGASE IN CULTURED CELLS

Manfiolli, A. O.[1]; Maragno, A. L. G. C.[1]; Baqui, M. M. A.[2]; Yokoo, S.[1]; Oliveira, E. B.[1]; Cunha, O. A. B.[1]; Gomes, M. D.[1] - [1]FMRP - USP - Bioquímica e Imunologia; [2]FMRP - USP - Biologia Celular

Ubiquitin (Ub)-dependent proteolysis provides a central regulatory function in many biological processes. The ubiquitination of the target protein is mediated by the E3 Ub-ligases, which represent a diverse family of proteins and complexes. The Skp1/Cul1/F-box (SCF) complex is the largest family of E3. Our data has been shown that FBXO25 protein is a component of a productive SCF with ub-ligase activity. The aim of this study is to characterize the tissue distribution and subcellular localization of FBXO25 in cultured cells. For this, we generated an antibody against the N-terminal of the protein, which was able to recognize specifically the protein in all major tissues of the adult mouse but not in striate muscle. In addition, immunofluorescence studies revealed that the FBXO25 is localized primarily to the nucleus of cultured cells. Striking, FBXO25 is found in prominent dot-like structures, which are generally adjacent to Cajal bodies and there is no overlapping with splicing speckles. The functional significance of this distribution is presently being studied. Finally, after Actinomycin D treatment, a transcription inhibitor, we observed in HeLa cells a dramatic reorganization of FBX025 from a pattern of large dots to a diffuse nuclear staining at the nucleus. This response contrasts with the behavior of splicing speckle proteins, which concentrate in enlarged speckles in the nucleoplasm. These data collectively suggest a role of FBXO25 on the transcriptional apparatus. **Supported by:** FAPESP and FAEPA

01.052

FBXO25, AN F-BOX PROTEIN HOMOLOGUE OF ATROGIN-1, IS NOT INDUCED IN ATROPHYING MUSCLE

Maragno, A. L. G. C.[1]; Baqui, M. M. A.[2]; Yokoo, S.[1]; Manfiolli, A. O.[1]; Sakagute, L. H.[1]; Gomes, M. D.[1] - [1]FMRP - USP - Bioquímica e Imunologia; [2]FMRP - USP - Biologia Celular e Molecular

Atrogin-1/MAFbx/FBXO32 is a muscle-specific ubiquitin-ligase (E3) that is dramatically increased in atrophying muscle. Here we have investigated the functional relationship between atrogin-1 and FBXO25 which shares 65% amino acid identity. Using a RT-PCR we demonstrated that FBXO25 is highly expressed in brain, kidney, and intestine, whereas atrogin-1 expression is largely restricted to striate muscle. FBXO25 was shown here to contain a functional F-box domain that binds to Skp1 and thereby to Roc1 and Cul1, the major components of SCF-type E3s. In addition, the productive SCF complex containing FBXO25 showed ubiquitin ligase activity. Immunofluorescence studies in transfected B16-F10 cells showed that FBXO25 colocalize in the nucleus with Skp1, thus, indicating that putative substrates of FBXO25 may be nuclear proteins. We investigated the differential expression of atrogin-1 and FBXO25 in fasted and dexamethasone-treated mice and also in rats with streptozotocin-induced diabetes. Although the atrogin-1 was strongly induced in muscle in all three models, no changes were observed in the expression of FBXO25. Therefore, here we have shown that FBXO25 is a novel F-box protein analogous to atrogin-1, which is not involved in muscle atrophy. These data contribute to elucidate the role of FBXO25 in the ubiquitin-proteasome pathway. **Supported by:** FAPESP and FAEPA.

01.053

DENERVATION INDUCES EXPRESSION OF FUNCTIONAL MUSCARINIC RECEPTORS IN RAT SKELETAL MUSCLE

Andrade-Lopes, A. L.[1]; Chiavegatti, T.[1]; Alves Amaral, G.[1]; Furlan, I.[1]; Godinho, R. O.[1] - [1]UNIFESP - EPM - Farmacologia

Studies from our lab have shown that expression of muscarinic acetylcholine receptors (mAChRs) is an attribute of noninnervated skeletal muscle cell since they are identified on rat myoblasts and denervated rat diaphragm (Diaph). To access the functionality of these receptors, we evaluated the effect of cholinergic agonists on activation of heterotrimeric G proteins by monitoring the exchange of GDP for the non-hydrolysable analogue of GTP [^{35}S]GTPgS at the Ga subunit. [^{35}S]GTPgS binding to rat Diaph membranes (n=4) depended on the incubation time (15 to 120 min), concentration of membrane (50 to 600 mg/mL) and GDP (10 to 100 mM). The optimal conditions were achieved incubating membranes (200mg/mL) with 40mM GDP for 2h. Denervation of rat Diaph for 7 days increased by 115% the total number of [^{35}S]GTPgS binding sites, in comparison to values obtained in innervated muscle (32,57 ± 1,22 fmol/mg protein, n=4). Activation of mAChRs with Oxotremorine-M and Carbachol (1-100 mM) increased in a dose-dependent manner the binding of [^{35}S]GTPgS to membranes of denervated Diaph (up to 130%) and cultured muscles (up to 115%), but did not modified the binding to innervated Diaph. Our results indicate that denervation of skeletal muscle increases the expression of G protein. In denervated rat Diaph, cholinergic agonists were able to stimulate G proteins, indicating that mAChRs may contribute to trophic influences of acetylcholine during neuromuscular synapse formation and repair. **Supported by:** FAPESP and CNPq

01.054

THE ENDOTHELIAL NITRIC OXIDE PRODUCTION INDUCED BY BRADYKININ AND ATP IS DIFFERENTLY REGULATED BY MELATONIN

Silva, C. L. M.[1]; Tamura, E. K.[2]; Cecon, E.[2]; Ferreira, Z. S.[2]; Bueno-Alves Jr., L.[2]; Markus, R. P.[2] - [1]UFRJ - Farmacologia Bás. Clínica; [2]IB - USP - Fisiologia

