01. Farmacologia Celular e Molecular

01.001

Efeito da desnutrição multifatorial na expressão e atividade das bombas de Ca^{+2} e expressão de FKBP12 no ducto deferente de rato. Muzi-Filho, H.[1]; Bezerra, C. G. P.[1]; Paixão, A. D. O.[2]; Castro-Chaves, C.[2]; Einicker-Lamas, M.[3]; Vieyra, A.[3]; Lara Morcillo, L. S.[1]; Cunha, V. M. N.[1]
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Introdução: A desnutrição afeta 174 milhões de crianças menores de 5 anos em todo o mundo, sendo caracterizada pelo baixo peso e estatura para a idade. A desnutrição gera um grave problema de saúde pública uma vez que resulta em seqüelas de implantação silenciosa que podem se manifestar na população adulta. No ducto deferente de rato (DDR) encontram-se expressos diferentes componentes celulares, tais como a imunofilina FKBP12, que regula a atividade dos canais liberadores de Ca^{2+} (CRC) e as bombas de Ca^{2+} PMCA e SERCA, importantes constituintes celulares responsáveis pela regulação da homeostasia do Ca^{2+}. O objetivo do presente trabalho foi estudar o efeito da desnutrição sobre a expressão e a atividade desses componentes celulares no DDR. **Métodos**: Foram estabelecidos dois modelos de desnutrição multifatorial: (1) ratas Wistar grávidas foram alimentadas com Dieta Básica Regional (DBR), sendo sua prole alimentada com uma dieta convencional (DBR-IU); (2) logo após o desmame, ratos provenientes de mães sadias foram submetidos à dieta DBR por 13 semanas (DBR-CR). Nos grupos controles, os ratos se alimentavam da dieta convencional. Os animais foram sacrificados e o DDR foi removido, lavado, homogeneizado e ultracentrifugado a 108.000 *g* para obtenção da fração FKBP(+). Para dissociar o complexo FKBP12-CRC, parte do homogeneizado ultracentrifugado foi tratada a 37^{o}C por 30 min antes de nova ultracentrifugação (fração FKBP(-)). Estas frações foram usadas para medida da captação de $^{45}Ca^{2+}$, atividade (Ca^{2+}+Mg^{2+})ATPásica e ensaios de *Western Blotting.* **Resultados**: Os ensaios de *Western Blotting* mostram que nos grupos DBR-IU e DBR-CR são observados redução da expressão de FKBP12 (68% e 69%, respectivamente) e aumento da expressão das bombas SERCA (71% e 65%) e PMCA (137% e 87%). Entretanto, o grupo DBR-IU apresenta diminuição da acumulação de $^{45}Ca^{2+}$ (51%) associada à manutenção dos níveis da atividade Ca^{2+}-ATPásica, enquanto o grupo DBR-CR mostra níveis de acumulação de $^{45}Ca^{2+}$ comparáveis ao controle (100%) associada ao aumento da atividade hidrolítica da enzima (82%). **Discussão**: Durante o período de desnutrição, mecanismos moleculares são alterados de forma que a homeostasia intracelular de Ca^{2+} no DDR é perturbada. No grupo DBR-CR o aumento da expressão da SERCA acarreta no aumento da atividade dessa enzima para se manter os níveis de transporte de Ca^{2+} no retículo sarcoplasmático (RS). No grupo DBR-IU o aumento da expressão da SERCA parece não ser suficiente para aumentar a atividade dessa enzima, o que resulta na diminuição do transporte desse íon. A diminuição da expressão de FKBP12 nesse grupo experimental pode agravar o baixo conteúdo do Ca^{2+} no RS e comprometer a capacidade contrátil do DDR. E o aumento da expressão de PMCA pode justificar o maior requisito do trabalho dessa família de bombas para remover o Ca^{2+} citoplasmático após o evento celular. Apoio Financeiro: Projeto Casadinho-CNPq; PROCAD-CAPES; FAPERJ Primeiros Projetos, Programa ALV

01.002

The extracellular Cys^{18}-Cys^{274} disulfide bond plays a functional role in angiotensin II AT_1 receptors. Martin, R. P.[1]; Rodrigues, E. S.[1]; Correa, S. A. A.[2]; Oliveira, L.[1]; Shimuta, S. I.[1] – [1]UNIFESP- Biofísica; [2]UNIFESP - Ginecologia

Previous studies on AT_1 receptor function have revealed that the N-terminal residues (Asp^1 and Arg^2) of angiotensin II (AngII) may modulate activation by binding at the receptor extracellular site and that C18S mutant AT_1 receptor, in which the extracellular Cys^{18}-Cys^{274} disulfide bridge was dissociated, became constitutively activated. To provide additional information about functioning of the AT_1 receptor extracellular site, binding assay was performed using ^3H- and ^{125}I-labelled AngII and increasing concentrations of the unlabelled AngII and its analogs in wild type (WT) and C18S mutant receptor. Besides the low levels of expression of the mutant receptor, it was shown that the ability of C18S receptor to bind [Sar^1]-AngII and AngII was reduced (IC_{50}(nM): 10 ± 1.6(5) and 46.7 ± 1.4(5), respectively), whereas [Lys^2]-AngII showed no binding when compared with WT receptor (IC_{50} (nM): 5.6 ± 1.7(5) for [Sar^1]-AngII, 1.5 ± 1.2(5) for AngII and 28.6 ± 1.4(4) for Lys^2AngII). On the other hand abolishment of ^{125}I-AngII binding was observed in the C18S mutant but not in WT receptor (IC_{50} (nM) 4.5 ± 1.8(4) for [Sar^1]-AngII, 1.9 ± 1.2(4) for AngII and 39.8 ± 1.6(4) for Lys^2AngII). These findings suggest that the Cys^{18}-Cys^{274} bridge acts against constitutive activation but is vital for agonist-mediated activation by allowing the binding of AngII N-terminal residues to the receptor extracellular site. The role of Asp^1 and Arg^2, as well as Tyr^4 amino acid residues of AngII molecule in these mechanisms are shown in a molecular model containing interactions between AngII and AT_1 receptor structures.
Apoio Financeiro: FAPESP e CNPq.

01.003

Evidences that kinin B_1 and B_2 receptors regulate the expression of Angiotensin II type I receptor. Rodrigues, E. S.; Martin, R. P.; Arantes Felipe, S.; Pesquero, J. B.; Shimuta, S. I. - UNIFESP - Biofísica

It was already shown that angiotensin II (AngII) mediated by AT_1 receptor may regulate the expression of kinin B_1 and B_2 receptors in rat vascular smooth muscle cells. It is also known that mice abdominal aorta express kinin B_1 and B_2 receptors as well as AngII AT_1 and AT_2 receptors. Our aim was to find out if the elimination of kinin receptors would interfere with the function and expression of AngII receptors. Recordings of isometric contractions were evoked by AngII and determination of gene expressions were carried out using real-time quantitative PCR. From concentration-response curves for AngII the potency (pD_2, considered -log EC_{50}) and efficacy (maximum effect) were determined in abdominal aorta isolated from wild type (WT, control) and mice deficient in B_1 receptor (B_1KO), mice deficient in B_2 receptor (B_2KO) and in both kinin receptors (B_1/B_2KO). The pD2 values were for AngII in WT: 7.8 ± 0.2(15), B_1KO: 7.6 ± 0.2(7), B_2KO: 7.6 ± 0.1(5) and B_1/B_2KO: 7.4 ± 0.5 (6); AngII-induced maximal effects were (% in relation to 1 µM NE): WT, 62 ± 5(20); B_1KO, 33 ± 3(4); B_2KO, 42 ± 0.9(8) and B_1/B_2KO, 43 ± 8(6). The values for gene expression of AT_1 receptor ($2^{-\Delta CT}$) were in: B_1KO, 0.039 ± 0.001(4); B_2KO, 0.029 ± 0.009(4) and B_1/B_2KO, 0.0426 ± 0.0056(3), whereas in WT it was 0.343 ± 0.191(4). Our finding that the expression of AT_1 receptor was significantly reduced in the absence of kinin B_1 or B_2 and both B_1 and B_2 receptors, may explain the reduction in the efficacy of AngII-induced contractile responses in abdominal aorta isolated from transgenic mice when compared to WT animal. These results indicate that the lower expression of AT_1 receptor could be due to the lack of interaction between B_1 and B_2 receptors and consequent crossed interactions between B_2 receptor and AT_1 receptor. According to our suggestion, it was already demonstrated that heterodimerization between kinin and AngII receptors induced low activation on kinin receptor and increase in AngII receptor activity. From these data it is suggested that interactions between B_1 and B_2 receptors and further crossed interaction with AngII receptor may regulate the expression level of AT_1 receptor. Apoio Financeiro: FAPESP, CAPES and CNPq

01.004

Avaliação farmacológica da reatividade vascular à DBK e BK em ratos com superexpressão de receptores B_1. Filippelli da Silva, R.; Rodrigues, E. S.; Martin, R. P.; Pesquero, J. B.; Shimuta, S. I. - UNIFESP-EPM - Biofísica

Ratos com superexpressão do receptor B_1 de cininas no endotélio (TGR(Tie2)) foram descritos como normotensos, com susceptibilidade maior à choques endotóxicos nos quais o agonista do receptor B_1 ($DesArg^9BK$, DBK) causava acentuada queda na pressão sanguínea e aumentada permeabilidade vascular em comparação com animais controle (WT). Em aorta isolada desse animal transgênico a DBK induz relaxamento desde o início de sua incubação, o que não é observado em aorta isolada de ratos WT (Merino e cols., 2008). Assim a reatividade a DBK foi estudada em aortas torácicas isoladas de ratos TGR(Tie2) e WT após um prolongado tempo de incubação, condição conhecida por induzir a síntese de receptores B_1. Investigou-se também se a superexpressão do receptor B_1 afetaria a reatividade à bradicinina (BK). Foram utilizadas aortas torácicas isoladas de ratos normais, (Sprague-Dawley) como controle e de animais transgênicos (TGR(Tie2)). Obteve-se registros de relaxamento induzido pela DBK e BK em anéis de aorta pré-contraídas pela adição de nor-epinefrina (NE) 10^{-6} M. A partir de curvas concentração-resposta determinou-se a potência (pD_2, log. negativo de EC_{50}, concentração suficiente para induzir 50% da resposta máxima) e eficácia (efeito máximo), utilizando-se o programa GraphPad-Prisma. Respostas à DBK foram registradas em anéis de aorta de animais WT incubadas por 5h forneceram valores de pD_2 = 8.0 ± 0.7 e eficácia = 20 ± 5% em relação ao tônus dado pela NE 10^{-6} M. Em anéis de TGR(Tie2) foram: pD_2 = 8.6 ± 0.3 e a eficácia = 73 ± 5.5%. Resultados de relaxamentos induzidos pela BK foram: pD_2 = 8.1 ± 1(WT) e 7.9 ± 0.4 (TGR(Tie2)) e para a eficácia foram: 21.5 ± 4% (WT) e 54 ± 6% (TGR(Tie2)). A potência da resposta relaxante à DBK após 5h de incubação em aortas de animais WT, pela expressão induzida de receptores B_1 foi semelhante aquela obtida em ratos transgênicos, indicando que a afinidade foi mantida nos receptores B_1 endógeno e exógeno. A maior eficácia da resposta a DBK nos ratos TGR(Tie2) era esperada, uma vez que o número de receptores nesses animais é maior, em contraste a da BK, pois na maioria das vezes que se altera a expressão de um receptor, observa-se um efeito compensador do segundo sub-tipo de receptor do mesmo agonista. Assim, a resposta mediada pelo receptor B_2 deveria ser reduzida e não aumentada em TGR(Tie2). Conclui-se que a afinidade do receptor B_1 em TGR(Tie2) não foi alterada e que a maior eficácia da resposta à BK poderia ser pelo aumento de receptores B2 ou uma alteração na transdução de sinal. Não se pode ainda descartar que as respostas à BK podem ter sido via formação de DBK mediando a ativação de receptores B_1. Apoio Financeiro: FAPESP, CNPq e CAPES

01.005

Kinin B_2 receptor gene product processing and expression in adult and foetal rats. Estevão de França, C.; Geroldo, E. A.; Lindsey, C. J. - UNIFESP Biofísica

Introduction. The rat bradykinin B_2 receptor gene consists of 4 exons separated by 3 introns, whereas mouse and human gene has 3 exons separated by 2 introns. Alternative splice has been reported for the rat B_2 encoding mRNA. One of the RNA species would contain exon 3 and the other mRNA species would not. Exon 3 has been proposed to contain an additional translation initiation codon, implicating in proteins of different sizes. In order to examine the process of the B_2 kinin receptor encoding messenger RNA (mRNA), cDNA amplicons where generated using primers directed to 5´ or 3´ flanking regions of different exons and introns. **Material and Methods**: In order to examine the processing of the B_2 kinin receptor encoding messenger RNA (mRNA), cDNA amplicons (RT_PCR) where generated using primers directed to 5´ or 3´ flanking regions of different exons and introns and analyzed for size in ethydium bromide stained agarose gels **Results**: No evidence was found for exon 3 expression in polyadenylated (ripe) mRNA in tissues of adult Wistar, Sprague-Dawley or spontaneously hypertensive rats (SHR) or 1 to 21 day rat embryos or foetuses.. Analyses intron-exon amplicons showed that introns 1 to 3 are removed sequentially and that "exon 3" removal follows that of intron 3. The lack of evidence for the expression of "exon 3" in ripe mRNA indicates that the structure of rat gene is similar to that of mouse, rabbit or human genes all consisting of 3 exons and 2 introns. Conclusion: The fact that it is removed late in the processing, after intron 3, suggests that the exon may represent an ancestral form of the gene probably expressed in earlier instances of phylogenetic evolution. Apoio Financeiro: FAPESP/CNPQ

01.006

Synthesis of a novel silicon phthalocyanine conjugated to bovine serum albumin for photodynamic therapy of cancer. Ribeiro, N. M.[1]; Cardoso, M. A. G.[2]; Soares, C. P.[3]; Ferrari, E. F.[4]; Beltrame Jr., M.[1] [1]UNIVAP- Síntese Orgânica; [2]UNIVAP- Imunologia; [3]UNIVAP - Biologia Celular e Tecidual; [4]UNIVAP- Fisiologia e Farmacodinâmica

Introduction: Macromolecular carrier systems have been developed in an attempt to optimize the delivery of agents neoplasic against[1]. Recently, bioconjugates of phthalocyanines with bovine serum albumin (BSA) have been successfully used to target particular cell types like tumor-associated macrophages and HeLa[2,3,4]. In this work we have attempted to develop an improve conjugation technique using a cationic phthalocyanine and BSA. **Materials and Methods**: The compound phthalocyaninato[bis(dimethylaminoethanoxy)]silicon (NzPc) was prepared as described by Ré *et al*.[5]. The preparation of the conjugated phthalocyanine was done using a solution of NzPc in *N,N*-dimethylformamide (DMF) (1.0 mM) and on its was added drop wise other solution of BSA (35 μM) in phosphate buffered saline (PBS). The pH was adjusted to 7.4 with HCl and the mixture was stirred at ambient temperature overnight. After time, the reaction mixture was chromatographed on a G-100 Sephadex column using aqueous NH_4HCO_3 (20 mM) as eluent. The conjugated phthalocyanine was collected as the first blue fraction and its was dialyzed and lyophilized to remove water and NH_4HCO_3[2]. SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) was used to verify the transformation in conjugates[1,4]. The phthalocyanine concentration was calculated from Q band absorbance in a diluted DMF solution with reference to the corresponding molar absorptivity[2]. **Results**: The analysis and characterization of NzPc was confirmed by results of Ré *et al.*. The formation of the conjugated phthalocyanine was verified by SDS-PAGE. Dialysis and lyophilization were sufficient to purify the conjugated phthalocyanine that is ready for *in vitro* assays. **Discussion**: The descript way was realized in 3 steps and it had confirmed its efficiency. The conjugated phthalocyanine was verified by SDS-PAGE, since the molecular weight of the conjugated was somewhat larger than that of BSA like demonstrated by SDS-PAGE method[1,4]. **Conclusions**: The synthesis of a novel silicon phthalocyanine conjugated to BSA that is a potential photosensitizer for Photodynamic Therapy of cancer will permit its studies *in vitro* assays. **References**: 1. Tanaka T. *et al.* International Journal of Pharmaceutics, 277, 39-61, 2004. 2. Huang J.D. *et al. Journal of Inorganic Biochemistry*, 100, 946-941, 2006. 3. Brasseur, N. *et al. Photochemistry and Photobiology* 69(3), 345-352, 1999. 4. Sutton J. M. *et al. Bioconjugated Chemistry* 13, 249-263, 2002. 5. Ré, M. I. *et al.*Minerva Biotecnológica, 18(1), 3-9, 2006.