Introduction: Melatonin (MT) is synthesized not only by pineal gland and some data suggested a production by endothelial cells (EC). We showed recently that MT inhibits the endothelial NO production induced by bradykinin (BK). Therefore our aim was to investigate if NAT, a step-limit enzyme in the MT synthesis, is expressed in the EC, and also if MT inhibits the endothelial NO production induced by other agonists. **Methods:**Cultured rat EC were characterized by flow cytometry. Total RNA was extracted from EC and rat pineal gland, and the mRNA of the enzyme NAT was quantified by real-time RT-PCR. The content of NO released by EC was measured by spectrofluorimetry using DAF-FM (5 uM). The cGMP content was measured by EIA. **Results:**In pineal gland it was observed the expression of mRNA of NAT but not in EC samples in the same experimental condition. The EC NO release was induced by 1 uM BK (119±6.4%), 100 uM carbachol (CCh, 106±0.58%), 100 uM ATP (120±2.6%) or 10 uM 2-MeSATP (126±7.6%). MT (1 nM) inhibited the BK-induced increase in NO (101±2.5%, $p<0.05$) and the formation of cGMP. Although MT also inhibited NO released evoked by CCh (95.7±1.03%, $p<0.05$) the same was not observed with ATP (115.2 ± 3.31%). **Discussion:**Although an extra-pineal MT production is possible this was not observed in EC. In addition the inhibitory effect of MT upon NO production induced by the agonists BK and CCh was not observed with ATP suggesting that depending on the signaling pathway MT differently regulates EC function. **Supported by:** FAPESP, CNPq, CAPES

01.055

INTERAÇÃO ENTRE BLOQUEADORES DA JUNÇÃO NEUROMUSCULAR: ESTUDOS DE ANÁLISE FRACIONAL E ISOBOLOGRÁFICOS

Souza, C. R.[1]; Silva, W. L.[1]; Serra, C. S. M.[1]; Oliveira, A. C. de[1] - [1]ICB - USP - Farmacologia

Introdução: O objetivo é encontrar associações de bloqueadores neuromusculares (BNM), que propiciem potenciação de efeitos. **Métodos:** A preparação foi a nervo ciático-músculo extensor longo dos dedos do rato, *in vitro*. Contrações indiretas foram geradas por pulsos elétricos de 1,5-3 V., duração de 0,5 mseg. e freqüência de 0,1 Hz. Foram estudados os BNM: atracúrio (ATR), cisatracúrio (CIS), mivacúrio (MIV), pancurônio (PAN) e vecurônio (VEC). Curvas dose-resposta forneceram, para cada BNM, individualmente ou associados dois a dois, concentrações inibitórias 50% (CI_{50}). Avaliou-se, para cada associação de BNM, por métodos de análise fracional e de isobolografia, se houve antagonismo ou sinergismo (por adição ou por potenciação) de efeitos. **Resultados:** As CI_{50} médias (mM) e seus erros padrões da média foram: ATR=2,97±0,19; CIS=0,61±0,02; MIV=1,22±0,25; PAN=0,80±0,01; VEC=0,88±0,05; ATR+CIS=2,10±0,13; ATR+MIV=1,43±0,09; ATR+PAN=1,62±0,14; ATR+VEC=2,28±0,09; CIS+MIV=0,49±0,02; CIS+PAN=0,60±0,03; CIS+VEC=0,81±0,07; MIV+PAN=1,49±0,07; MIV+VEC=0,97±0,03; PAN+VEC=0,80±0,03. Cada grupo consistiu de 3 a 5 experimentos, cada um correspondendo a uma curva dose-resposta completa que forneceu uma CI_{50}. Para $p<0,05$, nas associações ATR+CIS, ATR+PAN, CIS+PAN, CIS+VEC, MIV+VEC e PAN+VEC houve sinergismo por adição; nas ATR+MIV e CIS+MIV houve sinergismo por potenciação e nas ATR+VEC e MIV+PAN houve antagonismo. **Discussão:**Existem na junção neuromuscular receptores nicotínicos pré e pós sinápticos.Assim diferentes associações de BNM podem levar a interações de diferentes naturezas em decorrência de efeitos diferenciais pré e pós sinápticos dos BNM estudados. Apoio financeiro:Fapesp; Capes; CNPq; *Bolsistas PIBIC/CNPq.

01.056

HOT WATER TOGETHER WITH LOW DOSES OF N-NITROSODIETHYLAMINE CAUSES INFLAMMATION ASSOCIATED TO ESOPHAGEAL SQUAMOUS CELL CARCINOMA: A NOVEL MODEL

Rapozo, D. C. M.[1]; Blanco, T.[2]; Benjamim, C. F.[3]; Canetti, C.[4]; Barja Fidalgo, T. C.[5]; Fierro, I. M.[5]; Ribeiro-Pinto, L. F.[2] - [1]UERJ - Instituto de Biologia / Bioquímica; [2]UERJ - Bioquímica; [3]UFRJ - Farmacologia Básica e Clínica; [4]UFRJ - Instituto de Biofísica Carlos Chagas Filho; [5]UERJ - Farmacologia

Introduction Esophageal cancer is one of the most lethal and common cancers. Some areas from South America present a high incidence of this kind of cancer. Many etiological factors are associated with this disease in Brazil, like alcohol, tobacco and hot maté consumption causing thermal injury in the esophagus. However, there is no study on the effect of hot maté on experimental carcinogenesis. **Methods** The effect of thermal injury caused by hot water adiministration at 70ºC by gavage three times/week either with or without N-nitrosodiethylamine (NDEA) at 1ppm in the drinking water in Balb/C female mice that were 2 months old was analysed during five months. The control group received cold water at room temperature. Each group was composed by 5 animals. The evaluation was done histologically with hematoxylin-eosin. **Results** The animals that received cold water or only NDEA did not present tissue alterations. The group that received only water at 70ºC presented an initial epithelial necrosis that caused an acute inflammation that disappeared in 8 weeks. However, in the animals that were treated with water at 70ºC and NDEA, the inflammatory process became chronic and evolved to a hiperplasia-displasia-carcinoma sequence. With 4 weeks, 4 animals of this group presented dysplasia and all of them had associated inflammation. With 12 weeks of treatment, 2 animals presented dysplasia and 4 presented inflammation and with 16 weeks one animal presented carcinoma *in situ* and 5 animals showed inflammation. **Discussion** Our results suggest that the concomitant ingestion of low doses of NDEA and water at 70ºC leads to a chronic inflammation from the thermal injury caused by hot beverage administration, and this resulted in esophageal tumors in five months of treatment. **Supported by:** CNPq / FAPERJ / SR2 UERJ

01.057

ATIVAÇÃO DE RECEPTORES P_2 PROMOVE PROLIFERAÇÃO DAS CÉLULAS HEMATOPOÉTICAS DE CAMUNDONGOS

Paredes-Gamero, E. J.[1]; Oshiro, M. E. M.[1]; Ferreira, A. T.[1] - [1]UNIFESP - EPM - Biofísica

Introdução A hematopoese é o processo de proliferação e diferenciação das células-tronco hematopoéticas (CTH). Além das citocinas outros agonistas, como o ATP, podem participar deste processo. Nosso objetivo foi determinar se os receptores P2 participam da hematopoese. **Métodos** Foram utilizadas células da medula óssea (MO) de camundongos machos C57BL6. A proliferação foi quantificada pela contagem de células em culturas de MO de longa duração (CMOLD) e pela marcação com bromouridina (Brd-U). A citometria de fluxo foi usada para determinar o ciclo celular com iodeto de propídeo, e para quantificar os receptores P2. Medidas da concentração de Ca^{2+}_i ($[Ca^{2+}]_i$), nas áreas proliferativas em culturas de longa duração (APCLD), foram realizadas com o fluoróforo fluo3 por microscopia confocal (LSM510, Zeiss). **Resultados** O ATP (agonista dos P2), o ADP ($P2Y_1$ e $P2Y_{12}$) e o UTP ($P2Y_2$ e $P2Y_4$), foram capazes de promover proliferação nas CMOLD ou em células recém dispersas; as células Brd-U^+ tiveram seu ciclo celular alterado. Estes agonistas também promoveram aumento da $[Ca^{2+}]_i$ e diferenciação das células hematopoéticas (diminuição da expressão de ckit). Foi observada alta expressão do receptor $P2Y_1$, média do $P2Y_4$, e baixa dos $P2Y_2$ e $P2Y_{12}$ nas APCLD. As CTH ($ckit^+Sca1^+Lin^-$) expressaram o $P2Y_1$; as células diferenciadas Lin^+ expressaram os $P2Y_2$, $P2Y_4$ e $P2Y_{12}$. **Discussão** Vários trabalhos mostram o papel dos P2Y na proliferação de diversos tipos celulares; é provável que a proliferação das CTH pelo ATP e análogos ocorra principalmente pela ativação do receptor $P2Y_1$ e $P2Y_4$. **Apoio Financeiro:** FAPESP e FADA

01.058

AMYLOID-β (A β) PEPTIDE ACTIVATES NF-κB THROUGH NMDA-p21ras PATHWAY IN CEREBELLAR PRIMARY CELL CULTURE

Kawamoto, E. M.[1]; Lepsch, B. L.[1]; Cury-Boaventura, M. F.[2]; Sa Lima, L.[1]; Munhoz, C. D.[1]; Avellar, M. C. W.[3]; Mattson, M. P.[4]; Scavone, C.[1] - [1]USP - Pharmacology; [2]USP - Physiology; [3]UNIFESP - EPM - Pharmacology; [4]NIA - Neurosciences

Introduction: It has been shown Aβ cause synaptic dysfunction and render neurons vulnerable to excitotoxicity. NF-κB is a transcription factor, linked to survival and apoptosis, modulated by Aβ in neurons and glia. Our aim was evaluate some of the mechanisms by which this occurs. **Methods:** Cerebellar cell culture was treated with different concentrations of Aβ (500nM, 1μM, 2μM) in different time points (6, 12, 24 h). Cells were incubated with MK-801 (NMDA antagonist), Manumycin A (rasfarnesyltransferase inhibitor), PD98059 (MAPK inhibitor), LY294002 (PI3 kinase inhibitor) 20 min before Aβ. Nuclear extracts were isolated and EMSA used to measure changes in NF-κB activity in competition studies with specific and non-specific unlabeled double-strand oligonucleotide and super-shift assays with specific antibodies against NF-κB subunits. FACS assay to measure cell viability and Western blot to p65 were performed. **Results:** Aβ induces a time dose-dependent activation of NF-κB (peak of activation 12h/1μM), and both p50/p65 and p50/p50 dimers were involved. This activation was reverted by MK-801, Manumycin A and partially reduced by PD98059 and LY294002. FACS assay showed that none of these treatments caused cell death. **Discussion:** These results suggest that Aβ activates NF-κB by NMDA-p21ras through MAPK and PI3-kinase pathways in cerebellar cell culture. **Supported by:** FAPESP, CNPq, Bunka grant/Sumitomo Bank

01.059

IMPACT OF ADRENALECTOMY AND DEXAMETHASONE TREATMENT ON SPERM COUNT IN RATS.

Silva, E. J. R.[1]; Rodrigues, A.[1]; Kempinas, W. G.[2]; Avellar, M. C. W.[1] - [1]UNIFESP - EPM - Farmacologia; [2]UNESP - IB - Botucatu - Morfologia

Introduction: Testicular sperm are unable to interact with egg. Maturation of sperm and fertilization ability occurs during transit through epididymis. Steroid hormones play a key role on such events, however the role of glucocorticoid (GC) is poorly understood. Our aim was to evaluate the effect of adrenalectomy (ADX) and dexamethasone (Dex) on testicular and epididymal sperm count. **Methods:** Wistar rats (90 days old) were sham-operated or submitted to bilateral ADX for 1, 2, 7 and 15 days. Rats were also ADX and immediately treated with Dex (5 mg/kg, i.p.) for 7 days. Plasma corticosterone (C) and testosterone (T) levels were monitored by RIA (N=4-10). Testis (TE), caput/corpus (CP) and cauda (CD) epididymis were weighed and submitted to sperm counting (N=4-5). Results were analysed by ANOVA followed by Newman-Keuls test ($p<0.05$). **Results**: A significant reduction on plasma C, but not T levels, was observed with progression of ADX (1-15 days). Rat body weight and tissue relative weight (TE, CP and CD) did not change among the experimental groups. The relative number of homogenization-resistant spermatidis per testis and the daily sperm production was significantly reduced on ADX 2, 7 and 15 days when compared to sham-operated groups. The sperm transit time in CP was increased on ADX 7 days, while the relative sperm number in CD was reduced on ADX 7 days. These effects observed in ADX 7 days were reverted to control levels with Dex treatment, confirming the participation of GC. **Discussion**: Our results show that progression of ADX has effects on sperm parameters in both testis and epididymis, suggesting a role for GC on sperm production, transit and storage. **Supported by:** CAPES, FAPESP, CNPq.