01.007

Effect of ivermectin and synthetic derivatives on mammalian P-ATPases. Pimenta, P. H.; Silva, C. L. M.; Noël, F. - ICB-UFRJ - Farmacologia

Introduction: Ivermectin is a semi-synthetic analogue of the avermectins, a family of macrocyclic lactones produced by *Streptomyces avermitilis*. This almost twenty years-old highly effective antihelmintic agent is worldwide used in animals and humans, where it was approved for the treatment of onchocerciasis and, more recently, lymphatic filariasis and strongyloidiasis. When used in therapeutic doses (about 150 ug/kg), ivermectin reaches nanomolar concentrations in the plasma and is safe and well tolerated. However, since ivermectin is currently being tested at higher doses in experimental models of protozoan infections, the report that it inhibits the Ca^{2+}, Mg^{2+}ATPase of rabbit skeletal muscle in micromolar concentrations (Bilmen, *Biochem J* 366:255, 2002) raises the question of its safety at high doses. The aim of the present work was to investigate the effect of ivermectin and nine new synthetic analogues on the three main mammalian P-ATPases in order to address this question. Methods: Different rat organs were used for the preparation of fractions enriched in different ATPases (Pôças, PhD thesis, 2007): EDL (SERCA 1A), heart (SERCA 2A), kidney (Na^+,K^+ATPase, alpha1), brain (Na^+,K^+ATPase, alpha2/3) and stomach (H^+,K^+ATPase). The ATPasic activity was measured after 2h incubation following the Fiske & Subbarow method. Non-linear regression analysis was used for determination of the IC_{50}. **Results**: Ivermectin inhibits all the P-ATPases tested with IC_{50} in the micromolar range: 30 uM for SERCA 1A, 13 uM for SERCA 2A, 14 uM and 8 uM for Na^+,K^+ATPase (alpha1 and alpha2/3, respectively) and 8.5 uM for the H^+,K^+ATPase. The new synthetic analogues exhibit different patterns of inhibition, some being very similar to ivermectin whereas others have no effect at 50 uM. One of them, AM6, is particularly interesting since it has no significant effect on all the P-ATPases at the highest concentration tested (50 uM), although it is equipotent to ivermectin for inhibition of *Leishmania amazonensis* promastigotes and amastigotes (IC_{50} about 5 uM – Bartira, personal communication). **Discussion**: Present data show that ivermectin inhibits rat SERCAs, Na^+,K^+ATPase and H^+,K^+ATPase, but only in micromolar concentrations, in accordance with the lack of serious adverse effects when used at the legally approved low doses (nanomolar concentrations). On the other hand, our data indicate that higher doses of ivermectin could be potentially dangerous due to inhibition of enzymes essential for the maintenance of calcium homeostasis, such as SERCA2A and Na^+,K^+ATPase. Our data also indicate that the search for new analogues of ivermectin is rationale since the structural requirements for leishmanicide effect and ATPase inhibition are different, as demonstrated with AM6. Apoio Financeiro: CAPES, FAPERJ, CNPq

01.008

Pharmacologic evaluation of new alpha adrenoceptor antagonists. Chagas-Silva, F.[1]; Noël, F.[1]; Silva, R. O.[2]; Romeiro, L. A. S.[2]; Barberato, L. C.[3]; Oliveira, M. S.[2]; Silva, R. O.[2]; Silva, C. L. M.[1] - [1]UFRJ - Farmacologia Celular e Molecular; [2]LADETER-UCB. - Núcleo de Química Bioorgânica e Medicinal; [3]UCB - Desenvolvimento de Estratégias Terapêuticas

Introduction: The prostate smooth muscle tone depends largely on $alpha_1$-adrenoceptors, mainly $alpha_{1A}$ and $alpha_{1D}$ subtypes, and their blockade promotes muscle relaxation, which is useful in the treatment of benign prostatic hyperplasia. However, some adrenoceptor antagonists also act as antagonists of serotonin (5-HT) receptors, thus causing adverse effects. The objective of this study is to assess the affinity of new $alpha_1$-adrenoceptor antagonists (LDT) for $alpha_1$ and 5-HT receptors. **Methodology**: *Binding assays*: preparations from cortex of rats are used as preparations enriched in native 5-HT_{2A} receptors. 150 ug protein are incubated for 15-40 min at 37°C in the presence of 1 nM [^{3}H]-ketanserin as radioligand of 5-HT_{2A} receptors in the absence and presence of LDT62-LDT68 (1-50 uM). The reaction is stopped by addition of cold 5 mM TRIS-HCl buffer followed by filtration. *Functional studies*: isometric contraction experiments are performed as previously described (*Silva et al. 2002. Br J. Pharmacol. 135*:*293*), where rat aorta ($alpha_{1D}$- adrenoceptors and 5-HT_{2A} receptors) with intact endothelium are contracted with phenylephrine (PE) or 5-HT (1 to 10000 nM) before and after incubation with LDTs (10 uM; screening concentration) to assess the vasodilator effect. The results are analyzed by non-linear regression to calculate the parameters IC_{50} and E_{max}. **Results and Discussion**: LDT 62,63,64,65,66 and 68 compete with [^{3}H]-ketanserin with mean IC_{50} values (uM) of 11.9, 21.2, 12.3, 2.9, 1.8 and 1.8, respectively, indicating that they have a rather low affinity for 5-HT_{2A} receptors. The contraction induced by 5-HT is reduced by 10 uM LDT62 from 13.29 +/- 0.52 to 0.56 +/- 0.4 mN (n = 3). A similar effect is observed for the contraction induced by PE (E_{max}=11.9 +/- 0.3 and 1.7 +/- 0.8 mN, before and after treatment, respectively). On the other hand, LDT63 (10 uM) does not alter the contraction induced by 5-HT (E_{max}=17.6 +/-1.51 and 17.6 +/-2.7 mN, before and after treatment, respectively), although it reduces the FE-induced contraction (from 15.7 +/-0.8 to 1.3 +/-0.4 mN, n = 6). Based on our preliminary functional and biding data we suggest that LDT63, but not LDT62, has a higher affinity for alpha-adrenoceptors (including $alpha_{1D}$) than for 5-HT_{2A} receptors, indicating that small structural differences can modulate the pattern of selectivity for different G-protein coupled receptors of these biogenic amines. Apoio Financeiro: FAPERJ, CNPq.

01.009

Internalization of α_{1A}-adrenoceptors induced by oxymetazoline but not by norepinephrine in HEK293 cells. Akinaga, J.[1]; Ureshino, R. P.[2]; Smaili, S. S.[2]; Pupo, A. S.[1] [1]UNESP - Farmacologia; [2]UNIFESP - Farmacologia

Introduction: α_1-Adrenoceptors (α_1-ARs) are G-protein coupled receptors (GPCRs) important in cellular differentiation, proliferation and contraction of vascular and non-vascular smooth muscle. It is known that some agonists induce internalization of α_1-ARs. The α_1-AR agonist phenylephrine induces internalization of α_{1A}-ARs after 50 minutes of incubation (Chalothorn, 2002). The aim of this study was to compare the ability of two agonists from different chemical classes: oxymetazoline (OXY; an imidazoline derivative) and norepinephrine (NE; a phenethylamine derivative) to induce internalization of α_{1A}-AR fused to green fluorescent protein (α_{1A}-GFP) in HEK293 cells. **Methods**: HEK293 cells were transiently transfected with the cDNA encoding α_{1A}-AR/GFP fusion protein by calcium phosphate precipitation (20 mg DNA/4X10^5 cells). Cells were cultivated in plates treated with poly-D-lysine and images, in real time series, were acquired in a confocal microscope (LSM510, Carl Zeiss) with Plan-Neofluar 40X oil immersion objective. Argon Laser and HFT 488 nm filter were used for excitation and LP 505 nm filter for emission. Fluorescence data were extracted and were expressed as mean ± standard error of mean of three experiments (in relative arbitrary unities, r.u.). **Results**: The incubation of HEK293 cells expressing α_{1A}-GFP with NE (10 mM) for 30 minutes did not increased intracellular fluorescence. However, incubation of HEK293 cells expressing α_{1A}-GFP with OXY (10 mM) significantly increased intracellular fluorescence already after 5 minutes of incubation (basal, arbitrarily set as 1.0; 5 min: 2.1 ± 0.2 r.u.), indicating receptor internalization. There was a time-dependent increase in intracellular fluorescence after OXY incubation (after 10 min: 2.2 ± 0.2 r.u.; 15 min: 3.8 ± 0.6 r.u.; 20 min: 4.7 ± 0.8 r.u.; 25 min: 6.9 ± 1.1 r.u.; 30 min: 9.0 ± 1.7 r.u.). **Discussion**: It is surprising that OXY promptly increased intracellular fluorescence in HEK293 cells expressing α_{1A}-GFP, whereas NE was unable to increase the same parameter even after 30 minutes of incubation. These results suggest that α_1-ARs agonists may present different efficacies in inducing α_{1A}-AR internalization. **References**: Chalothorn *et al.*; *Mol. Pharmacol.* 61(5): 1008-1016; 2002 Apoio Financeiro: FAPESP (05/57569-9)

01.010

Sustained pharmacological βIIPKC inhibition is cardioprotective in late-stage hypertrophy and end-stage heart failure in two rat models. Ferreira, J. C. B.[1]; Koyanagi, T.[1]; Inagaki, K.[1]; Fajardo, G.[2]; Churchill, E.[1]; Budas, G.[1]; Zambelli, V. O.[3]; Kihara, Y.[4]; Bernstein, D.[2]; Brum, P. C.[5]; Mochly-Rosen, D.[1] [1]Stanford University School of Medicine - Chemical and Systems Biology; [2]Stanford University School of Medicine - Pediatrics; [3]Instituto Butantan - Fisiopatologia; [4]Hiroshima University Hospital - Cardiovascular Medicine; [5]USP - Biodinâmica do Movimento Humano, Escola de Educação Física

Introduction: Previously, we found that βIIPKC levels increase during the transition from compensated hypertrophy to dysfunction in hearts from hypertensive Dahl salt-sensitive rats. **Methods**/**Results**: We show here that a six-week treatment with βIIV5-3, a βIIPKC-specific inhibitor peptide that our lab has previously designed, prolongs survival in this model of hypertrophy and delays the transition from compensated hypertrophy to cardiac dysfunction in these hypertensive rats. We also show that a six-week treatment with βIIV5-3 in a post-myocardial infarction model also increased survival and improved fractional shortening from 14 ± 2% in control-treated animals to 27 ± 2%. Importantly, this chronic but mild inhibition of βIIPKC using βIIV5-3 (60 ± 8% inhibition of translocation and 62 ± 9% inhibition of catalytic activity relative to control-treated animals) did not affect the activity of any other PKC isozymes, including αPKC and the closely related, βIPKC. We also found that in the post-myocardial infarction model, exercise tolerance was improved and cardiac hypertrophy was decreased in the βIIV5-3-treated group *vs*. controls. Changes in the levels of the Ca^{2+}-handling proteins, SERCA2 and the Na^{+}/Ca^{2+} exchanger, seen in the control-treated heart failure rats were not observed in the βIIPKC-treated rats, suggesting that βIIPKC regulates calcium levels in the myocardium. In contrast, treatment with the selective inhibitor of βIPKC, an alternative splicing variant of βIIPKC, had no beneficial effects. Finally, we show that βIIV5-3 treatment improved calcium handling in isolated rat cardiomyocytes and enhanced contractility in isolated rat hearts. Conclusion: In conclusion, our data using two distinct *in vivo* models of heart failure (late-phase hypertrophy and end-stage ischemic heart disease) suggest that βIIPKC activation contributes to the pathology associated with heart failure and may be a potential therapeutic target for the treatment of this disease. Apoio Financeiro: Supported by FAPESP 06/56321-6, CAPES 2177-07-2

01.011

Interactions of some norepinephrine uptake inhibitors with α_1-adrenoceptor subtypes. Nojimoto, F. D.; Pupo, A. S. UNESP - Farmacologia

Introduction: Norepinephrine (NE) uptake inhibitors such as amitriptyline (AMI) and imipramine (IMI) are largely used as antidepressants and many of its side effects are related to the antagonism of α_1-adrenoceptors (α_1-ARs). However, it is unknown whether these uptake inhibitors present selectivity for any of the α_1-ARs subtypes. **Objective**: To investigate the interactions of AMI and IMI with the three α_1-ARs subtypes natively expressed in the rat vas deferens (α_{1A}), spleen (α_{1B}) and aorta (α_{1D}). **Methods**: The vas deferens (RVD), spleen (RS) and thoracic aorta (RTA) from male Wistar rats (16-20 weeks) were maintained in organ baths for digital recording of isometric contractions in response to NE in the absence and presence of increasing concentrations of AMI and IMI. The experiments were done in presence of a cocktail of inhibitors containing cocaine 6 μM, corticosterone 10 μM, yohimbine 0.1 μM or idazoxan 3 μM and propranolol 0.1 μM to block neuronal and extraneuronal uptake, α_2- and □-ARs, respectively) **Results and Discussion**: In RVD, both AMI (0.3 to 10 μM) and IMI (1 to 10 μM) induced rightward shifts in the concentration-response curves (CRCs) to NE, but AMI 10 μM reduced the maximal contraction by ≅25%. The estimated pK_B values for AMI 0.3, 1 and 3 μM (8.0, 7.6 and 7.7, respectly, n= 4) suggest that AMI has significant affinity for α_{1A}-ARs. IMI behaved as a competitive antagonist at the α_{1A}-ARs of the RVD yielding Schild plots with pA_2 = 7.1 ± 0.04 and slope= 0.93 ± 0.09 (n=5). In RS, AMI and IMI were unable to affect the contractions induced by NE, suggesting that they are inactive at α_{1B}-ARs (n=6). In RTA, AMI (0.3 to 3 μM) and IMI (3 to 30 μM) induced rightward shifts in the CRCs to NE. The estimated pA_2 and slope for AMI were 7.05 ± 0.04 and 1.23 ± 0.07, respectively (n=4), and for IMI (n=4) 7.5 ± 0.04 and 1.28 ± 0.05 respectively, indicating competitive antagonism at α_{1D}-ARs**.** **Conclusion**: The results suggest that AMI and IMI are competitive antagonists of α_1-ARs presenting selectivity for α_{1A}- and α_{1D}-ARs subtypes. Both AMI and IMI are inactive at α_{1B}-ARs. It is important to confirm the affinities of AMI and IMI for α_1-ARs subtypes in radioligand binding studies employing recombinant human receptors to confirm the selectivity of these uptake inhibitors. Apoio Financeiro: FAPESP (06/58828-0)

01.012

The role of protein kinase C (PKC) and phosphoinositide 3-kinase (PI3K) pathway in the up-regulation of bradykinin B_1 receptor in the rat portal vein. Basei, F. L.[1]; Cabrini, D. A.[2]; Antunes, V. L.[1]; Nascimento, A. F. Z.[1]; Figueiredo, C. P.[1]; Bader, M.[3]; Medeiros, R.[1]; Calixto, J. B.[1] [1]UFSC - Farmacologia; [2]UFPR - Farmacologia; [3]Max - Delbrück - Center for Molecular Medicine, Berlin, Germany. - Hypertension

Introduction: The bradykinin B_1 receptor (B_1R) is normally absent under physiological conditions, but is highly inducible during inflammatory conditions or following tissue damage. Under prolonged *in vitro* incubation of damaged tissues occur the activation of several intracellular signaling pathways, such as mitogen activated protein kinase (MAPK) and nuclear factor-κB (NF-κB), and consequently the increase of B_1R expression (*Medeiros et al., Circ Res 94*:*1375, 2004*). Previously, we have demonstrated the participation of PKC and PI3K on B_1R expression in the rat portal vein following tissue injury (Basei, FL; *39º Cong Bras Farmacol Ter Exp*, p.65, 2007). Herein, we attempted to further evaluate the involvement of these intracellular pathways on B_1R expression in the rat portal vein following tissue injury. **Methods**: Portal vein was isolated from normal rats and incubated *in vitro* in the absence or in the presence of PI3K (Ly294002, 10 μM or Wortmannin, 10 nM) or PKC (GF109203x, 1 μM or Ro318220, 10 μM) selective inhibitors. The induction of B_1R expression following tissue damage and *in vitro* incubation was assessed through the analysis of des-Arg^9-BK-induced contraction. Also, changes in the B_1R mRNA expression were evaluated using quantitative RT-PCR. The effect of PI3K and PKC inhibitors on tissue damage-induced NF-kB, c-Jun and CREB activation was assessed by immunohistochemistry. Finally, the profile of PI3K and PKC activation was evaluated by western blotting. **Results**: The PKC (GF109203x or Ro318220) and PI3K (Ly294002 or Wortamannin) inhibitors significantly and selectively reduced the contractile response caused by the B_1R agonist des-Arg^9-BK, as well as the B_1R mRNA expression following tissue damage and *in vitro* incubation. Under these conditions we found the activation of transcriptional factors CREB, AP-1 and NF-κB (5-, 1.5- and 2-fold compared with basal group, respectively). Either GF109203x or Ly294002 inhibitor significantly decreased AP-1 activation induced after tissue damage and incubation *in vitro* (77 ± 6 and 90 ± 6%, respectively). Moreover, both inhibitors decreased the CREB activation at basal levels. Finally, the tissue damage in the rat portal vein elicited a time-dependent activation of both PKC and PI3K pathways. **Conclusions**: Altogether these results indicate that tissue damage following *in vitro* incubation of the rat portal vein induces the activation of PI3K and PKC pathways which, consecutively, play a critical role in the up-regulation on the B_1R expression. Apoio Financeiro: CNPq, CAPES and FAPESC.