01.060

NUCLEAR FACTOR kappa B (NFκB) RHYTHM IN RAT PINEAL GLAND – NEW MOLECULAR BASIS FOR THE CONTROL OF TIMING IN INJURED ORGANISMS

Cecon, E.[1]; Fernandes, P. A. C. M.[1]; Ferreira, Z. S.[1]; Markus, R. P.[1] - [1]IB - USP - Fisiologia

INTRODUCTION: NFκB, a key factor in inflammatory responses, is constitutively expressed in the pineal (Ferreira, J.Pin.Res,38:182,2005). Here we investigated its regulation by circadian timing and the effect of its activation and inhibition on the trancription of the key enzyme in melatonin synthesis. METHODS: Nuclear NFκB content of pineals from Wistar rats (2 months, light/dark 12/12h) treated or not with propranolol (20mg/Kg, 1h before lights OFF, 2 days) was assesed by EMSA. TNFa (30ng/ml, 30min-48h) and corticosterone (CORT, 1μM, 48h) effects on noradrenaline (NA, 100nM, 5h)-induced transcription of *aa-nat* mRNa (quantified by real time RT-PCR) was determined in cultured denervated glands. RESULTS: Activation of NFκB showed a diurnal rhythm with maximal peak (5 times increase) at CT9 (9 hours after lights ON) and lowest peak at CT12, just after lights OFF. Constant lighting and the block of β-adrenoceptors inhibited NFκB nuclear translocation *in vivo*. Transcription of the *aa-nat* gene was transiently inhibited by TNFa (30min, 31.5±3.8 times vs 17.5±3.2; $p<0.05$), and tonically potentiated by 3 fold with corticosterone *in vitro*. DISCUSSION: Constitutive NFκB activation is blocked by nocturnal sympathetic traffic, as it is restored by lighting or propranolol. NA-induced transcription of *aa-nat* is modulated by substances that acts through NFκB pathway (TNFα and CORT). These data strongly suggest a new molecular pathway for understanding the rhythmic changes in injured organisms and the "feed-back" of inflammatory mediators on the pineal activity. **Supported by:** FAPESP, CNPq, Pró-Reitoria de Pesquisa USP

01.061

FIBRONECTIN AND LAMININ EXPRESSION DURING THE PLACENTATION IN DIABETIC RATS

Giachini, F. R. C.[1]; Carriel, V.[2]; Nigro, D.[1]; Carvalho, M. H. C.[1]; Fortes, Z. B.[1]; Zorn, T. M. T.[3]; San Martin, S.[2]; Tostes, R. C. A.[1] - [1]USP - Farmacologia; [2]Universidade de Valparaíso - Escola de Medicina; [3]USP - Histologia

Introduction: The establishment of the maternal-fetal unit involves remodelation of extracellular matrix components (EMC) and changes in this process may be associated with altered placental morphology. We evaluated whether maternal diabetes affects the placental expression and distribution of fibronectin (FBN) and laminin (LAM). **Methods:** Diabetes was induced in female Wistar rats by a single injection of alloxane (40mg/kg iv) in the second day of pregnancy. Pregnancy was interrupted at days 14, 17 or 20, and the placental FBN/LAM expression and distribution were evaluated by RT-PCR and immunohistochemistry (IHC), respectively. **Results:** Placentas from normoglicemic rats exhibited increased FBN mRNA expression at day 17, decreased gene expression of the a1 and b2 LAM subunits at day 20 and no changes in b1 LAM mRNA expression. The changes in FBN and LAM gene expression were attenuated in placentas from diabetic rats. The IHC analysis showed FBN and LAM expression in almost all placental structures. However, FBN expression in the labyrinth region was observed exclusively in placentas from diabetic rats. A significant decrease in LAM expression was confirmed in term placentas from both groups. **Discussion:** High glucose levels may contribute to altered expression and distribution of FBN and LAM in placentas from diabetic rats. These alterations may be associated with placental structural and functional abnormalities in diabetes. **Supported by:** CNPq and DIPUV

01.062

GROWTH HORMONE STIMULATES OSTEOGENESIS ACTIVITY OF ALVEOLAR BONE DERIVED-OSTEOBLASTS FROM ADOLESCENT DONORS

Crippa, G. E.[1]; Beloti, M. M.[1]; Rosa, A. L.[1] - [1]FORP - USP - Cirurgia

Introduction: The aim of this study was to investigate the effect of GH in human osteoblasts derived from alveolar bone of adolescent (13-15 years old) and adult (36-39 years old) donors. Methods: Primary culture was obtained by enzimatic digestion of adolescent (n=5) and adult (n=4) alveolar bone fragments and were cultured in osteogenic medium. First passage cells were cultured in 24-well culture plates (2×10^4 cells/well) in osteogenic medium containing GH (100, 200 and 300 ng/ml). At day 7, cell proliferation, total protein, collagen and alkaline phosphatase (ALP) activity were evaluated. Bone-like nodule formation was evaluated at day 21. All experiments were done in quintuplicate and data were compared by Kruskal-Wallis test. Results: All evaluated parameters were not affected by GH in cultures of osteoblasts from adult donors. In cultures of osteoblasts from adolescent donors, GH caused concentration-dependent increase in cell proliferation ($p=0.026$), total protein content ($p=0.0053$), collagen synthesis ($p=0.003$), ALP activity ($p=0.03$) and bone-like nodule formation expressed as area of bone-like nodules ($p=0.03$). Discussion: These results indicate that GH effect on osteoblasts is donor age-dependent. While GH had no effect on responses of osteoblasts from adult donors, events related to both proliferation and differentiation were increased when osteoblasts from adolescent donors were cultured in presence of GH. **Supported by:** FAPESP (04/13756-7; 03/09767-0).

01.063

INHIBITION OF HUMAN MONOCYTE APOPTOSIS BY ATL-1, A SYNTHETIC ANALOG OF 15-EPI-LIPOXIN A_4: INVOLVEMENT OF ERK-2 AND PI3-KINASE

Simões, R. L.[1]; Da-Fe, A. R.[1]; Fierro, I. M.[1] - [1]UERJ - Farmacologia e Psicobiologia

Introduction: Mononuclear cells are produced in the bone marrow and circulate in the blood stream for 24-48h before undergoing spontaneous apoptosis, a form of programmed cell death. Monocyte apoptosis may be a central regulatory event in the resolution phase of the inflammatory process. Lipoxins, a distinct class of arachidonic acid metabolites, are generated under a variety of conditions, such as infection and inflammation. In this study, we investigated the effects of ATL-1, a synthetic analog of 15-epi-lipoxin A_4, in human monocyte survival and apoptosis. **Methods and Results:** Addition of ATL-1 (100 nM) to human peripheral blood monocytes cultured in the absence of serum for 48h increased survival by approximately 40% above control, as evaluated by the MTT assay. To investigate whether this effect was a consequence of monocyte apoptosis reduction by ATL-1, we used flow citometry analysis. Incubation of the cells with ATL-1 (100 nM) reduced in 43% spontaneous apoptosis induced by 48h serum starvation. Treatment of these cells with PD98059 (10 μ M) and LY294002 (3 μ M), an ERK-2 and PI3-kinase inhibitors respectively, blocked ATL-1 effects in monocyte apoptosis, suggesting an involvement of the MAPk and PI3-kinase/Akt pathway in this event. Furthermore, ATL-1 inhibited caspase-3 activation, an important protein involved on apoptosis. **Conclusion:** These results demonstrate a cytoprotective effect of ATL-1 in monocytes, which might contribute to the elucidation of the mechanisms associated with the resolution phase of the inflammatory process. **Supported by:** UERJ/SR-2; FAPERJ; CNPq

01.064

DIHYDROCUCURBITACIN B, A CUCURBITACIN-RELATED COMPOUND FROM *Wilbrandia ebracteata* COGN WITH ANTITUMORAL PROPERTIES

Siqueira, J. M. Jr[1]; Gazola, A. C.[2]; Farias, M. R.[2]; Rivard, N.[3]; Brum-Fernandes, A. J.[4]; Ribeiro-do-Valle, R. M.[1] - [1]UFSC - Farmacologia; [2]UFSC - Ciências Farmacêuticas; [3]Université de Sherbrooke - Immunology; [4]Université de Sherbrooke - Rheumatology

INTRODUCTION: We have characterized *Wilbrandia ebracteata* COGN (WE) as a source of biologically active substances, known as cucurbitacins. Our present purpose was to investigate whether an isolated compound from WE, dihydrocucurbitacin B (DHCB), has *in vitro* antitumoral activity, using B16F10 cells (murine melanoma).**METHODS**: To access the effect on proliferative and cell viability status, we used the H^3-Thymidine and MTT assays. The cell cycle phases where observed by flow cytometry. The DHCB effect on the expression of cell cycle components was observed by western blot assays. To investigate the effect on apoptosis we used the Annexin V-FITC and propidium iodide detection, by flow cytometry. We also evaluated the effects on the cytoskeleton (actin filaments dyed with rhodamine-phalloidine) and the formation of focal adhesion points (using anti-paxillin) by immunofluorescence. **RESULTS:** Our experiments demonstrated that the DHCB addiction was able to significantly reduce the proliferative status (47.83 - 98.55%; 0.1-100 μg/mL, compared to control) without important effects on cells viability (24.02 - 48.43%; 0.1-100 μg/mL, compared to control). In the investigation of cell cycle phases, we observed that DHCB promotes an alteration to G2/M phases (26.34% to control, 52.59% to DHCB 10μg/mL, at 16 h) accompanied by polyploid cells augmentation (1.17% to control, 59.62% to DHCB 10μg/mL, at 48 h). The western blot analysis for the cell cycle components demonstrated decreased expression for Cyclin-A, Cyclin-E and mainly in the Cyclin-B1. The immunofluorescence assays demonstrated that DHCB promotes a remarkable alteration in the cell cytoskeleton accompanied by formation of the focal adhesion points. The apoptosis detection demonstrated that DHCB did not induce the formation of apoptotic cells. **DISCUSSION**: Our present results shown that dihydrocucurbitacin B, a cucurbitacin-related compound from WE, demonstrated a cytostatic but not cytotoxic effect on B16F10 cells and this effect is related at least in part, with the important alterations in the cytoskeleton able to inhibit the cytokinesis but not the caryokinesis. Others experiments in our laboratory are in course to investigate the *in vivo* activity of this compound. **Supported by:** CAPES, CNPq and FAPESC.

01.065

EFFECT OF LEUKOTRIENE B4 ON SMOOTH MUSCLE CELL MIGRATION AND PROLIFERATION: ROLE OF PI3K, ERK AND INTEGRINS

Moraes, J. A. de[1]; Assreuy, J.[2]; Barja Fidalgo, T. C.[3] - [1]UERJ - Farmacologia Bioquímica e Celular; [2]UFSC - Farmacologia; [3]UERJ - Farmacologia

A local vascular injury can lead to local inflammation, with lesion of endothelial cells, extracellular matrix exposition and aggregation/adhesion of circulating leukocytes. The release of inflammatory mediators amplifies the process, and induces smooth muscle cells (SMC) adhesion and proliferation, that can form a neointima, leading to vessel occlusion (restenosis). To elucidate the molecular mechanisms involved in restenosis, we have studied the mechanisms involved in SMC migration and proliferation induced by Leukotriene B4 (LTB4), an inflammatory mediator. LTB4 was chemotactic for a SMC murine cell line (A7R5) as evaluated in Boyden chambers, after 6 hours incubation *in vitro*. LTB4 stimulated integrin-related signaling pathways, inducing focal adhesion kinase (FAK) phosphorylation, its association to PI3K, as well as the nuclear translocation of Erk-2 and NFKB, as evaluated by western blotting. Confirming the involvement of PI3K and Erk-2, LY294002, an inhibitor of PI3K, and PD98059, an inhibitor of Erk-2 pathway, inhibited SMC chemotaxis induced by LTB4. Pre-treatment of SMC with kistrin, a selective ligand of avb3 integrin, prevented the effect of LTB4 on SMC chemotaxis and Erk-2 and NFkB nuclear translocation. LTB4 also stimulated SMC proliferation, as evaluated by MTT assay, and its effect was blocked by integrins ligands. The data suggest that the effect of LTB4 on migration and proliferation of SMC could be modulated by integrin signaling activation, suggesting that these adhesion molecules may be important target for therapeutic intervention in restenosis. **Apoio Financeiro: Supported by:** FAPERJ, CNPq, IFS-Sweden

01.066

ANDROGEN DEPRIVATION INDUCES REMODELING OF NUCLEAR DOMAINS AT THE RAT LEVATOR ANI MUSCLE.