01.013

Efeito do tratamento agudo com agonista de adrenoceptor β_2 e com inibidor inespecífico de fosfodiesterase na concentração plasmáticas de AMP cíclico em ratos. Aparecida-Santos, E.; Birbrair, A.; Bergantin, L. B.; Andrade-Lopes, A. L.; Chiavegatti, T.; Godinho, R. O. UNIFESP – Farmacologia

Introdução: O AMP-cíclico (AMPc) modula diversos processos fisiológicos. Estudos anteriores do nosso grupo demonstraram a existência de um transportador capaz de promover o efluxo do AMPc da fibra muscular esquelética, sugerindo que este poderia funcionar como pró-hormônio e exercer ações autócrinas e/ou parácrinas através do seu metabólito adenosina (via extracelular AMPc - adenosina) (Godinho & da Costa, 2003; Chiavegatti *et al*., 2008). Embora vários medicamentos tenham como principio ativo fármacos que aumentam o AMPc intracelular, não há estudos avaliando seus efeitos no efluxo do AMPc. Assim, este trabalho teve como objetivo determinar o efeito das drogas utilizadas na terapêutica do fenoterol (agonista de adrenoceptor β2) e da pentoxifilina (inibidor não-seletivo de fosfodiesterases) na concentração plasmática de AMPc. **Métodos**: Ratos Wistar machos adultos foram submetidos a gavagens diárias com água (10 ml/kg), durante 14 dias. No 15º dia, os animais foram tratados com salina (n=7), fenoterol (10 mg/kg, n=6) ou pentoxifilina (25 mg/kg, n=6). Após 2 h os animais foram anestesiados, o sangue foi coletado da aorta abdominal (3 mL) e o AMPc plasmático foi quantificado por método radiométrico. A atividade da acetilcolinesterase (AChE) plasmática, determinada por ensaio colorimétrico, foi usada como controle interno dos ensaios. **Resultados**: Ao final de 14 dias de adaptação, não houve variação significante no peso dos animais (294,0 ± 8,3 g). O tratamento agudo por 2 h com pentoxifilina não alterou a atividade da AChE nem a concentração plasmática de AMPc, em relação aos valores controles (AChE = 7593 ± 314 UA/min/ml; AMPc = 2,2 ± 0,2 pmoles/mL). Por outro lado, o tratamento agudo com fenoterol aumentou em 211% a concentração plasmática de AMPc, sem modificar a atividade da AChE. **Discussão**: Os resultados obtidos demonstram que a ativação da adenilil ciclase pelo agonista de adrenoceptor β2 fenoterol aumenta a concentração plasmática de AMP cíclico. Considerando as características hidrofóbicas do AMPc cuja formação é exclusivamente intracelular, nossos dados demonstram a existência do efluxo deste nucleotídeo cíclico *in vivo*, indicando a possível ativação da via extracelular AMPc - adenosina. A ineficácia da pentoxifilina pode ser justificada tanto pela administração aguda como pela baixa dose baixa utilizada, já que seus efeitos terapêuticos são evidenciados após tratamento crônico. Estudos complementares serão realizados para determinar o efeito do tratamento sub-crônico dessas drogas nas concentrações dos metabólitos do AMP cíclico (AMP e adenosina) no plasma. Apoio Financeiro: FAPESP e CNPq

01.014

Mutagenesis in Angiotensin II AT_1 receptor reveals critical structural features for activation of different signaling pathways. Reis, R. I.[1]; Restini, C. A.[2]; Santos, E. L.[3]; Pecher, C.[4]; Schanstra, J.[4]; Bascands, J. L.[4]; Bendhack, L. M.[5]; Pesquero, J. B.[3]; Costa-Neto, C. M.[1] [1]FMRP-USP - Bioquímica e Imunologia; [2]USP - Física e Química; [3]UNIFESP - Biofísica; [4]Inserm U388 - Université Paul Sabatier; [5]FCFRP- SP

In addition to the classical signaling pathway by activation of G protein, the binding of AngII to the AT1 receptor also leads to activation of mitogen-activated protein kinase (MAPK) pathways, such as ERK1/2 phosphorylation. Up to date, studies of AT1 structural requisites involved in activation of different functional pathways are still limited. We have performed single mutations in the transmembrane helixes of the AT1 receptor aiming at to comparatively investigate the behavior of these mutants concerning the activation of distinct signaling pathways. Wild type and mutant-transfected cells were used to perform extracellular acidification rate, calcium mobilization and ERK1/2 phosphorylation assays. Our results showed that although some of the mutants were not able to induce neither extracellular acidification nor intracellular calcium mobilization, the same mutants were fully capable of inducing ERK phosphorylation. Our data contribute to provide evidence that different structural features from the receptor are required to trigger distinct functional events. Apoio Financeiro: FAPESP, CAPES, CNPq, FAEPA

01.015

Analysis of kallikrein-kinin system participation in induced muscle atrophy. Parreiras-e-Silva, L. T.[1]; Gomes, M. D.[1]; Godinho, R. O.[2]; Costa-Neto, C. M.[1] [1]FMRP-USP - Bioquímica e Imunologia; [2]UNIFESP - Farmacologia

The kallikrein-kinin system (KKS) is classically involved in blood pressure control and regulation of circulatory homeostasis. After binding to their receptors, B_2 or B_1, kinins also participate in inflammatory response through activation of NFκB, which induces expression of cytokines. Muscle atrophy is a multifactorial process and the molecular mechanisms involved in it depend on the type of muscle tissue and associated pathologies. Muscle fibers loose mass due to induction of catabolic signals mediated by the ubiquitin-proteasome system, other proteolytic enzymes and also by inflammatory cytokines. Therefore, we decided to analyze participation of the KKS in induced levator ani muscle and gastrocnemius models of atrophy. To induce levator ani muscle atrophy, Wistar rats and Balb-C mice were gonadectomized, euthanized at 2, 7, 15 and 30 days after surgery, and then the levator ani muscles were collected. Muscles from non-gonadectomized rats were used as controls. Total RNA from each muscle was extracted, cDNA was produced and relative expression of B_2 and B_1 mRNAs were analyzed by PCR. The expression level of atrogin-1, an E3-ubiquitin ligase from the ubiquitin-proteasome pathway was used as a molecular marker of atrophy. Expression of these genes was also analyzed in a murine model of atrophy induced by fasting during 2 days. Gastrocnemius muscles from fasted mice were extracted and processed as described above. We showed that the expression level of B_2 receptor mRNA was not altered during atrophy, whereas the B_1 receptor mRNA was up regulated at second day after castration, reaching a peak at the seventh day in mice; and at the fifteenth day in rats. These data suggest the participation of B_1 kinin receptor in muscle atrophy, possibly due to an activation of NFκB, induction of inflammatory cytokines and also components of the ubiquitin-proteasome system, which are currently being investigated. Apoio Financeiro: FAPESP, CAPES, CNPq, FAEPA

01.016

Estudo de alterações no sistema adrenérgico da musculatura lisa de ratos por tratamento crônico com anfetamina e etanol. Mendes, C. S.; Jurkiewicz, A.; Zanuto, J. G. C.; Jurkiewicz, N. H. - UNIFESP Farmacologia

Introdução: A interação entre anfetamina (AMPH) e etanol (ETOH) no sistema adrenérgico periférico (SAP) tem sido pouco estudada, apesar dessas drogas serem largamente utilizadas em associação para fins não terapêuticos. Há indícios de que existe interação entre AMPH e ETOH no aumento de risco de dependência (Hajnalka *et al*., Silveria *et al*.), mas não há indícios de que em nível periférico elas possam interagir modificando seus efeitos sobre o sistema adrenérgico. O sistema nervoso adrenérgico periférico desempenha papel fundamental na mediação de importantes mecanismos fisiológicos como do processo de contração do ducto deferente (Pupo *et al*.) que é fundamental para o processo da ejaculação (Tomasi *et al*.), da ativação do eixo hipotalâmico-adrenal-pituitário (Feldman *et al*.) e também da regulação da expressão de receptores de membrana (Quintas *et al*.). O objetivo principal desse estudo foi verificar se o SAP é influenciado pelo tratamento simultâneo com AMPH e ETOH. Para isso estudamos a resposta da musculatura lisa do ducto deferente ao bário, noradrenalina, dopamina, fenilefrina e tiramina *in vitro* após o tratamento crônico *in vivo* de ratos com AMPH e ETOH. **Métodos**: utilizamos ratos "Wistar" de 35 dias de idade, que foram tratados ao mesmo tempo com ETOH (3 ml/kg a 25%, gavado) e AMPH (5 mg/kg, subcutânea) ou com somente uma dessas drogas, durante 10 dias. Aos 45 dias de idade, os animais eram sacrificados e tinham os ductos deferentes retirados para estudo de contração isométrica, no sistema PowerLab, induzida pelos agonistas supracitados. Cada grupo tinha seu correspondente grupo controle. **Resultados**: verificamos que o tratamento simultâneo com AMPH e ETOH modificou o efeito máximo do bário, noradrenalina e fenilefrina, porém, não modificou a afinidade aparente de nenhuma das drogas utilizadas. Encontramos também uma diminuição significativa nos estoques endógenos de noradrenalina para o grupo tratado com ETOH e AMPH, porém esse resultado foi o mesmo do grupo tratado apenas com AMPH. O tratamento com ETOH apenas, não modificou nenhum dos parâmetros estudados. **Discussão**: os resultados podem indicar que o uso simultâneo do ETOH e AMPH não aumenta o risco de alterações do SAP, comparado ao uso da AMPH, somente, mas pode provocar modificações dose-dependente na neurotransmissão desse sistema. **Referências Bibliográficas**: Feldman, S.; *Brain Research Bulletin*, v.52(6); 531; 2000. Hajnalka A.. J.; *Devl. Neuroscience*, v.25; 265; 2007. Pupo, A. S.; *Annals New York Academy of Sciences*, USA, v.812; 290; 1997. Quintas, L. E.; *Biochem. Pharmacol*., v. 64; 1431; 2002. Silveira, M. M.; *Alcohol*, v.32; 145; 2004. Tomasi, P. A.; J. *Impotence Research*, v.17; 297; 2005. Apoio Financeiro: CNPq

01.017
PREMIO INOVAÇÃO

01.018

Functional vascular alterations induced by schistosomiasis. Oliveira, S. D. S.[1]; Farsky, S.[2]; Waisman, K.[2]; Noel, F.[1]; Silva, C. L. M.[1.] [1]UFRJ - Farmacologia Celular e Molecular; [2]USP - Análises Clínicas e Toxicológicas

Introduction: Schistosomiasis is an intravascular disease caused by *Schistosoma mansoni,* that is related to an endothelial dysfunction of large vessels (Silva *et al,.* 2007. *Vasc. Pharmacol*. 46:122). The objective of this study was to evaluate if the disease alters the aortic contraction induced by 5-HT and ET-1, both agonists involved in the physiopathology of schistosomiasis. Another aim of the present work was to investigate the function of endothelial cells from microcirculation. **Methods**: *Isometric tension*: Control (CO) and infected (INF) Swiss mice were anaesthetized, killed and the aorta was removed. Aortic rings were fixed to an isometric transducer and immersed in an aerated Krebs solution (KS; 37ºC). A first contraction was induced with phenylephrine (1 uM) and at the plateau acetylcholine (1 uM) was added to access the endothelium-dependent relaxation (Silva *et al,.* 2007. *Vasc. Pharmacol*. 46:122-128). One hour after washing, it was induced a second contraction using 5-HT (10^{-9}-10^{-5} M), ET-1 (10 nM, in the presence of L-NNA 250 uM) or caffeine (32 mM, in the presence of a zero-Ca^{2+} KS containing 0.1 mM EGTA). *Endothelial cell culture*: Mesenteric vessels were removed, washed in DMEM, cut into small segments and immersed in DMEM enriched with 20% fetal calf serum. Confluent cells (first passage) were used to NO measurements using the fluorescent indicator DAF-FM (2.5 uM), in the absence (basal) and presence of ATP (100 uM) or bradykinin (1 uM) for 45 min (Silva *et al*., 2007, *Br. J. Pharmacol*. 151:195). *Intravital microscopy*: Animals were anaesthetized, the cremaster muscle was exposed and moisted with a warmed Ringer-Locke solution containing 1% gelatin. Transilluminated images were obtained with an Axioplan optical microscopy. In addition animals were treated with LPS (1 mg/kg i.p.) and 3h later the peritoneal exsudate was collected to determine leukocyte number. **Results and Discussion**: The 5-HT-induced contraction was greater in the INF (15.4+/-0.75 mN, n=10) than in the CO group (6.5+/- 0.6 mN, n=6, $P<0.05$). These data are similar to the one observed with noradrenaline (Silva *et al.* 2007. *Vasc. Pharmacol*. 46:122). Caffeine also induced a greater contraction in the INF than in the CO animals (2.16 +/- 0.25 and 1.04 +/- 0.08 mN, respectively, n = 10, $P<0.05$), suggesting that the aortic Ca^{2+} mobilization is enhanced in INF animals. These data are compatible with previous results where the inhibition of Ca^{2+} mobilization (10 uM ryanodine) reduced 5-HT-induced contraction only in the INF group. On the other hand, the contraction induced by ET-1 was similar in both groups (CO: 5.82 +/- 0.49 mN (10), INF: 4.75 +/- 0.8 mN (8)). The blood flow is largely impaired in the INF animals suggesting an endothelial dysfunction. In this group the leukocyte migration in response to LPS challenge was higher than in the CO group (20.9 +/- 2.15 and 3 +/- 0.45 x 10^6 cells, respectively, $P< 0.05$). In conclusion, these results suggest that the contractile vascular alterations induced by schistosomiasis are specific for some receptors, and also that the disease exerts a pro-inflammatory status that potentiates the LPS effect. Apoio Financeiro: FAPERJ, CNPq

01.019

Efeito da ciclosporina A e do artemether no *Schistosoma mansoni*: agente causador da esquistossomose no Brasil. Gonçalves, J. P.[1]; Azevedo, R. P.[1]; Peruchetti, D. B.[1]; Ribeiro, M. C.[1]; Valverde, R. H. F.[2]; Vieyra, A.[2]; Lanfredi, R.[2]; Cunha, V. M. N.[1] [1]ICB-UFRJ - Farmacologia Celular e Molecular; [2]IBCCF-UFRJ