Pires-Oliveira, M.[1]; Bueno, M. A.[1]; Furlan, I.[1]; Chiavegatti, T.[1]; Godinho, R. O.[1] - [1]UNIFESP - EPM - Pharmacology

Introduction: Atrophy of skeletal muscle has been associated to remodeling of myonuclear domain (ND) and decreased myonuclei number. Since apoptosis has been previously reported in androgen-responsive tissue following hormonal deprivation, this study investigated the effect of gonadectomy (GDX) of adult male rats on muscle fiber size, neuromuscular junction (NMJ) remodeling and myonuclei organization of the androgen-dependent *levator ani* muscle (LA). **Methods:** Male Wistar rats (n=4) were gonadectomized for 2-3 (G2-3), 7 (G7), 15 (G15) or 30 (G30) days. Cryosections of LA and *extensor digitorum longus* (EDL) muscles were submitted to HE and Hoechst dye 33258 stains and NMJ AChE histochemistry. Images were acquired using a CCD camera and muscle fiber, nuclei and NMJ sizes were determined using the public domain NIH Image J 1.32j program. **Results:** There was significant remodeling of LA fibers after hormonal deprivation, not limited to a linear loss of muscle mass (G7=22%, G15=36% and G30=47%), but also to an exponential decline of NMJ size (G7=24%, G15=31% and G30=38%) and myonuclei length (G2-3 =5%, G7=10%, G15=10% and G30=13%) in comparison to LA control values. Apoptotic myonuclei were detected after 2-day GDX. None of these alterations were seen in EDL. **Discussion:** These results indicate that LA atrophy induced by GDX is accompanied by a reduction in nuclei length and apoptosis of individual nuclei along the muscle fibers which might contribute to the maintenance of ND size and appropriate transcriptional activity of atrophied fibers. **Supported by:** CNPq, FAPESP.

01.067

EFEITOS DE LIGANTES DE PBR *IN VITRO* SOBRE AS FASES DO CICLO DE CÉLULAS DO TUMOR DE EHRLICH

Sakai, M.[1]; Massoco, C. O.[2]; Dagli, M. L. Z.[1]; Palermo-Neto, J.[1] - [1]FMVZ - USP - VPT- Patologia; [2]Oncocell Biotecnologia Ltda - Desenvolvimento Vacinas

Introdução: Sabe-se que o tratamento *in vivo* com diazepam induz maior crescimento do tumor de Ehrlich em camundongos e que receptores periféricos para benzodiazepínicos (PBR) relacionam-se com a proliferação de tumores. Este estudo avaliou a presença de PBR no Tumor Ascítico de Ehrlich (TAE), bem como os efeitos do tratamento *in vitro* com diazepam (DZ), RO5-4864 (RO) e PK 11195 (PK) sobre as fases do ciclo do TAE. **Métodos: PBR**: Células fixadas foram incubadas com anti-PBR e anti-IgG FITC. **Tratamento:** 2 x 10^5 células foram tratadas com etanol 0,01%, DZ, PK e RO em concentrações entre 100nM e 1microM e mantidas em estufa a 5% de CO_2, a 37^{o}C, por 5 dias. **Ciclo celular:** Células foram incubadas com solução de Iodeto de Propídeo + RNase durante 30 minutos para posterior leitura no citômetro de fluxo. **Resultados:** A presença de PBR em células do TAE foi demonstrada por citometria (85,53 ± 12,60 %). A % de células em fase G0-G1 apresentou-diminuída nas células tratadas com DZ 100nM (27,84±5,50) e 300nM (23,00±1,00), PK 100nM (28,11±5,93) e 300nM (30,54±4,12), RO 300nM (23,99±4,55), 600nM (19,40±4,50) e 1microM (23,47±4,11) quando comparadas com o controle (37,7±3,84). Por outro lado, a % de células em fase S-G2-M apresentou-se aumentada com o DZ 100nM (67,69±5,04) e 300nM (72,95±1,04), RO 300nM (66,54±2,66), 600nM (75,15±5,31) e 1microM (71,58±5,55) quando comparadas com o controle etanol (59,26±4,96). **Conclusão:** Este trabalho descreve a presença de PBR em células do TAE. O tratamento com ligantes de PBR *in vitro* diminuiu a porcentagem de células em fase G0-G1 e aumentou em S-G2-M. Sugere-se que este mecanismo seja responsável pelo aumento do crescimento tumoral *in vivo* demonstrado anteriormente. **Apoio Financeiro:** FAPESP (05-01388-6 e 04/14128-0), CNPq

01.068

EXPRESSION OF SERCA1 AND Na^+/K^+-ATPase ISOFORMS IN *BOTHROPS JARARACUSSU* VENOM-INDUCED NECROSIS/REGENERATION OF MICE FAST-TWITCH EDL MUSCLE: EFFECT OF HEPARIN TREATMENT

Schaffazick, N.[1]; Amaral, L. S.[2]; Fonseca, T. F.[2]; Calil-Elias, S.[2]; Melo, P. A.[2]; Noel, F.[2]; Quintas, L. E. M.[2]; Cunha, V. M. N.[2] - [1]UFRJ - Farmacologia; [2]UFRJ - Farmacologia Básica e Clínica

Introduction: Normal fast twitch skeletal muscles express high levels of SERCA1 pump and Na^+/K^+-ATPase a2 isoforms as well as low levels of Na^+/K^+-ATPase a1 isoform. The *in situ* injection of *B. jararacussu* venom causes a rapid necrosis and subsequent regeneration in skeletal muscle. The myotoxic activity of the venom is inhibited by heparin. The aim of this work was to study the expression of SERCA1 and Na^+/K^+-ATPase a1/a2 isoforms during the regeneration of murine *extensor digitorium longus* (EDL) from venom-induced necrosis and after heparin treatment. **Methods:** 50µl of venom (1.0µg/g) or saline (control) were injected in mice right EDL. Heparin (10µg/g) was injected i.v. after 15 and 240 min. **Results**: Preliminary Western blot assays showed immunoblots of muscle homogenates indicated that SERCA1 protein density decreased about 70%, 24h after venom ± heparin administration and returned to control levels after 7 days. Na^+/K^+-ATPase a isoforms were reduced by 40% in 24h and did not change after 7 days. Heparin did not affect this pattern but appears to decrease the proteolysis induced by the venom after 24 h. **Discussion:** Our preliminary data indicate that the quantity of all full-length ATPases decreased in response to *B. jararacussu* venom. Although heparin (10µg/g) seems to decrease the proteolysis at early stages, the regeneration profile of ATPases expression seems to be not affected by this polyanion. **Supported by:** FAPERJ, CNPQ