Introdução: Ciclosporina A (CsA) é um fármaco imunossupressor enquanto Artemether (ART) é eficaz no tratamento da malária. Sabe-se que a CsA e seus análogos com baixa atividade imunossupressora demonstram elevada atividade contra o *S. mansoni* e o ART contra infecções causadas por *S. japonicum*. O objetivo deste trabalho foi avaliar o efeito da CsA e do ART na atividade das bombas de Ca^{2+} presentes no parasito, relacionando-os com processos dependentes de Ca^{2+}, assim como, a motilidade e o comprimento do corpo dos vermes adultos machos de *S. mansoni*. **Métodos**: Vermes adultos machos foram recuperados de camundongos suíços após 45 dias de infecção. Grupos de 5 vermes foram utilizados para observação dos efeitos da CsA 100 µM e ART 335 µM por 30 min. Para a análise da sobrevida do *S. mansoni* em cultivo na presença de CsA e ART, grupos de 10 vermes adultos foram mantidos em meio de cultura RPMI 1640 a 37°C e 5% CO_2 durante 10 dias. A morfometria dos parasitos e a microscopia eletrônica de varredura foram realizadas após 15 min de incubação em meio de Tyrode ou morte dos vermes em cultivo (Mafra & Lanfredi, *J. Parasitol*. 84(3):582, 1998). A atividade SERCA ATPásica foi determinada de acordo com Cunha *et al*., Comp. Biochem. Physiol. 114B(2): 199, 1996. **Resultados**: Após 15 min de incubação em meio de Tyrode, ART aumentou a motilidade e o comprimento do corpo dos vermes ($P<0,05$; n=12; Rank Sum test). Em cultivo, os vermes incubados com CsA ou Praziquantel 1 µM sobreviveram 4 dias e com ART, 6 dias. A análise morfométrica revelou que ART alongou o corpo dos vermes tanto em meio de Tyrode quanto em meio de cultura ($P<0,05$; n=20; One Way ANOVA) e CsA 100 µM não alterou o comprimento deste parasito. Na fração subcelular P_4, CsA aumentou a razão de contribuição da atividade Ca^{2+}-ATPásica resistente em relação à sensível a tapsigargina (TgR/TgS). CsA aumentou o nível de fosforilação da Ca^{2+}-ATPase resistente à Tg detectada na região de 110 kDa e danificou estruturas de superfície localizadas no tegumento do verme. Já ART 335 µM estimulou a atividade Ca^{2+}- ATPásica sensível à Tg (TgS) em relação ao seu controle ($P<0,05$; n=3; Teste *t* pareado). **Conclusões**: Esses dados mostram que a CsA e o ART são capazes de matar os vermes adultos machos de *S. mansoni* em cultura até 6 dias de incubação; o alvo molecular da CsA é provavelmente uma Ca^{2+}-ATPase localizada no tegumento do *S. mansoni* semelhante a SPCA de mamíferos; e, como o ART parece estimular a atividade SERCA ATPásica(TgS), é possível que esta ação contribua para o relaxamento muscular do verme adulto macho promovido por este fármaco. Apoio Financeiro: CAPES, FAPERJ

01.020

Melatonin inhibits lipopolysaccharide (LPS) induced-relaxation of rat aorta by blocking activation of the transcription factor nf-κb in endothelial cells. Tamura, E. K.[1]; Cecon, E.[1]; Silva, C. L. M.[2]; Markus, R. P.[1] [1]IB-USP- Fisiologia; [2]UFRJ - Farmacologia Básica e Clínica

Introduction: Melatonin (MEL) inhibits constitutive (TAMURA, J Pineal Res, 41:267, 2006) and LPS-induced nitric oxide (NO) production in primary cultures of rat endothelial cells. LPS-induced vasodilation is mediated by NO, which is produced by inducible nitric oxide synthase (iNOS) via activation of the NF-kB pathway (Kleinert, *Eur J Pharmacol*, 500:255, 2004). MEL blocks NF-kB activation in murine macrophages (Gilad, *FASEB J*, 12:685, 1998). Here we investigated whether MEL inhibition NF-kB nuclear translocation in endothelial cells may lead to the reduction of rat aorta relaxation induced by an endothelial-dependent LPS effect. **Methods**: Effect of MEL (1 nM, 1 and 10 microM, 1 min) on LPS (1 mg/ml)-induced nuclear translocation of NF-kB in primary cultured endothelial cells, obtained according to TAMURA (J Pineal Res, 41:267, 2006), was measured by electromobility shift assay (EMSA). Effect of MEL (10 microM, 10 min) on the relaxation of rat aortic rings (3 mm) endothelium-intact and endothelium-denuded pre-contracted with phenylephrine (10 microM) was accessed by recording isometric contraction (SILVA, Brit J Pharmacol, 135:293, 2002). Experiments were done in the presence or absence of PDTC (25 microM, 1h), an inhibitor of the NF-kB pathway. LPS was added in the peak of the vasoconstriction, and the tension was recorded for 2 h. **Results**: LPS only induced relaxation in endothelium-intact rings. The maximal relaxation in the absence and presence of LPS was 48 ± 3.1% (N= 13), and 74.8 ± 4.2% (N= 14), respectively. MEL 10 microM (-25.4 ± 15.3, n= 3), but not 1 nM and 1 microM reduced totally the amount of LPS-induced nuclear translocation of NF-kB (133.9 ± 50%, N= 3). MEL (45.6 ± 6.4%, N= 10) and PDTC (29 ± 3.5%, N= 8) significantly inhibited the LPS-induced relaxation. **Discussion**: Here we put together two different approaches to prove that LPS-induced vasodilation is blocked by melatonin due to its inhibition of the NF-kB pathway. The fact that melatonin blocks the increase in nuclear concentration of NF-kB induced by LPS, and that both melatonin and the inhibitor of NF-kB impairs LPS-induced relaxation of arterial rings with endothelium are the basis for our conclusion. Finally, we propose that the effect of melatonin on vascular reactivity is one of the mechanisms that underlay the protective effect of this indolamine against LPS. Apoio Financeiro: CAPES, CNPq and FAPESP

01.021

Effect of TAK-778 on resorption activity of osteoclastics cells derived from human bone marrow. Bellesini, L. S.[1]; Oliviera, F. S.[2]; Crippa, G. E.[2]; Rosa, A. L.[2] [1]FMRP - Farmacologia; [2]FORP - Cirurgia e Traumatologia Buco-Maxilo-Facial e Periodontia

Introduction: Drugs prescribed in the treatment and prevention of bone loss can stimulate the bone formation or inhibit the resorption. There are evidences that TAK-778 enhances bone formation at least partially by stimulating osteogenesis. However, until now none is know about the effect of TAK-778 on osteoclast, so the aim of the present study was to investigate the influence of TAK-778 on *in vitro* osteoclastic activity of cells differentiated from human bone marrow. **Methods**: Mononuclear cells were isolated from human bone marrow by the Ficoll-Paque method and were cultured in 96-well culture plate (8×10^4 cells/well) and in BD BioCoat Osteologic MultiTest Slides (10^5 cells/well) in the presence of recombinant M-CSF, soluble RANK ligand and 1,25-$(OH)_2D_3$ for 18 days. At day 18^{th}, TAK-778 was added to the culture medium and the cells were cultured until 21^{st} day. The osteoclastic differentiation was confirmed by tartrate-resistant acid phosphatase (TRAP) staining. Resorbing activity was evaluated by images analysis considering the resorption pits area. The experiments were done in triplicate and submitted to Mann-Whitney test. **Results**: Image analysis indicated the formation of TRAP positive multinucleated osteoclasts in control and TAK-778 group of cells. There was no difference between the resorption activity of osteoclasts cultured in the presence or absence of TAK-778 ($p>0.05$). **Conclusion**: The TRAP positive staining and resorption activity confirmed the osteoclastic phenotype expression in the cultures. However, the resorption activity of osteoclasts was not affect by TAK-778. **Acknowledgements**: FAPESP (06/03402-9 and 06/02868-4) for financial support and Takeda Chemical Industries for TAK-778 supplied. Apoio Financeiro: FAPESP (06/03402-9 and 06/02868-4)

01.022

Effect of purmorphamine on gene expression of human alveolar bone cell culture. Oliveira, F. S.; Crippa, G. E.; Bellesini, L. S.; Beloti, M. M.; Rosa, A. L. [1]FORP - Cirurgia e Traumatologia Buco-Maxilo-Facial e Periodontia

Introduction: Purmorphamine induces osteogenesis in mesenchymal progenitor cells. The expression of the bone transcription factor RUNX2 (Runt-related transcription factor 2) and the bone marker ALP (alkaline phosphatase) are upregulated by purmorphamine in osteoblastic cells. This study investigated the effect of purmorphamine on osteogenesis in cultures of human osteoblastic cells derived from alveolar bone fragments. **Methods**: This experiment was carried out using cells from one adolescent donor obtained under approval of Committee of Ethics in Research. Osteoblastic cells were obtained from explants by enzymatic digestion and cultured in osteogenic medium. Subconfluent cells in primary culture were enzymatically harvested and first passage cells were subcultured in 25 cm^2 tissue culture flasks (2.4 x 10^4 cells/flask) for 7 days. Cells were exposed to purmorphamine (2 µM) or vehicle (dimethyl sulphoxide) for 7 days, and for the last 4, 3 and 2 days of subculture. At 7 days, gene expression of ALP, osteocalcin (OC), osteopontin (OPN), RUNX2 and Msh homeobox 2 (MSX2) were evaluated. All experiments were done in triplicate and submitted to Mann-Whitney test. **Results**: Gene expression of: RUNX2 was upregulated at 2, 3 and 4 days (1.27-fold), and at 7 days (1.79 days). OPN was upregulated at 2 (1.70-fold), 4 (0.87-fold) and 7 days (1.65-fold). MSX2 was upregulated only at 2 days (1.38-fold) and ALP only at 4 days (1.07-fold). OC was not affected at all. **Discussion**: These results suggest that purmorphamine stimulates the expression of osteoblastic markers in human alveolar bone cells. Acknowledgements: FAPESP for financial support. Apoio Financeiro: FAPESP

01.023

Modulation of the contraction of rat vas deferens by TRPM8 activation. Vladimirova, I.[1]; Philyppov, L.[1]; Kulieva, E.[1]; Sotkis, A.[2]; Boldyrev, A.[2]; Naidenov, V.[1]; Jurkiewicz, A.[3]; Shuba, Y.[2] [1]Bogomoletz - Physiology; [2]International Center of Molecular Physiology NASU; [3]UNIFESP – Farmacologia

Introduction *and Methods*: The influence of menthol (100 uM) on the contractile responses evoked by high potassium (60 mM), noradrenaline and carbachol were examined in the smooth muscle preparations from prostatic and epididimal portions of the rat *vas deferens.* **Results and Discussion**: All agonist-induced contractions were greatly increased in the prostatic and suppressed in the epididimal portions of the *vas deferens* from orchidectomized rats as compared with those from normal animals. Menthol did not change the basal tonus of isolated strips of the *vas deferens,* but decreased the magnitudes of agonist- and KCl-induced contractions in both groups of animals. The same effects were observed with more specific agonist of TRPM8 channel, icilin (10 uM) suggesting TRPM8 involvement. The inhibitory action of menthol and icilin was not dependent on the presence of the epithelium. Real-time RT-PCR showed two-fold higher TRPM8 expression in the smooth muscle tissue (without epithelium) from epididimal vs. prostatic parts of the *vas deferens* of control rats. Following orchidectomization TRPM8 expression increased 1.5-fold in epididimal portion and almost 3-fold in the prostatic portion. The enhancement of TRPM8 expression was paralleled by the increase in androgen receptor expression. **Conclusion**: We conclude that TRPM8 activation modulates contractility of the *vas deferens* in androgen-dependent manner, which may play role in the control of its physiological contractile function, however, the mechanism of such modulation needs further investigation. Keywords: *Vas deferens,* Contraction, TRPM8, Menthol, Icilin Apoio Financeiro: Supported in part by INTAS 05-1000008-8223 grant.

01.024

Influência da idade sobre a monoamino oxidase (MAO) no sistema nervoso periférico de ratos.

Zanuto, J. G. C.; Mendes, C. S.; Jurkiewicz, N. H.; Jurkiewicz, A. UNIFESP - Farmacologia

Introdução: O presente estudo tem por objetivo verificar os possíveis efeitos do tratamento agudo e semi-agudo *in vivo* com inibidores da MAO (pargilina e iproniazida) que atuam como perturbadores sobre a reatividade farmacológica da musculatura lisa de ratos. **Métodos**: Estudamos a contração muscular induzida *in vitro* por drogas adrenérgicas (noradrenalina), na presença e na ausência de antagonistas (prazosin, fentolamina, WB4101). Primeiramente foi feita uma curva de noradrenalina; em seguida utilizamos bloqueadores de captação neuronal e extraneuronal e adrenoceptores beta que poderiam interferir no resultado, para estudarmos os adrenoceptores alfa, também fizemos o deslocamento com os antagonistas. Através destas curvas analisamos parâmetros de afinidade aparente como pA_2 (para antagonistas) e pD_2 (para agonistas), alem do efeito contrátil produzido pelo agonista indireto tiramina. Utilizamos ratos de 30, 90 e 150 dias, tratamentos: (1) Tratamento agudo, com animais tratados com uma dose de 100 mg/kg/dia de pargilina (IMAO B) ou iproniazida (IMAO A). (2) Tratamento semi-agudo de animais tratados por três dias com dose de 20 mg/kg/dia de pargilina (IMAO B) ou iproniazida (IMAO A). Todos os grupos controle foram tratados com solução salina. Os animais foram sacrificados duas horas após a última dose. **Resultados**: Os dados são referentes a animais tratados com iproniazida. Primeiramente nos animais de 90 dias: o tratamento agudo (1) revelou diferenças estatisticamente significantes (DES) nos parâmetros pA_2 (controle: 7,78 ± 0,13, tratado: 8,20 ± 0,11 $p < 0,05$)e pD_2 (controle: 6,31 ± 0,11, tratado: 7,08 ± 0,04, $p < 0,05$). Os animais de 150 dias submetidos ao tratamento agudo (1) apresentaram DES nos parâmetros pD_2 (controle: 6,20 ± 0,11, tratado: 7,75 ± 0,14 $p < 0,05$) e tiramina (controle: 53,6 ± 4,71, tratado: 76,40 ± 7,52, $p < 0,05$). Nos tratamentos semi-agudos (2): Tivemos DES para os parâmetros pA_2 (controle: 8,28 ± 0,03, tratado: 7,93 ± 0,12 $p < 0,05$)e pD_2 (controle: 6,43 ± 0,20, tratado: 7,49 ± 0,22, $p < 0,05$) e também na tiramina (controle: 45,30 ± 3,38, tratado: 60,70 ± 2,54 $p < 0,05$) dos animais de 90 dias. **Discussão**: Os resultados revelaram que os tratamentos com IMAO afetam a resposta adrenérgica do sistema nervoso periférico. No entanto os IMAO A apresentam influência maior que os IMAO B. A inibição da degradação das catecolaminas por IMAOs resultou em uma resposta adrenérgica maior aos estímulos e um aumento da sensibilidade à noradrenalina. A diferença de idade nos casos já analisados, 90 e 150 dias, não mostrou diferenças, porém em experimentos prévios vimos que em animais de 30 dias temos alterações nas respostas. Na continuação desse trabalho faremos dosagens de catecolaminas e caracterização por biologia molecular dos adrenoceptores alfa do DD. Apoio Financeiro: CNPq

01.025

Transcriptome analysis of *Bothrops alternatus* snake venom gland, with emphasis on metalloproteinases. Cardoso, K. C.[1]; Silva, M. J.[2]; Lacerda G. G. L.[2]; Menossi, M. T.[2]; Hyslop, S.[1] [1]UNICAMP – Farmacologia; [2]UNICAMP – Engenharia genética

Introduction: Snake venom glands produce a complex mixture of proteins (mainly enzymes) and peptides. In this work, we constructed a cDNA library of the venom gland from the snake *Bothrops alternatus* to analyze the transcriptional activity and general venom composition. **Methods**: The venom glands were dissected from adult snakes three days after venom milking. Total RNA was extracted using Trizol reagent (Invitrogen), and a cDNA library was produced using CloneMiner cDNA library kits (Invitrogen). The cDNA isolated from colonies was sequenced (ABI 3700 Applied Biosystems) using M13 forward primers. The ESTs (expressed sequence tags) were analyzed using appropriate bioinformatics tools in combination with BLAST annotation. **Results**: EST analysis revealed a mixture of venom and cellular proteins, the latter associated mainly with general metabolism, cytoskeleton and protein biosynthesis and degradation. The venom components consisted predominantly of snake venom metalloproteinases (SVMPs), bradykinin-potentiating peptides, phospholipases A_2, serine proteinases and C-type lectins. Class PIII metalloproteinases were the most abundant group of enzymes and showed high similarity with metalloproteinases (HF2, HF3, bothropasin and jararhagin) from *Bothrops jararaca* venom gland, and metalloproteinase from *Echis ocellatus*, halysase from *Gloydius halys,* and a metalloproteinase-disintegrin-like protein from *Agkistrodon contortrix laticinctus*. **Conclusions**: Class PIII metalloproteinases were the most abundant venom proteins in the *B. alternatus* cDNA library. The predominance of SVMPs in this analysis agreed with the marked hemorrhagic activity of this venom. Apoio Financeiro: CNPq, FAPESP and UNICAMP.

01.026

Efeitos pré- e pós-sinápticos de inibidores da acetilcolinesterase na neurotransmissão em musculatura lisa. Pereira, J. D.; Caricati-Neto, A.; Jurkiewicz, A.; Jurkiewicz, N. H. [1]UNIFESP – Farmacologia

Objetivos: O presente trabalho aborda os efeitos de inibidores de acetilcolinesterase (AChE) na reatividade farmacológica e neurotransmissão em ducto deferente de rato. O protocolo do trabalho envolve a comparação de novos anticolinesterásicos sintetizados pelo grupo do Professor Antônio Garcia, da Universidade Autônoma de Madri. O novo composto, chamado de 12118 possui estrutura híbrida, derivada da molécula do anticolinesterásico tacrina e da 1,4 diidropiridina nimodipina, inibidor de canal de cálcio do tipo L (Marco-Conteles *et al*., J. Medic. Chem. 49, 7607-7610, 2006). O objetivo da síntese foi obter fármacos mais potentes como candidatos para o tratamento da Doença de Alzheimer, utilizando a estratégia já existente de inibição de colinesterase. **Materiais e métodos**: O experimento para mensurar a ação dos anticolinesterásicos fisostigmina, tacrina 12118 e de nimodipina em ducto deferente de rato consistiu na realização de uma curva dose-resposta para bário, duas para acetilcolina (ACh) e duas curvas para ACh com incubação prévia respectivamente por 10 ou 60 minutos com o inibidor de AChE. Foram avaliados os parâmetros farmacológicos efeito máximo (Emax), dose eficaz 50 (DE50), afinidade dos agonistas (pD_2), relação de doses (Dose Ratio, DR) para avaliar o deslocamento para a esquerda (potencialização) da resposta da ACh pelo inibidor de AChE e responsividade relativa (rô) para avaliar o efeito máximo de ACh. Para estudar o papel do cálcio foram feitas curvas cumulativas para cálcio em solução nutritiva livre de cálcio na presença dos inibidores de AChE ou nimodipina. Foram feitas curvas por estimulação transmural a 60V e 3ms, nas freqüências de 0.1, 0.5, 1, 2, 5 10 e 20 Hz, a fim de estudar o efeito sobre a contração neurogênica. Além disso, foi medida a atividade da enzima AChE na presença dos inibidores, conforme método de Ellman modificado em microplaca para confirmar a inibição por cada composto. **Resultados**: Observou-se maior potenciação das curvas de ACh pela fisostigmina, seguido da tacrina e 12118. Entretanto, o Emax das curvas de ACh caiu drasticamente após a fisostigmina (C= 1,40 ± 0,20 g, F= 0,60 ± 0,07 g) e 12118 (C= 1,30 ± 0,20 g, 12118= 0,60 ± 0,01 g). Os experimentos para Ca^{+2} evidenciaram apenas uma inibição significativa com a nimodipina (controle) e 12118. Em relação à contração neurogênica, observamos somente bloqueio da fase tônica por 12118. Os experimentos pelo método de Ellman ainda não são conclusivos. **Conclusões**: A potenciação da ACh é coerente com o mecanismo de inibição da AChE pelos antagonistas. Entretanto ainda merece explicação a redução observada no Emax. Conforme os experimentos de contração, o composto 12118 parece ser também inibidor de canal de cálcio além de anticolinesterásico. Apoio Financeiro: Capes, CNPq

01.027

Different pattern of activation induced by aspirin-triggered lipoxins in mononuclear cells. Da-Fe, A. R.[1]; Cezar-de-Mello, P. F. T.[1]; Villela, C. G.[1]; Barja-Fidalgo, T. C.[2]; Fierro, I. M.[2] [1]UERJ - Farmacologia e Psicobiologia; [2]UERJ - Farmacologia

Introduction: Monocytes are circulating peripheral blood cells involved in a wide range of pathophysiological events including the inflammatory process in the vascular wall that can lead to atherosclerosis. Lipoxins (LX) are arachidonic acid metabolites generated during cell-cell interactions under a variety of conditions. These lipids and their stable analogs bind to a specific receptor named ALX displaying potent inhibitory actions in several key events in inflammation. In this work, we investigated whether ATL-1, a stable 15-epi-LXA_4 analog, could modulate different processes of monocyte activation. **Methods**: U937 cells, a monocytic cell line, were cultured in RPMI-1640 medium with 10% FBS, and maintained in growth by passage every 2-3 days. The viability of the cells (97-98%) was tested by trypan blue exclusion procedure. ALX expression was evaluated using RT-PCR analysis and western blot assay. Cultured cells were incubated with vehicle or ATL-1 (1-100 nM) for 15 min to investigate ERK-2 and p38 MAPK activation, which was detected by western blot analysis. The induction of HO-1 after treatment of the cells with the analog (1-100 nM) was analyzed by both RT-PCR and western blot assays. **Results**: The presence of a functional LX receptor in U937 cells was confirmed with protein expression associated with mRNA levels. ATL-1, in a concentration-dependent manner, stimulated ERK-2 phosphorylation, a MAPK involved in the proliferation and cell survival. In contrast the p38 MAPK pathway in monocytes appears not be modulated by lipoxins. Additionally, the incubation of the cells with the analog for different periods of time (1-12h) induced the expression of the stress-inducible enzyme heme oxygenase (HO)-1, which has been shown to play important roles in the modulation of inflammation with anti-oxidative and anti-apoptotic properties. **Conclusion**: Recruitment of monocytes into atherogenic foci is required for the onset and progression of atherosclerosis. A better knowledge of the intracellular mechanisms modulating the activation of these cells can lead to new approaches in order to control this pathological condition. Apoio Financeiro: FAPERJ, CNPq, SR-2/UERJ

01.028
PRÊMIO INOVAÇÃO

01.029

ERK-2 and PI3-KINASE modulate lipoxin A_4 inhibition of human monocyte apoptosis. Niconi-de-Almeida, Y.[1]; Simões, R. L.[2]; Da-Fe, A. R.[2]; Barja-Fidalgo, T. C.[3]; Fierro, I. M.[3] [1]UERJ - Farmacologia e Psicobiologia; [2]UERJ - Farmacologia e Psicobiologia; [3]UERJ – Farmacologia

Aim: Human monocytes play a central role in several steps of the immune response. The regulation of their survival is critical to population control and to the resolution phase of the inflammatory process. Lipoxins are members of the eicosanoid family of bioactive lipid mediators that exhibit selective stimulatory but nonphlogistic activities in mononuclear cells. In this study, we investigated the effects of 15-epi-16-(*para*-fluoro)phenoxy-LXA_4 (ATL-1), a synthetic analog of 15-epi-lipoxin A_4, in human monocytes survival and apoptosis. **Methods**: Monocytes were isolated by density centrifugation method with a purity of >90%. Cultured cells were incubated with ATL-1 (1-100 nM) in the absence of serum for 48h to evaluate survival (MTT assay) and apoptosis (flow cytometer, analyzing the number of annexin-V-positive cells, a marker of apoptotic cells and DNA fragmentation, a characteristic of the apoptotic process). In some experiments, cells were pre-treated with PD98059 (PD-10 mM), an ERK-2 inhibitor, or LY294002 (LY-3 mM), a PI3-kinase inhibitor, to evaluate the signaling pathways involved on this process. A western blot assay was used to investigate Akt phosphorylation and ERK nuclear translocation. **Results**: ATL-1 concentration-dependently increased monocyte survival, as a consequence of cell apoptosis reduction by the analog. Treatment of the cells with PD or LY blocked ATL-1 effect, indicating the involvement of ERK-2 and PI3-K, both pathways associated with cell survival. We found that ATL-1 (10-100 nM) stimulated significant activation of Akt already at 1 min after treatment, demonstrating that the kinase phosphorylation was very rapid. The treatment of the monocytes with LY for 15 min completely inhibited this event, confirming that ATL-1 is acting through Akt activation via PI3-K activity. Furthermore, ERK-2 phosphorylation was induced by different concentrations of the analog. This effect was inhibited by the treatment with LY, indicating that ATL-1 promoted cell survival through ERK-2 activation downstream the PI3-K/Akt pathway. In addition, a late induction of ERK-2 nuclear translocation was observed with 24h post-incubation, suggesting that ERK-2 translocation is an important step on ATL-1 signaling, possibly involved on monocyte survival promoted by ATL-1. **Conclusion**: These results demonstrate a cytoprotective effect of ATL-1 in monocytes and might contribute to the elucidation of the mechanisms associated with the resolution phase of the inflammatory process. Apoio Financeiro: FAPERJ, CNPq, SR-2/UERJ.

01.030

Determinação da atividade antiagregante plaquetária de novos compostos tienilacilidrazônicos candidatos a antitrombóticos. Nunes, P. C. G.[1]; Motta, N. A. V.[1]; Oliveira, S. G. T.[1]; Kummerle, A. E.[2]; Barreiro, E. J.[2]; Miranda, A. L. P.[2]; Brito, F. C. F.[1] [1]UFF - Instituto Biomédico - LAFE - Fisiologia e Farmacologia; [2]UFRJ - Farmácia - Fármacos - LASSBio

Introdução: A despeito do grande número de estudos visando novos agentes antiagregante plaquetários, poucos avanços foram obtidos e o número de pacientes que carece de agentes mais seguros e eficazes aumenta a cada ano (Troxler *et al*., 2007). A relevante atividade antiagregante plaquetária de compostos tienilacilidrazônicos frente a diversos agonistas fisiológicos foi anteriormente descrita (Brito *et al*., SBFTE 2003; Brito *et al.*, SBFTE 2004). Buscando a elucidação dos grupamentos farmacofóricos da série e a otimização de sua atividade farmacológica, novos compostos foram sintetizados e avaliados quanto a sua atividade antiagregante plaquetária. Neste trabalho avaliamos a atividade antiagregante plaquetária desses compostos na agregação plaquetária induzida por ácido araquidônico (AA) em plasma rico em plaquetas (PRP) citratado de coelhos, a fim de contribuirmos para a elucidação da relação estrutura x atividade dessa série de compostos e para o avanço das terapias antiagregante plaquetárias. **Método**: Os compostos tienilacilidrazônicos (LASSBio 123, 897, 1028, 1029, AA 1,T e AA 2,T) foram avaliados quanto a sua atividade antiagregante plaquetária através do método turbidimétrico de Born & Cross (1963, J. Physiol, 168: 178), solubilizados em DMSO, e a agregação plaquetária foi estimulada pelo AA (200 µM). A potência antiagregante plaquetária foi avaliada através da determinação da CI_{50} dos compostos tienilacilidrazônicos (0,1-100 µM) nessas condições. Os experimentos foram realizados em duplicata, com um n de 3 a 5 experimentos independentes. Os resultados foram expressos em média ± SEM e foram analisados pelo teste "t" de Student ($p< 0,05$*). As curvas de CI50 foram determinadas utilizando o programa GraphPrism, v.4.0, através de regressão não-linear. **Resultados**: Nos estudos realizados para a determinação da potência antiagregante plaquetária frente ao AA, os compostos tienilacilidrazônicos apresentaram valores de CI_{50} iguais a 23,4 ± 0,02 µM (LASSBio 123); 7,0 ± 0,7 µM (LASSBio 897); 34,4 ± 0,004 µM (LASSBio 1028); 10,4 ± 0,05 µM (LASSBio 1029); 1,1 ± 0,08 µM (AA 1,T) e 5,7 ± 1,8 µM (AA 2,T). **Discussão**: Os resultados obtidos demonstram a potente atividade antiagregante plaquetária dos novos derivados tienilacilidrazônicos e corroboram com resultados anteriores obtidos para essa série, apontando o núcleo tienilacilidrazônico como importante grupamento farmacofórico para a atividade antiagregante plaquetária. Podemos destacar os compostos, AA 1,T e AA 2,T, como novos candidatos a protótipos de agentes antiplaquetários e/ou antitrombóticos. Apoio Financeiro: FAPERJ

01.031

Effect of *Lonomia obliqua* venom on vascular smooth muscle cell. Moraes, J. A. de[1]; Rodrigues , G. S.[2]; Assreuy, J.[3]; Guimarães, J. A.[4]; Barja-Fidalgo, T. C.[5] [1]UERJ - Farmacologia Bioquímica e Celular; [2]UERJ - Farmacologia Bioquímica e Celular;; [3]UFSC - Farmacologia; [4]UFRGS - ; [5]UERJ - Farmacologia

Envenomation caused by human contact with the caterpillar *Lonomia obliqua* is characterized by a hemorrhagic clinical profile. After an initial inflammatory response, the venom causes disseminated intravascular coagulation, hypotension, fibrinolysis and hemorrhage. In parallel to blood coagulation induced-disturbances and the intense fibrinolytic activity, proteases and other active enzymes of *L. obliqua* venom and end-products could also cause direct effects on vascular cells. Together with the endothelium, the smooth muscle vascular cells are considered key constituent in vascular inflammatory processes. The present study was carried out to evaluate the *in vitro* effects of *L. obliqua* venom on smooth muscle cell activation and functionality. *L. obliqua* caterpillar bristles extract (LOCBE) was obtained as described (Bohrer et al., *Toxicon* 2007 49:663-9). The effects of LOCBE (1-30 µg/mL) were evaluated on rat thoracic aorta smooth muscle cell (SMC) linage (A7r5; ATCC): Chemotaxis assays were performed in Boyden chambers, after 4h of incubation; Changes in actin cytoskeleton were analyzed by fluorescence microscopy after phaloidin staining; Cell proliferation was measured though ^{3}H-thymidine incorporation after 48h incubation; Reactive Oxygen Species (ROS) production was evaluated using a DHR probe; and Focal Adhesion Kinase (FAK) and Erk phosphorylation were evaluated by immunoblotting. SMC migration and proliferation are crucial steps for the development of vascular obstruction- associated states. The treatment of A7r5 cells with LOCBE (1-30 ug/mL) did not affect cell viability as assessed by Trypan blue. Incubation for 4h with LOCBE (1-10 ug/mL) induced SMC chemotaxis in vitro, and this migratory effect was preceded (1-30 min incubation with 1 ug/ml LOCBE) by profound alterations in the actin cytoskeleton dynamics. Corroborating these data, FAK, a key kinase involved in cell migration, was rapidly and strongly phosphorylated in cells treated with venom (1-30 min). Incubation of SMC with LOCBE (1-30 µg/ml) for 48h significantly increased (50%-60%) cell proliferation, even at concentrations as low as 1 ug/ml. An important mechanism underlying SMC proliferation is the production of ROS, which are involved in the redox modulation a panel of other signaling molecules. Incubation (30 min) of A7r5 cells with LOCBE 1 ug/mL, induced ROS production. Finally, LOCBE induced Erk-1/2 phosphorylation, indicating that the proliferative effect may be modulated by MAP kinases. In resume, our data show, for the first time, that *L. obliqua* venom can directly interact and activate vascular SMC, changing cell functionality. These effects may contribute to the complex symptomatology observed during envenomation. Apoio Financeiro: CAPES, CNPq, FAPERJ

01.032

Heme modulates SMC proliferation via NADPH oxidase activation: counter-regulatory role for HO-1 system. Moraes, J. A. de[1]; Assreuy, J.[2]; Arruda, M. A.[3]; Barja-Fidalgo, T. C.[3] [1]UERJ - Farmacologia Bioquímica e Celular; [2]UFSC - Farmacologia; [3]UERJ – Farmacologia