01.069

ACTIVATION OF THE TRANSCRIPTION FACTOR NF-κB BY THE NON-CODING T-CELLS TRANSCRIPT (NTT) IN HUMAN MELANOMA CELLS

Delgado Andre, N.[1]; De Lucca, F. L.[1] - [1]FM - USP - Biochemistry and Immunology

Introduction: We have recently found that the non-coding transcript in T-cells (NTT) is also expressed in human melanoma cells. The function of NTT transcript is still unknown. We also showed that NTT activates the RNA-dependent protein kinase (PKR). It is known that PKR activation leads to the phosphorylation of the I-κBβ, an inhibitor of the transcription factor NF-κB, and its subsequent degradation. In this study, we investigated whether NTT transcript is able to induce NF-κB activation through degradation of I-κBβ in human melanoma cells. **Methods**: RNA was extracted from human melanoma cells (278 cell line) and used for cDNA synthesis.The primers for amplification were designed based on the sequence of NTT (GenBank Access No. U54776). The PCR product (426 bp) was subsequently cloned into pGEM-T vector and sequencing. The NTT transcripts obtained by *in vitro* transcription were incubated with human melanoma cells and the degradation of I-κBβ was evaluated by Western blot analysis. Cells incubated with medium were used as control. **Results and Discussion**: We found that NTT transcript induces a significant degradation of I-κBβ. It is well established that nuclear translocation of NF-κB is preceded by a decrease of in the level of cytoplasmatic I-κBβ, indicating that its degradation is a key step in NF-κB activation. Thus, our findings suggest that NTT transcript is involved in the activation of NF-κB. It is possible that this effect is mediated by PKR since its activation results in phosphorylation of I-κBβ which serves as a molecular tag, leading to its ubiquitination and subsequent degradation. The activation of NF-κB by NTT transcript is relevant since promoters of many cytokines contain binding sites for NF-κB. **Supported by:** CAPES; FAPESP

01.070

VALIDAÇÃO DO MODELO EXPERIMENTAL DE TUMOR DE PULMÃO POR VIA INTRABRÔNQUICA(TPIB) UTILIZANDO-SE DROGAS COM DIFERENTES MECANISMOS DE AÇAO EM RATOS

Bezerra, N. P.[1]; Simao, A. F. L.[2]; Mourao, L. T. C.[1]; Miranda, S. P.[1]; Gomes Neto, A.[1]; Almeida, P. R. C.[1]; Albuquerque Ribeiro, R.[1] - [1]UFC - Fisiologia e Farmacologia; [2]Universidade Feeral do Ceara - Fisiologia e Farmacologia

INTRODUÇÃO: O objetivo foi avaliar o efeito anti-tumoral de um quimiterápico convecional(Paclitaxel-Pcl), um inibidor seletivo de COX-2(Celecoxib-Cxb), um anti-angiogênico(Talidomida-Tal) e um inibidor da Tirosina-quinase do EGFR,(Gefitinib-Gfb), através do estudo da sobrevida mediana em dias(sm) e volume tumoral(vt) em ratos com TPIB. **MÉTODOS:** Usaram-se ratos Wistar fêmeas (n=127), em modelo de TPIB com implantação de 4 x 10^5 células de carcinossarcoma de Walker. Foram divididos em 5 grupos: Pcl, (8mg/kg, ip) administrado no 3º dia pós-implante em dose única; Cxb (15mg/kg) 12/12hs, por gavagem-(pgav) iniciando 12h pré-implante; Tal (45mg/kg/dia, sc) e Gfb (25mg/kg, pgav) iniciando no 1º dia pós-implante; e controle (salina pgav). Os dados de vt foram comparados pelo teste de Mann-Whitney, a sm pelo de Kaplan-Meier e a diferença de sm entre os grupos pelo de *Log-Rank*. **RESULTADOS:** Não houve diferença no vt nos grupos tratados. A sm aumentou significativamente ($p<0,05$) nos animais tratados com Pcl (29), Tal (13,64) e Gfb (13,43), em relação ao controle (10,92). Não houve aumento da sm dos tratados com Cxb (11,57). **DISCUSSÃO:** O aumento da sm dos tratados com Pcl, Tal e Gfb sugere efeito anti-tumoral no modelo desenvolvido, provavelmente por ação citotóxica, anti-angiogênica e inibidora da transduçao dos sinais conseqüentes à ativação do EGFR, validando o modelo para o teste de novas drogas em tumor no pulmão. **Apoio Financeiro:** CNPQ

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ROLE OF INTEGRIN aDß$_2$ IN THE EARLY PHASE OF PULMONARY INFLAMMATION CAUSED BY SILICA IN MICE.

Ameixoeira, V.[1]; Ferreira, T. P. T.[1]; Farias-Filho, F. A.[1]; Lima, J. G. M.[1]; Carvalho, V.[1]; Bunting, M.[2]; Prescott, S.[3]; Zimmerman, G.[4]; Castro-Faria-Neto, H. C.[1]; Silva, P. M. R. e[1] - [1]FIOCRUZ - Fisiologia e Farmacodinâmica; [2]University of Utah - Program in Human Molecular Biology and Genetics; [3]Huntsman Cancer Institute - Pathology; [4]University of Utah - Internal Medicine

Introduction: Beta2 integrins play an essential role in leukocyte trafficking and activation during inflammation. In this study, we investigated the potential contribution of aDß$_2$ (CD11d/CD18), one of the most recently-identified member of the leukocyte integrin family, to the early phase of silicosis in mice. **Methods:** C57/Black 6 and aDß$_2$ knockout mice were instillated nasally with crystalline silica and the analyses performed on day 7. Total and differential leukocytes were evaluated in the BAL and lung morphology analyzed by histological techniques. **Results:** Intranasal silica into the wild type mice led to an increase in the leukocyte numbers in the BAL, mainly macrophages and neutrophils, phenomenon which paralleled with a marked leukocyte infiltration and numerous granulomas presented in the lung tissue. The aDß$_2$ knockout mice exhibited a less intense macrophage infiltration (51%) in the BAL, though a more pronounced inflammatory response was noted in the lungs. Attenuation of tissue collagen deposition was also noted in the knockout mice. **Conclusion**: Our findings indicate that aDß$_2$ integrin seems play a role in the early phase of lung inflammation caused by silica in mice and also offer evidence that macrophages are important and effective part of the process of immediate immunity responses such as silicosis. **Supported by:** CNPq,PAPES4/FIOCRUZ