Cardiovascular diseases represent the major cause of mortality and morbidity in western countries. Among these conditions, atherosclerosis is the most prominent one. A hallmark of atherosclerosis is the atheromatous plaque formation, characterized by oxidized LDL infiltration, foam cell formation and, especially, vascular smooth muscle cell (VSMC) accumulation. VSMC dysfunction and exacerbated proliferation have been implicated in the pathogenesis of atherosclerosis and other cardiovascular pathological situations, including restenosis. The pathological effect of VSMC in response to different stimuli that are able to induce VSMC migration and proliferation, leads to fibrous cap (atherosclerosis) or neointima (restenosis) formation. Notably, these cardiovascular diseases occur mainly in sinuous vessels, and are associated to turbulent blood flow, what may lead to hemolysis and consequent free heme accumulation. Our group has characterized heme as a proinflammatory molecule that mediates its effect via NADPH oxidase complex activation and reactive oxygen species (ROS) production. In this work we aim to elucidate the putative role of free heme in VSMC physiology and the molecular mechanisms underlying the affected processes. A7r5 (VSMC) cells were obtained of rat thoracic aorta originally from the ATCC. Cell proliferation was measured using thymidine (H3) incorporation assay. ROS production was evaluated by DHR probes analysis. Protein expression were analyzed by immunoblotting. The cell images were obtained from a fluorescence or confocal microscopy. We observed that free heme is able to induce VSMC proliferation in a ROS-dependent manner. Heme-induced ROS production relies on NADPH oxidase activation, once diphenyleneiodonium (DPI), a NADPH oxidase inhibitor completely abolished this effect. Accordingly, heme induces p47phox subunit translocation to plasma membrane, and DPI pre-treatment abolished heme proliferative effect. Additionally, heme activates proliferation-relationed signaling routes, such as MAP kinases and the redox-sensitive signaling pathway of NFkB. It was also observed a critical crosstalk between NADPH oxidase and heme oxygenase (HO-1) system, once heme induces HO-1 expression and VSMC pre-treatment with HO inhibitors increased heme proliferative effect. In this work we show, for the first time, that free heme induces VSMC proliferation via NADPH oxidase activation, which is elegantly counter-regulated by HO-1 activity. Thus, we present a putative new mediator implicated on the pathogenesis of atherosclerosis and other correlated conditions. We do hope that the depiction of the signaling events underlying this process leads to the development of novel and more effective therapeutic interventions in restenosis/atherosclerosis. Apoio Financeiro: CAPES, CNPq, FAPERJ

01.033

Rat alveolar macrophage phagocytosis is induced by ATL-1, a synthetic analog of 15-EPI-LIPOXIN A_4: a possible role in infectious diseases. Simões, R. L.[1]; Niconi-De-Almeida, Y.[1]; Da-Fe, A. R.[1]; Canetti C.[2]; Fierro, I. M.[1] [1]UERJ - Farmacologia e Psicobiologia; [2]UFRJ - Biofísica

Aim: Mononuclear cells play a central role in the initiation, development and outcome of the immune response. In the lung periphery, the alveolar macrophage (AM) is the resident defender of mucosal sterility, patrolling the alveolar epithelial surface and clearing organisms by phagocytosis and intracellular killing. The antimicrobial activities of this sentinel of innate immunity are regulated by a number of factors including cytokines, chemokines and lipids. Lipoxins are generated during cell-cell interactions under a variety of conditions, such as infection and inflammation. LXA_4 was recovered in the bronchoalveolar and nasal lavage fluids of patients with various respiratory diseases and might be a potential mediator or modulator of inflammation in the lung. In the present study, we sought to assess the effect of ATL-1, a synthetic analog of 15-epi-lipoxin A_4, on FcR-mediated AM phagocytosis of IgG-opsonized erythrocytes, as well the signaling pathways involved in this process. Methods: Rat resident alveolar macrophages were obtained by lung lavage and cultured overnight in a 96-wells plate before stimulation. Sheep red blood cells were opsonized with a subagglutinating concentration of anti-sheep erythrocyte IgG antibody. Phagocytosis was determined by a microcolorimetric assay. AM were stimulated with different concentrations of ATL-1 (1-100 nM) for 15 minutes at 37º C. In some experiments, the cells were treated with wortmanin (100 nM), a PI3-kinase inhibitor, to evaluate the signaling pathways involved in this process. A western blot assay was used to investigate Akt and ERK-2 phosphorylation. **Results**: ATL-1 concentration-dependently induced FcR-mediated AM phagocytosis. The PI3-kinase/Akt is a well-known pathway involved in AM phagocytosis. The pre-treatment of the AM with wortmanin for 10 minutes significantly reduced the ATL-1-induced phagocytosis. However, phosphorylation of Akt, a downstream protein in the PI3-K pathway, was not observed. In addition, ATL-1 stimulated significant ERK-2 phosphorylation in these cells, which was obvious within 10 and 100 nM, indicating the involvement of the ERK-2 pathway in the analog effect. **Conclusions**: These data suggest that ATL-1 rapidly promotes AM phagocytosis *in vitro* and support a role for LX as potential mediators of inflammation and infectious processes in the lung. Apoio Financeiro: FAPERJ, CNPq, SR-2/UERJ

01.034

NADPH oxidase regulates proliferation pathways in melanoma cells. Ribeiro Pereira, C.[1]; Barja-Fidalgo, T. C.[2]; Arruda, M. A.[2] [1]UERJ - Farmacologia e Psicobiologia; [2]UERJ – Farmacologia

NADPH oxidase-derived reactive oxygen species (ROS) have emerged as critical mediators of several cell functions as migration, growth, proliferation and survival. Recent evidence has show that NADPH oxidase activity is essential to melanoma proliferation and survival. However, the mechanisms by which NADPH oxidase regulates these signaling pathways are not completely understood. In this study, we investigate the role of NADPH oxidase-derived ROS on the signaling events that coordinate melanoma cell proliferation. **Materials and Methods**: Human melanoma cells (MV3) were incubated in the absence or in the presence of the NADPH oxidase inhibitor diphenyleneiodonium (DPI) and cell growth was assessed by MTT assay. The confocal immunofluorescence microscopy was used to detect focal adhesions and actin cytoskeleton dynamics. Whole cell extracts were obtained for immunoblotting as well as imunopreciptation technique. **Results**: We reported that NADPH oxidase inhibition by DPI reduced melanoma growth and induced changes in cell shape with cell spreading decrease, rounding up and detachment. These phenomena were accompanied by rearrangement of actin network and decreased focal adhesion kinase (FAK) phosphorylation in Tyr^{397} residue as well as FAK association to actin and c-Src, indicating that inhibition of ROS generation would down-modulate integrin-mediated signaling, what often results in a particular type of apoptosis (*anoikis*). Confirming this hypothesis, we observed that the inhibition of NADPH oxidase activity induced apoptosis in MV3 cells, with caspase-3 activation and DNA cleavage, as accessed by FACS. Moreover, NADPH oxidase inhibition effect on MV3 growth was completely abolished by the pre-treatment of these cells with a protein tyrosine phosphatase, inhibitor sodium orthovanadate. In conclusion, our results strongly suggest that ROS generated by NADPH oxidase complex convey cell survival signals in melanoma cells through the FAK-Src pathway, probably inhibiting protein tyrosine phosphatase activity. The understanding of NADPH oxidase role as well as its regulation in tumorigenesis may lead to the development of more successful strategies in order to control cancer growth. Apoio Financeiro: FAPERJ, CNPq, SR2-UERJ

01.035

Atividade antioxidante *in vitro* do extrato metanólico de *Desmodium incanum* (Fabaceae). Bertuzzi, D.[1]; Bertoldi, K.[1]; Moysés, F. S.[1]; Elsner, V.R.[1]; von Poser, G. L.[2]; Ritter, M. R.[3]; Siqueira, I. R.[1] [1]UFRGS - Farmacologia; [2]UFRGS - Produção de Matéria-Prima; [3]UFRGS – Botânica

Recentemente, um levantamento etnobotânico na região de Porto Alegre descreveu o uso de infusões de *Desmodium incanum* DC. (Fabaceae, "pega-pega") no tratamento de afecções renais. O objetivo foi estudar a atividade antioxidante *in vitro* de *Desmodium incanum*. O material vegetal, coletado em Porto Alegre, foi submetido à maceração com metanol, sendo o extrato conduzido à secura em evaporador rotatório. Diferentes concentrações do extrato foram incubadas com as fontes geradoras de radicais livres. Os radicais ânion superóxido foram gerados pelo sistema xantina e xantina oxidase (XO); a modulação da atividade da enzima XO foi avaliada pela formação de ácido úrico. A atividade quelante de metais foi testada pela incubação com cloreto ferroso e ferrozina. A atividade seqüestradora de peróxido de hidrogênio foi observada pelo decaimento a 230 nm. Os resultados foram analisados por ANOVA seguida de Tukey. O extrato de *Desmodium incanum* inibiu significativamente a atividade da enzima xantina oxidase nas concentrações finais de 0,1 a 0,5 mg/ml ($p < 0,0001$). No entanto, não demonstrou atividade seqüestradora dos radicais ânion superóxido nas concentrações inferiores a 0,1 mg/ml. Além disso, a formação do complexo ferro-ferrozina não foi completa na presença dos extratos, demonstrando habilidade de quelar íons ferrosos (0,5; 1; 1,5 e 2 mg/ml, $p < 0,0001$). O extrato não apresentou atividade seqüestradora de peróxido de hidrogênio. O extrato metanólico de *Desmodium incanum* apresentou atividades antioxidante e quelante de ferro, mecanismos de ação que podem estar envolvidos na ação sugerida pela população usuária. Apoio Financeiro: PIBIC CNPq/UFRGS; BIC/FAPERGS, BIC/UFRGS

01.036

Isolation and characterization of mesenchymal cells associated to skeletal muscle. Brunetta-Fonseca, L.[1]; Andrade-Lopes, A. L.[1]; Pires-Oliveira, M.[1]; Bueno, M. A.[1]; Semedo, P.[2]; Câmara, N. O. S.[3]; Godinho, R. O.[1] [1]UNIFESP - Farmacologia; [2]UNIFESP - Bioquímica; [3]ICB-USP

Introduction: Adult skeletal muscle repair and fiber replacement depend on activation of distinct mononucleated quiescent cells: the satellite cells (SC), committed to myogenic lineage and the multipotent muscle-derived stem cells (MDSC). Considering that mesenchymal cells from bone marrow can be enriched by differential plastic adhesion, in this study we evaluated the efficiency of preplate technique in the enrichment of MDSC. Besides, we characterized the MDSC phenotype by flow cytometry and the cellular response to classical differentiation inductors. **Materials and Methods**: Mononucleated muscle-associated cells were obtained from soleus and extensor digitorum longus muscles of adult male Wistar rats. Muscle fragments were kept in DMEM plus 15% fetal calf serum (DMEM-15). After 72 h, mononucleated cells were preplated on plastic flasks in DMEM-15 for 2h and the adherent cells (**Ade**$^{+}$) were maintained in DMEM-10. Cells that did not adhere after 24 h (**Ade**$^{-}$) were seeded in DMEM-10 plus 2% horse serum. Both cell populations were subjected to Hoechst 33342 and alkaline phosphatase (ALP) staining. **Ade**$^{+}$ cells were treated with 1 μM dexamethasone (DEXA), 500 μM IBMX, 2 mM pentoxyfilline or vehicle for at least 15 days. Also, after 7 passages **Ade**$^{+}$ cells were incubated with fluorescent antibodies against CD90, CD45 and CD34 or with TRICT-α-bungarotoxin (TRICT-α-BTX) and used for flow cytometry analysis. **Results**: **Ade**$^{-}$ cultures were separated in 2 subpopulation of cells based on Hoechst 33342 exclusion: ALP^{+}/Hoechst^{+} and ALP^{+}/Hoechst^{-} cells. Either **Ade**$^{-}$ or **Ade**$^{+}$ formed cell agglomerates (myospheres). In fact, **Ade**$^{+}$ cells were highly positive for mesenchymal stem cell marker CD90 (99.7%), while hematopoietic stem cell marker CD45 and hematopoietic/SC marker CD34 were expressed only in 12.8% and 9.2% of cells, respectively. Muscular nicotinic receptors were expressed in 11.4% of **Ade**$^{+}$ cells (TRICT-α-BTX^{+} cells). Treatment of **Ade**$^{+}$ cells with DEXA induced myogenic differentiation and 104% myotube hypertrophy (control diameter = 19.8 ± 0.8 μm, n=66 fibers). The phosphodiesterase inhibitors IBMX and pentoxyfilline reduced the proliferation of **Ade**$^{+}$ cells, leading to an early myogenic differentiation. **Conclusion**: The preplate technique was effective in enriching **Ade**$^{+}$ with ALP^{+} and CD90^{+} mesenchymal stem cell. **Ade**$^{+}$ cells respond to classical differentiation inductors, but while DEXA induces myogenic differentiation, phosphodiesterase inhibitors seem to remove **Ade**$^{+}$ cells from the cell cycle. Therefore, the skeletal muscle is a viable source of multipotent cells whose differentiation may be induced by pharmacological treatment. Apoio Financeiro: FAPESP and MCT/CNPq, DECIT/MS, Fundo Setorial de Biotecnologia (CT-Biotecnologia) # 552178/05-5.

01.037

Chronic denervation selectively increases adenylyl-cyclase activity in slow twitch skeletal muscle. Bergantin, L. B.; Andrade-Lopes, A. L.; Chiavegatti, T.; Godinho, R. O. [1]UNIFESP – Farmacologia

Introduction: Activated adenylyl-cyclase (AC) synthesizes cyclic AMP (cAMP) from ATP, which in turn regulates skeletal muscle physiological processes such as contractility, metabolism and expression of synaptic proteins. After chronic denervation, it is described a progressive muscle atrophy and a decrease of synaptic proteins expression, such as nicotinic acetylcholine receptor (nAChR) and acetylcholinesterase (AChE). Although activation of receptors coupled to Gs/AC/cAMP signaling pathway increases skeletal muscle mass and modulates several synaptic proteins expression, it is not known whether AC activity is directly modulated by neural influences. The aim of this work was to investigate possible changes at typical fast and slow twitch muscle AC activity after skeletal muscle chronic denervation. **Methods**: Adult male Wistar rats had their tibial nerve sectioned which causes denervation of extensor digitorium longus (EDL, fast twitch) and soleus (SOL, slow twitch) muscles. After 14 days, the muscles were removed, homogenized and used for analysis of AC activity (n = 3-4) by radiometric assay to determine cAMP. Denervation was validated by detection of reduced AChE activity. **Results**: EDL and SOL atrophied by 31% and 51% following 14-day-denervation. Denervation did not change EDL AC kinetic parameters (Vmax = 5.1 ± 1.2 pmol cAMP/ h/ µg protein and Km = 0.61 ± 0.24 mM) but increased by 198% and 72% SOL AC Vmax (3.4 ± 0.4 pmol cAMP/h/µg protein) and Km (0.33 ± 0.03 mM), respectively. **Discussion**: Motoneuron innervation modulates the expression of numerous muscle genes which, at least in part, confers fiber-type specific innervation responsiveness. Our results show that the signaling molecule AC is selectively upregulated at slow twitch muscle soleus indicating that it may contribute for differences observed on nerve-dependent modulation of fast and slow twitch skeletal muscle events. Apoio Financeiro: CNPq & FAPESP

01.038

Secreção constitutiva da endopeptidade EC 3.4.24.15 (EP24.15) em cérebro de ratos e modulação pela calmodulina I em células HEK293. Russo, L. C.[1]; Castro, L. M.[2]; Scavone, C.[1]; Ferro, E. S.[3] [1]ICB-USP - Farmacologia; [2]ICB-USP Biologia Celular e do Desenvolvimento; [3] ICB-USP Farmacologia

Introdução: A endopeptidase EC 3.4.24.15 (EP24.15) é uma enzima envolvida no metabolismo de neuropeptídeos como bradicinina, opióides, neurotensina, GnRH, secretada por uma via secretória não convencional. Em trabalhos anteriores, descrevemos que a interação da EP24.15 com a proteína 14-3-3 epsilon favorece sua secreção estimulada pelo ionóforo de cálcio A23187. No presente trabalho, caracterizamos a interação da calmodulina I (CaM) com a EP24.15, bem como avaliamos a relevância funcional dessa interação para a secreção não convencional da EP24.15. Também investigamos a secreção da EP24.15 por fatias de tecido cerebral de ratos. **Métodos**: Os ensaios *in vitro* de interação física EP24.15-CaM foram realizados em colunas de afinidade Glutationa-Sepharose seguida de western blot. Células HEK293 foram transfectadas individualmente ou em combinações com os plasmídeos codificantes para EP24.15, CaM, 14-3-3 ε ou controle mock e submetidas a ensaios imunohistoquímicos ou de secreção constitutiva ou estimulada por diferentes agentes farmacológicos. Avaliamos ainda a secreção constitutiva e estimulada da EP24.15 em fatias de cerebelo, córtex ou o restante das áreas cerebrais dissecados de cérebros de ratos Wistar, na ausência e/ou presença de glutamato (50 μM) ou A23187 (7,5 μM) e forskolin (10 μM). As dosagens da atividade enzimática da EP24.15 foram realizadas por de ensaios fluorimétricos utilizando o substrato QFS padrão. **Resultados**: A EP24.15 interage *in vitro* com a apo ou Ca^{2+}-CaM, enquanto a co-localização EP24.15-CaM foi confirmada por imunohistoquímica em células HEK293, sendo estimulada pelo tratamento das células com forskolin (10 mM). A super-expressão das combinações de CaM, EP24.15 e 14-3-3 ε em células HEK293 aumenta em 100% ou mais a secreção estimulada da EP24.15 quando comparada à mock, sem interferir na secreção constitutiva. A secreção da EP24.15 em fatias de cérebros de ratos ocorre de forma constitutiva, não sendo alterada após o tratamento das fatias com glutamato. Em associação, os agentes farmacológicos A23187 e forskolin causam um aumento de aproximadamente 30% na secreção estimulada da EP24.15 apenas após 45 min de tratamento. **Discussão**: Descrevemos originalmente a interação *in vitro* e *in vivo* entre a CaM e EP24.15, e caracterizamos a participação funcional da CaM na secreção estimulada da EP24.15 em células HEK293. A presença mútua da CaM e 14-3-3 ε promovem um aumento ainda mais significativo na secreção estimulada da EP24.15. Em cérebro de ratos observamos que a secreção da EP24.15, distintamente do observado em cultura de células, ocorre principalmente pela via constitutiva. Apoio Financeiro: Capes, CNPq e FAPESP

01.039

Efeitos do LPS na atividade da enzima Na^+,K^+-ATPase em hipocampo de ratos jovens e idosos.

Vasconcelos, A. R.; Scavone, C.; Kawamoto, E. M. ICB-USP - Farmacologia

Introdução: A enzima Na^+,K^+-ATPase é uma proteína de membrana que, através da utilização da energia proveniente da hidrólise de uma molécula de ATP, mantém as concentrações do meio intracelular de K^+ elevadas e as de Na^+ baixas através do transporte de três íons Na^+ para o meio extracelular e dois íons K^+ para o meio intracelular. A enzima é uma proteína tetramérica constituída por duas subunidades a e duas subunidades b. A subunidade a apresenta três isoformas, que podem ser classificadas em dois tipos tomando-se como base a sua localização predominante: Isoforma Comum (a_1) e Isoforma Cerebral (a_2 e a_3). O glicosídeo ouabaína é um inibidor específico da Na^+,K^+-ATPase. O seu sítio de ação está localizado na subunidade a. As isoformas a_2 e a_3 são sensíveis a concentrações de ouabaína 1000 vezes menores (3 μM) do que as necessárias para inibir a isoforma a_1 (3 mM). Portanto, através da medida de atividade da ATPase total na ausência e na presença de ouabaína (3 μM e 3 mM) é possível determinar as atividades das isoformas a_1 e $a_{2/3}$ da Na^+,K^+-ATPase. Neste projeto nós comparamos os efeitos do LPS sobre a atividade das isoformas a_1 e $a_{2/3}$ Na^+,K^+-ATPase no hipocampo de ratos jovens e idosos. **Métodos**: Foram administradas doses intravenosas de salina estéril livre de pirógenos ou de LPS dissolvido em salina em ratos machos Wistar jovens (4 meses) e idosos (24 meses). Após 2 horas, os ratos foram sacrificados e seus hipocampos foram retirados. As amostras de tecido foram homogeneizadas e utilizadas para medir a atividade da enzima Na^+,K^+-ATPase. O método adotado na determinação da atividade da enzima se baseia na quantificação de moléculas de fosfato (Pi) livres provenientes da hidrólise de ATP por espectrofotometria, após a reação de complexação com molibdato de amônio, formando um cromóforo cuja absorbância é determinada através da leitura em comprimento de onda de 700 nm. **Resultados**: A atividade total da enzima Na^+,K^+-ATPase diminuiu no grupo de ratos jovens que receberam as injeções de LPS em relação ao grupo controle. Este fato não foi observado nos ratos velhos. O mesmo padrão é observado na atividade das isoformas a_2 e a_3. Em contrapartida, a atividade da isoforma a_1 aumentou no grupo de ratos jovens tratados com LPS. Essa alteração também não ocorreu nos ratos velhos. **Discussão**: Os dados obtidos mostram que no envelhecimento ocorre uma perda da modulação da enzima Na^+,K^+-ATPase induzida pelo LPS, o que sugere a presença de uma menor capacidade de resposta inflamatória do sistema nervoso central. Esta ausência de resposta pode estar relacionada à maior susceptibilidade de desenvolvimento de processos neurodegenerativos no envelhecimento. Apoio Financeiro: FAPESP, CNPq

01.040

Caracterização farmacológica do inibidor de fosfodiesterase Tipo-9, BAY 73-6691, em corpo cavernoso de camundongos. Pereira, M. N.; Teixeira, C. E.; Nucci, G. de; Claudino, M. A.; Antunes, E. UNICAMP – Farmacologia

Introdução: A disfunção erétil é essencialmente devida a prejuízos na via de sinalização óxido nítrico (NO)-GMPc ao nível de tecido erétil. Ao ser liberado de fibras nitrérgicas ou do endotélio sinusoidal, o NO se difunde para células musculares lisas adjacentes ativando a guanilil ciclase solúvel (GCs), levando conseqüentemente à formação de GMPc e relaxamento vascular. Nos corpos cavernosos, o GMPc é degradado principalmente pela fosfodiesterase-5 (PDE-5). Estudos recentes mostram que a isoforma PDE-9 apresenta grande afinidade pelo GMPc, mas nenhum estudo foi conduzido para se avaliar a importância desta isoforma em tecidos eréteis. No presente estudo, investigamos o efeito de um inibidor da PDE-9, BAY 73-6691, nas respostas relaxantes diretas e indiretas mediadas pelo NO em corpos cavernosos de camundongos *in vitro*. **Métodos**: Camundongos C57BL/6 (20-25 g) foram anestesiados e submetidos à penectomia. Segmentos de corpos cavernosos foram montados em banhos para órgão isolado e suspensos entre um transdutor de força e uma unidade fixa. A tensão aplicada aos tecidos (2-2.5 mN) foi periodicamente ajustada até estabilização dos tecidos (60 min), após o qual construiu-se curvas concentração-resposta à acetilcolina (ACh) e ao nitrito de sódio acidificado (NaOH acid.), e curvas freqüência-resposta à estimulação elétrica (EFS; 1-32 Hz), na presença e na ausência do BAY 73-6691. **Resultados**: A potência (pEC_{50}) da acetilcolina não foi alterada na presença do BAY 73-6691 (6,92 ± 0,10) comparada ao grupo controle (6,78 ± 0,09). Entretanto, o relaxamento máximo (E_{max}) da acetilcolina foi significativamente maior na presença do BAY 73-6691 (75,1 ± 3,8%) comparado ao grupo controle (62,2 ± 2,3%). O BAY 73-6691 não alterou a potência do doador de NO (NaOH acid; 5,7 ± 0,3) comparado ao grupo controle (5,6 ± 0,2); porém, ampliou o relaxamento máximo induzido por este agente (64,2 ± 5,7%) comparado ao grupo controle (52,3 ± 2,4%). A amplitude do relaxamento induzido por EFS não foi alterada na presença do BAY 73-6691; no entanto, o BAY 73-6691 aumentou significativamente o tempo de duração dos relaxamentos em resposta ao EFS (8 Hz: 13,3 ± 0,6 s e 19,7 ± 2,9 s na ausência e na presença de BAY 73-6691, respectivamente). **Conclusão**: O BAY 73-6691 amplifica a resposta relaxante nitrérgica e dependente de endotélio em corpo cavernoso de camundongos, possivelmente pelo aumento dos níveis intracelulares de GMPc, favorecendo a via de sinalização NO-GMPc. Apoio Financeiro: FAPESP

01.041

Proinflammatory genes and activation of NF-kB through NMDA-Src-MAP kinase pathway by ouabain in cerebellar primary cell culture. Sá Lima, L.; Munhoz, C. D.; Lepsch, B. L.; Kawamoto, E. M.; Yshii, L. M.; Scavone, C. ICB-USP- Farmacologia

Introduction: The Na,K-ATPase enzyme is responsible for maintaining the electrochemical gradient of sodium and potassium ions and play an important role in the regulation of ionic homeostasis in tissue and cells. This enzyme can also act as a signal transducer and a transcription activator by interacting with membrane proteins and organized cytosolic cascades of signaling proteins. Our aim was to study if Ouabain, an inhibitor of Na,K-ATPAse, can regulate NF-kB in primary cell culture. Methods: Cerebellar cell culture was treated with 2 hours with Ouabain (10 uM). Cells were incubated with PP-1 (Src-family tyrosine kinase inhibitor)(10 uM), PD98059 (MAP-Kinase inhibitor)(10 uM), MK-801 (NMDA receptor antagonist)(10 uM), Manumycin (Rasfarnesiltransferase inhibitor)(5 uM) 20 min before Ouabain. Nuclear extracts were isolated and gel mobility shift assay used to measure changes in NF-kB activity. RNA were also isolated and RT-PCR are used to measure mRNA expression of α3Na,K-ATPase, IKB-α, BAX, BCL-2, TNF-α, IL-1β, BDNF and GAPDH. RT-PCR results were expressed as mRNA levels divided by GAPDH values. Statistic comparisons were performed by ANOVA-Newman-Keuls test – $P<0.05$. **Results**: Ouabain induces NF-kB after 2 hours (% of control n=10; Ouabain = 139.8 ± 3.7 n=13). This activation was completely reduced by PP-1+OUA (98.7 ± 10,9 n=7), PD98059+OUA (83.6 ± 4.4 n=7), MK-801+OUA (83.7 ± 3.1 n=12) and Manumycin+OUA (78.8 ± 5.4 n=9). Ouabain increased TNF-α (OUA=1.5 ± 0,3 n=4; control=0.8 ± 0.1 n=9), IL-1β (OUA=1.3 ± 0.1 n=3; control=0.7 ± 0.1 n=5) and BDNF (OUA=1.4 ± 0.1 n=5; control=0.5 ± 0.2 n=5) mRNA expression after 2 hours incubation when compared with control group. Therefore, no difference was observed in mRNA levels of α3Na,K-ATPase, IKB-α, BAX and BCL-2. **Discussion**: These results suggest that Ouabain induced genes involved in the inflammatory response by activation of transcription factor NF-kB via NMDA-Src-MAPK pathway in cerebellar cell. Apoio Financeiro: FAPESP, CNPq and *Procontes-USP.

01.042

Amyloid beta-peptide activates nitric oxide synthase and sodium, potassium pump activities in rat hippocampal slices. Kawamoto, E. M.; Lepsch, B. L.; Munhoz, C. D.; Sá Lima, L.; Scavone, C. ICB-USP – Farmacologia

Introduction: Beta-amyloid peptide (Ab) has been shown to cause synaptic dysfunction and can render neurons vulnerable to excitotoxicity and oxidative stress. Nitric oxide synthase (NOS) is an enzyme which has been linked to both survival and apoptosis. Sodium, potassium pump (Na^+, K^+-ATPase) plays an important role to maintain cell ionic equilibrium. Disruption of NOS and Na^+, K^+-ATPase activities could lead to oxidative stress process which could be detrimental to the cells. Our aim was to evaluate the signaling pathways of Ab in relation to NOS and Na^+, K^+-ATPase activities in rat hippocampal slices. **Materials and Methods**: Ab fragment 1-40 was incubated for 7 days at 37°C. Hippocampal slices which were obtained from 4-months male rats were treated with Ab in different concentrations (200 nM, 1 uM, 2 uM) for 1 h. MTT reduction assay was used to evaluate tissue viability. Nitric oxide synthase (NOS) and sodium pump (Na^+, K^+-ATPase) activities were performed to measure Ab effects in this preparation.MK-801(1 uM) and L-NAME (100 uM) were used to study the participation of N-Methyl-D-Aspartate receptor and the NOS in the effects mediated by Ab. **Results**: Ab peptide induced activation of NOS in all the concentrations tested. MK-801 partially inhibited Ab-induced NOS activation. L-NAME blocked totally the Ab-induced NOS activation. Ab peptide also induced activation of Na^+, K^+-ATPase. MK-801 did not inhibit Ab-induced Na^+,K^+-ATPase activation and L-NAME blocked totally the Ab-induced NOS activation, **Discussion**: Our results suggest that Ab induces a dose-dependent activation of NOS and Na^+, K^+-ATPase activities in rat hippocampal slices. NMDA receptor is involved on NOS activation and NO influences Na^+, K^+-ATPase activity. The non-response of MK-801 on Na^+, K^+-ATPase activity could be explained by the partial inhibition of NOS activity by the antagonist. Financial support: FAPESP, CNPq and Bunka grant/Sumitomo Bank.

01.043

Identification of endogenous substrates of thimet oligopeptidase (EC 3.4.24.15) in human embryonic kidney cells. Berti, D. A.[1]; Morano, C.[2]; Cunha, F. M.[3]; Zhang, X.[2]; Sironi, J.[2]; Russo, L. C.[4]; Klitzke, C. F.[5]; Klitzke, C. F.[5]; Ferro, E. S.[4]; Fricker, L. D.[2] [1]ICB-USP - Biologia Celular e do Desenvolvimento; [2]Albert Einstein College of Medicine - Molecular Pharmacology; [3]UNIFESP Biologia Molecular; [4]ICB-USP - Farmacologia; [5]Instituto Butantan - CAT/CEPID

Introduction: Thimet oligopeptidase (EC3.4.24.15; EP24.15) is an intracellular enzyme that has been proposed to metabolize peptides within cells and affect antigen presentation. However, only a small number of endogenous substrates of EP24.15 have been previously reported. In the present study, we have identified EP24.15 substrates in human embryonic kidney 293 (HEK293) cells using several approaches. **Experimental Procedures**: Cellular peptides were extracted from HEK293 cells and incubated *in vitro* with purified EP24.15. Then, the peptides were labeled with isotopic tags and analyzed by mass spectrometry to obtain quantitative data on the extent of cleavage. A related series of experiments tested the effect of overexpression of EP24.15 on the cellular levels of peptides in HEK293 cells. Finally, synthetic peptides that corresponded to ten of the cellular peptides were incubated with purified EP24.15 *in vitro* and the cleavage monitored by HPLC and mass spectrometry. **Results and Discussion**: Collectively, these studies have identified a large number of peptides that likely represent the endogenous substrates of EP24.15 in HEK293 cells. Although there is no apparent recognition motif, the optimal EP24.15 substrates were 9-11 amino acids in length, with the cleavage occurring a minimum of 2 amino acids from either the N- or C-terminus. Because the optimal size of EP24.15 substrates is similar to the size range of peptides used by cells for major histocompatibility class I antigen presentation (8-10 amino acids), these findings support the proposal that EP24.15 primarily functions in the degradation of peptides that could be used for antigen presentation. However, EP24.15 also converts some peptides into products that are 8-10 amino acids, thus contributing to the formation of peptides for antigen presentation. Apoio Financeiro: Capes, CNPq e FAPESP, FINEP, National Institutes of Health

01.044

Intracellular peptides as natural regulators of cell signaling. Cunha, F. M.[1]; Berti, D. A.[2]; Klitzke, C. F.[3]; Ferreira, Z. S.[4]; Markus, R. P.[4]; Ferro, E. S.[5] [1]UNIFESP - Biologia Molecular; [2]ICB-USP-Biologia Celular e do Desenvolvimento; [3]Instituto Butantan - CAT/CEPID; [4]IB-USP – Fisiologia ; [5]ICB-USP

Introduction: Protein degradation by the ubiquitin proteasome system releases large amounts of oligopeptides within cells. Methods: To investigate possible functions for these intracellularly generated oligopeptides, we fused them to a cationic TAT peptide sequence using reversible disulfide bonds, introduced them into cells, and analyzed their effect on GPCR signal transduction. **Results and Discussion**: A mixture containing four of these peptides (20-80 μM) significantly inhibited the increase in the extracellular acidification response triggered by angiotensin II (ang II) in CHO-S cells transfected with the ang II type 1 receptor (AT1R-CHO-S). Subsequently, either alone or in a mixture, these peptides increased luciferase gene transcription in AT1R CHO-S cells stimulated with ang II and in HEK293 cells treated with isoproterenol. These peptides without TAT failed to affect GPCR cellular responses. All four functional peptides were shown *in vitro* to competitively inhibit the degradation of a synthetic substrate by thimet oligopeptidase. Overexpression of thimet oligopeptidase in both CHO-S and HEK293 cells was sufficient to reduce luciferase activation triggered by a specific GPCR agonist. Moreover, using individual peptides as baits in affinity columns, several proteins involved in GPCR signaling were identified, including alpha-adaptin A and dynamin 1. These results suggest that before their complete degradation, intracellular peptides similar to those generated by proteasomes can actively affect cell signaling, likely representing additional bioactive molecules within cells**.** Apoio Financeiro: CNPq e FAPESP