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ANALYSIS OF MUTATIONS IN *TP53*, *APC*, *K-RAS* AND *DCC* GENES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Soares Lima, S. C.[1]; Rapozo, D. C. M.[2]; Braunstein, A. G.[3]; Carvalho, A. T.[4]; Paiva, D. D.[5]; Barja Fidalgo, T. C.[6]; Ribeiro-Pinto, L. F.[3] - [1]UERJ - Bioquímca; [2]UERJ - Instituto de Biologia / Bioquímica; [3]UERJ - Bioquímica; [4]UERJ - Medicina Interna; [5]UERJ - Patologia e Laboratórios; [6]UERJ - Farmacologia

Introduction Colorectal cancer is the fourth most frequent type of neoplasia in the Brazilian population with an increasing incidence each year. Among the best known risk factors for colorectal cancer are the inflammatory bowel diseases such as Ulcerative Colitis (UC) and, to a lesser degree, Cronh Disease (CD). Many genetic alterations are present in colorectal cancer, and the most common genes affected are *TP53*, *APC*, *K-ras* and *DCC*. **Methods** Biopsies were colleted from 6 defined areas of the colon from 34 patients with UC and from 10 patients with CD that did not have dysplasia. Mutations in exons 5, 6, 7 and 8 of the *TP53*; around codons 1061 and 1309 of the *APC* ; and in codons 12 and 13 of the *K-ras* gene were analyzed by PCR-SSCP/direct sequencing. The deletion of *DCC* was evaluated by PCR-duplex. A total of 264 biopsies were analyzed. **Results** Mutations in *TP53* gene, *APC* gene, and *K-ras* gene as well as deletion of the *DCC* gene were not detected in patients with UC. However a mutation was found in codon 1141 of the *APC* gene of two patients with CD, with one being somatic and the other germinative. **Discussion** The mutation in both patients was an exchange of thymine for cytosine. This resulted in an exchange of the amino acid leucine for serine. Our results suggest that in patients without dysplais, mutations in *TP53, APC, K-ras* and *DCC* genes are rare in those with UC, but mutations in *APC* gene may not be rare in those with CD. **Supported by:** FAPERJ, CNPq and SR2/UERJ

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PKR ACTIVATION BY A NON-CODING RNA EXPRESSED IN LYMPHOCYTES OF MICE BEARING B16 MELANOMA

Murad, J. M.[1]; Sousa, T. A.[1]; Delgado Andre, N.[1]; De Lucca, F. L.[1] - [1]FM - USP - Biochemistry and Immunology

Introduction: In recent years, non-coding RNAs (ncRNAs) have become an exciting area of research. It is known that ncRNAs, named regulatory RNAs, play an important role in the regulation of gene expression in eukaryotic cells. However, little is known about ncRNAs in lymphocytes. We have already shown that total RNA isolated from activated lymphocytes is able to activate the RNA-dependent protein kinase (PKR). We hypothesized that total RNA contains ncRNAs that are able to bind PKR with subsequent activation of this protein kinase. PKR is a serine/threonine kinase containing two RNA-binding domains within the N-terminal region. In this study, we took advantage of the ability of RNAs to bind PKR in order to identify ncRNAs in lymphocytes activated during B16 melanoma development. **Methods**: The presence of ncRNAs was investigated by using the differential display reverse transcription-PCR. RNAs that coimmunoprecipitated with PKR were reversed transcribed, re-amplified, cloned, sequenced and the secondary structure was determined. The transcripts obtained by *in vitro* transcription were used in PKR assay. PKR was analyzed by SDS-PAGE followed by autoradiography. **Results and Discussion**: We detected a highly structured transcript of 220 nt with no open reading frame (ORF) which is able to activate PKR and it is only expressed in lymphocytes of mice bearing B16 melanoma. Therefore, the transcript 220 nt may be included in the class of ncRNAs that act by modifying protein activity. We have previously shown that PKR activation is accompanied by degradation of the inhibitor I-κBα of the transcription factor NF-κB. Thus, our data suggest that regulation of gene expression in activated lymphocytes by transcript 220 nt could be mediated by PKR through activation of the transcription factor NF-κB. **Supported by:** FAPESP

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REDUCED EXPRESSION OF IL-3 MEDIATES INTESTINAL MAST CELL DEPLETION IN DIABETIC RATS.

Carvalho, V.[1]; Barreto, E.[2]; Farias-Filho, F. A.[1]; Cordeiro, R. S. B.[1]; Martins, M. A.[1]; Silva, P. M. R. e[1] - [1]FIOCRUZ - Fisiologia e Farmacodinâmica; [2]UFAL - Genética e Biologia Molecular

Introduction: Rats turned diabetic by treatment with alloxan were shown to be refractory to systemic anaphylaxis, in a clear association with hyporesponsiveness of intestinal tissue. In this study, we investigated the relationship between intestinal refractoriness to antigen challenge and potential mast cell alterations under diabetic conditions. The involvement of the mast cell growth factor interleukin-3 (IL-3) was also evaluated. **Methods:** Diabetes was induced by means of a single i.v. injection of alloxan into Wistar rats and the analyses were made 21 days later. Mast cell evaluation was performed by histological techniques and expression of IL-3 by immunohistochemistry. **Results:** We found that staining of ileum fragments with PAS-alcian blue revealed a significant reduction in mast cell numbers in the ileum from diabetic rats as compared to those from controls. The immunohistochemical analysis revealed a strong labeling for IL-3, which was distributed more or less along the smooth muscle layer and from crypts to villus. Diabetic animals exhibited a weaker labelling for IL-3 in the ileum tissue. In addition, the administration of insulin into diabetic rats restored mast cell depletion as well as the expression of a normal pattern for IL-3. **Conclusion:** Our findings indicate that there is a causative relationship between down-regulation of mast cell numbers and expression of growth factors IL-3 under diabetic conditions, phenomena clearly sensitive to insulin treatment. **Supported by:** CNPq, FAPERJ, PAPES3/FIOCRUZ, CAPES