01.045

FBXO25 promotes ubiquitination of polyglutamine-expanded huntingtin for proteasomal degradation and suppresses polyglutamine-expanded huntingtin aggregation. Manfiolli, A. O.; Yokoo, S.; Teixeira, F. R.; Gomes, M. D. FMRP-USP - Bioquímica e Imunologia

Introduction: FBXO25 is one of several human F-box proteins that serve as specificity factors for a family of ubiquitin (ub) ligase composed of Skp1, Cul and F-box protein that is involved in targeting proteins for destruction across the ub-proteasome system (UPS). The aim of the study was to examine the subcellular localization of endogenous FBXO25 in cultured cells and the expression of the FBXO25 protein in mouse tissues. Also, we investigate the association of FBXO25 with other subnuclear components and the effects of inhibiting the transcription process on the nuclear distribution of this enzyme. The nuclear ub-ligase activity of FBXO25 was probed using an assay for nuclear aggregation of polyglutamine (polyQ)-containing proteins in cultured cells. **Methods and Results**: Confocal analysis revealed that the endogenous FBXO25 was partially concentrated in a novel dot-like nuclear domain that is distinct from clastosomes and other well-characterized structures. These nuclear compartments contain a high concentration of ub conjugates and other components of the UPS. We propose to name these compartments FANDs for FBXO25-associated nuclear domains. Interestingly, inhibition of transcription by actinomycin D drastically affected the nuclear organization of FANDs, indicating that they are dynamic compartments of the cell. To explore the possibility of FBXO25 being involved in preventing polyQ-containing proteins aggregation, we expressed huntingtin (htt) exon-1 (Ex1) with 103 glutamines (Q) fused to EGFP in HEK293T cells and processed them for confocal microscopy. The results showed that FBXO25 colocalized with EGFP-httEx1-103Q aggregates largely in the intranuclear region. Additionally, we analyzed the effect of overexpression of FBXO25 on the nuclear aggregation of polyQ-containing proteins in cultured cells. We expressed in HEK293T cells EGFP-httEx1-74Q in combination with full-length wild-type (WT) or inactive version of FBXO25 in which the F-box had been deleted (ΔF). Full-length FBXO25, but not the FBXO25ΔF protein, strongly reduced the level of aggregated EGFP-httEx1-74Q in the filter retardation assay. **Discussion**: The major conclusions from this study are that a protein that participates in ubiquitination reactions, FBXO25, is localized in a novel subnuclear compartment, the FAND, which is disrupted by inhibition of transcription with subsequent relocation of FBXO25. Our results also suggest that FANDs recruit polyQ-containing proteins and prevent their accumulation in the nucleus, supporting the notion that FBXO25 is a functional ub-ligase and that FANDs are competent sites of polyubquitination in the nucleus.

01.046

Androgen deprivation shrinks skeletal muscle nuclear domains and increases atrophy-related ubiquitin ligase atrogin-1 expression. Pires-Oliveira, M.[1]; Maragno, A. L. G. C.[2]; Furlan, I.[1]; Gomes, M. D.[2]; Godinho, R. O.[1] [1]UNIFESP - Farmacologia; [2]FMRP-USP - Bioquímica e Imunologia

Introduction: Skeletal muscle fibers are multinucleated cells with contractile function. Each myonuclei controls a cytoplasmic region known as a nuclear domain (ND). Disuse or denervation-induced skeletal muscle atrophy is associated with increased expression of ubiquitin ligases such as atrogin-1 and proteolysis, leading to loss of cytoplasm and ND remodeling. Adjustment in ND, in response to changes on functional demand or hormone blood levels might affect the compartmentalized expression of acetylcholinesterase (AChE) and nicotinic receptors (nAChR) at the neuromuscular junction (NMJ). Androgens have a long known anabolic action whose mechanisms are not yet fully understood. Therefore, this study investigated the actions of androgen on myofiber size, atrogin-1 expression, NMJ morphology and ND organization of the androgen-dependent levator ani muscle of the adult male rat. **Methods**: Male Wistar rats were sacrificed as normal controls (N) or after castration (C) for 2 to 90 days. LA or extensor digitorum longus muscle (EDL) fiber bundles were subjected to AChE histochemistry and nAChR histofluorescence. Brightfield or confocal *z*-series images were acquired using a CCD camera and muscle fiber diameter, ND size, NMJ length and volume were determined using NIH ImageJ 1.32j program. Atrogin-1 expression was determined by qRT-PCR and immunoblotting and caspase-3 activity was determined through a fluorometric assay. **Results**: C induced atrophy of the LA, up to 70% after 90 days (N=164.3 ± 6.3 mg). Also, there was an exponential decrease on myofiber diameter, up to 61% after 60 days (N=35.3 ± 0.4 μm). Atrogin-1 mRNA increased 31-fold after 2-day C, returning to N levels after 30 days whereas the protein increased by 270% after 30 days. T administration (4 mg/kg, *sc*) reduced atrogin-1 mRNA expression to N levels in 24 h. Apoptotic myonuclei were detected after just 2-day C in LA fibers, but cytoplasm/nucleus ratio decreased 83% after 60 days of castration (N=4.2 ± 0.3 x 10^4 μm^{-3}). Interestingly, there was no change on caspase 3 activity in LA from 2 to 30 days of C. NMJ length and volume decreased progressively, up to 35% (30 days) and 46% (90 days), respectively (N=28.2 ± 0.7 μm; 1057 ± 59 μm^3). No similar changes were seen in EDL after castration up to 90 days. **Discussion**: These results show that LA atrophy induced by castration is preceded by atrogin-1 overexpression and apoptosis of nuclei along the fibers does not seem to be effective to maintain ND integrity. Testosterone deprivation induces NMJ shrinkage, but this adaptation of junctional ND occurs in just 7 to 15 days, while loss of muscle mass is markedly more prolonged. Apoio Financeiro: Supported by CNPq, FAPESP.

01.047

Modulation of MAS receptor expression levels in rats after audiogenic kindling, a model of temporal lobe epilepsy. Pereira, M. G. A. G.[1]; Becari, C.[2]; Salgado, M. C. O.[2]; Garcia-Cairasco, N.[3]; Costa-Neto, C. M.[1] [1]FMRP-USP - Bioquímica e Imunologia; [2]FMRP-USP - Farmacologia; [3]FMRP-USP - Fisiologia

Since 1971, when Ganten and coworkers showed the renin activity in central nervous system (CNS), and finally when Dzau and coworkers in 1986 detected the mRNA of renin and angiotensinogen in CNS, the existence and functional relevance of all components of the renin-angiotensin system in the brain have been widely demonstrated. The angiotensin converting-enzyme (ACE), AT_1 and AT_2 receptors represent the classical AngII signaling pathway. Nevertheless, alternative pathways have been described in the literature. Recently, it has been shown that the *mas* receptor can be activated by the peptide Ang(1-7). Epilepsy is characterized by diverse mechanisms involving alteration of excitatory and inhibitory neurotransmission that result in hyperexcitability of the CNS. It is reasonable to infer about a possible role for the Ang(1-7) through *mas* receptor in that pathology, since it is involved with learning and memory process in the hippocampus. Hence, we decided to study the *mas* receptor expression in the Wistar audiogenic rat (WAR) strain, that is a model of temporal lobe epilepsy (TLE) which is displayed after multiple inductions of audiogenic seizures with recruitment of limbic areas. To this, we analyzed the modulation of *mas* receptor expression level by semi-quantitative RT-PCR in hippocampus of WAR and Wistar (control animals) neonates, and adults were subjected to none, single or multiple seizures (TLE model). The analyses in WAR and Wistar neonates showed that transcripts levels are not different when both strains are compared, but it is up-regulated about 5-fold in WARs with TLE. Our data demonstrated that the role of *mas* receptor in epileptic seizures is not innate, but has a important regulation and possible participation in the TLE model. We can then speculate that the Ang(1-7) through *mas* receptor may participate in seizure mechanisms by activating specific neurochemical pathways in WARs. Apoio Financeiro: FAPESP, CAPES, CNPq and FAEPA

01.048

Signaling pathways involved in the induction of heme oxygenase-1 by lipoxins. Vieira, A. M.[1]; Nascimento da Silva, V.[2]; Arruda, M. A.[2]; Barja-Fidalgo, T. C.[2]; Fierro, I. M.[2] [1]UERJ-DFP; [2]UERJ – Farmacologia

Introduction: Lipoxins (LX) and aspirin-triggered LX (ATL) are eicosanoids generated via transcellular biosynthetic routes during inflammation, that elicit distinct anti-inflammatory and pro-resolution bioactions. Heme oxygenase-1 (HO-1) is an enzyme responsible for the catabolism of heme generating metabolites that lead to anti-inflammatory, antiapoptotic and antiproliferative effects. We have previously reported that ATL induce HO-1 expression in endothelial cells (EC), what confers cell protection against pro-oxidant insults. However, the cellular mechanisms underlying this effect are presently unknown. **Objective**: In this study, we investigated the possible signaling pathways involved in the induction of HO-1 in EC using an aspirin-triggered lipoxin A_4 stable analog, 15-epi-16-(*para*-fluoro)-phenoxy-lipoxin A_4 (ATL-1). **Materials and Methods**: ECV 304 cells were cultured on glass coverslips overnight before incubation with vehicle or ATL-1 (100 nM) for 18h. In some experiments, the cells were pre-treated with LY294002 (LY-3 mM); PD98059 (PD-10 mM); SB203580 (SB-1 mM); SP600125 (SP-10 mM) or H89 (10 mM), for 30 min and exposed to ATL-1. Protein levels were detected by western blot analysis. **Results**: ATL-1 induced HO-1 expression in EC after 18h of incubation and this induction was inhibited by the pre-treatment of the cells with PD, a specific inhibitor of p42/44 MAPKinase. We next examined the involvement of the c-AMP-dependent protein kinase A (PKA) pathway in the effect of the analog. Cells were exposed to the specific inhibitor H89, which significantly decreased HO-1 induction. In contrast, the selective p38 MAPK pathway inhibitor SB203580 failed to affect the induction activity of HO-1 by ATL-1. In addition, neither the c-Jun NH_2-terminal kinase (JNK) pathway, nor the PI3-Kinase/PKB pathway appears to be involved in the analog action, since inhibition of both pathways with SP and LY, respectively, did not affect the enzyme expression. Other signaling pathways that could be modulating HO-1 expression induced by ATL in EC, including the AP-1 transcription factor, are currently under investigation. **Conclusions**: Taken together, our results indicate the involvement of the ERK and c-AMP-PKA pathways in the effect of ATL-1 and contribute to the elucidation of the mechanism of action of lipoxin which are a promising strategy for the treatment of various diseases and resolution of inflammation. Apoio Financeiro: Supported by FAPERJ, CNPq and SR-2/UERJ

01.049

The anti-apoptotic effect of leukotriene B_4 in neutrophils: a role for NADPH oxidase-derived ROS and NF-kappaB. Barcellos-de-Souza, P.[1]; Canetti C.[2]; Barja-Fidalgo, T. C.[1]; Arruda, M. A.[1]
[1]UERJ - Farmacologia; [2]UFRJ - Biofísica

Introduction: Leukotriene B4 (LTB_4), an arachidonic acid-derived lipid mediator, is a known proinflammatory agent released in many inflammatory situations. Several studies describe that LTB_4 is able to activate numerous biological responses in human neutrophils (PMN), such as calcium influx, chemotaxis and degranulation as well as reactive oxygen species (ROS) generation by the multimeric complex nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. It has been previously shown that LTB_4 delays neutrophils spontaneous apoptosis through the activation of classical pro-survival signaling pathways such as phosphoinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinases (MAPKs), which in turn may corroborate to the onset of a chronic inflammatory condition. Recently, ROS have emerged as second-messengers, coordinating a myriad of intracellular signaling cascades, and thus modulating several biological phenomena, among them, apoptosis. In this study, we aim to elucidate the putative role of NADPH oxidase-derived ROS in the antiapoptotic effect of LTB_4.
Methodology: PMN were isolated from whole blood of healthy volunteers by Ficoll-Paque™ density. ROS production was evaluated by cytochrome *c* reduction assessed by a spectrophotometer one hour after LTB_4-stimulation. Apoptosis was determined according to cell morphological changes observed in optical microscopy after 20-hour incubation. Protein expression was evaluated by western blot analysis of whole cell or nuclear extracts (1-60 min.).
Results and conclusion: Our data show that NADPH oxidase-derived ROS are critical to LTB_4 (100-300 nM) pro-survival effect on human neutrophils. This event depends on redox modulation of NF-kappaB translocation and IkappaB-alpha phosphorylation/degradation. We have also observed that LTB_4-induced Bad degradation requires NADPH oxidase activity. The effect of NADPH oxidase in LTB_4-induced mitochondria stability is under investigation. Our results strongly indicate that LTB_4-induced antiapoptotic effect in neutrophils occurs via ROS-dependent signaling routes and we do believe that a better knowledge of molecular mechanisms underlying neutrophils spontaneous apoptosis may contribute to design better strategies to control chronic inflammatory conditions. Apoio Financeiro: FAPERJ; CNPq; SR-2/UERJ; ABC-UNESCO-LOREAL

01.050

Epidermal growth factor receptor is expressed in rat epididymis and sperm. Patrão, M. T. C. C.; Avellar, M. C. W. [1]UNIFESP - Farmacologia

Introduction: The epididymis, an organ involved in sperm maturation, is divided into 4 regions (initial segment, IS; caput, CP; corpus, CO; cauda. CD). Each region is subjected to different microenvironment, which can drive differential pattern of mRNA and protein expression and tissue function. We have previously described a region-specific distribution of epidermal growth factor (EGF) along rat epididymis. Since little is known about the function and regulation of EGF and its receptor (EGFR) in this tissue, our aim was to characterize EGFR expression in rat epididymis and sperm. EGF effect on the activation of EGFR in isolated epididymis was also evaluated. **Methods**: Epididymides from adult Wistar rats were isolated and incubated *in vitro* in absence and presence of EGF (100 ng/ml; 1, 5 and 15 min). EGF effect on EGFR activation was then assessed by Western blot using semi-purified membrane extracts and polyclonal antibody against total EGFR (anti-EGFR) or its active receptor conformation (phosphorylated EGFR; pEGFR). EGFR and pEGFR expression was assessed by immunohistochemistry using longitudinal paraffin epididymis sections. EGFR expression on sperm collected from testis and different epididymal regions (IS+CP, CO and CD) was assessed by immunofluorescence studies. Negative controls were performed using primary antibody preadsorbed with excess of its repective blocking peptide. **Results**: Western blot using anti-EGFR showed that EGFR is expressed in the epididymis in its expected size (~175 kDa). Anti-pEGFR also revealed a significant increase in EGFR activation after 1-15 min EGF stimulation. Immunohistochemistry confirmed differential EGFR expression in the epithelium, interstitial cells and in sperm present in epididymal tubules along rat epididymis. Interestingly, pEGFR immunostaining was also differentially detected in these same cell types along epididymis, suggesting constitutive levels of activated receptor in this tissue. Immunofuorescence studies indicated significant changes in the pattern of EGFR expression in maturing sperm. In fact, EGFR was observed mainly in acrosome region of testicular sperm, along the head, middle piece and part of the principal piece of sperm from IS+CP, over the entire sperm from CO and located only in acrosome region of CD sperm. **Discussion**: Collectively, the data indicate that EGFR is differently expressed along rat epididymis and able to be activated in response to *in vitro* EGF stimulation. Constitutive expression of pEGFR also suggests that epididymal cells expressing EGFR are submitted to autocrine and/or paracrine effects of EGF produced in the epididymis or testis, respectively. More importantly, the dynamic in EGFR immunolocalization in the maturing sperm may suggest an important role for EGF/EGFR in events leading to sperm maturation and, consequently, to male fertility. Apoio Financeiro: FAPESP, CAPES, CNPq, Fogarty International Center

01.051

Metabolic programming induced by maternal protein deprivation activates NF-kB pathway and nitric oxide production by macrophages in adult progeny. Renovato-Martins, M.[1]; Salama Rodrigues, C.[2]; Vargas da Silva, S.[2]; Barja-Fidalgo, T. C.[2] [1]UERJ - Farmacologia e Psicobiologia; [2]UERJ - Farmacologia

Several studies have demonstrated that maternal nutritional imbalance during critical time windows of development leads to persistent effects on metabolism and cell homeostasis in offspring. Clinical observations and epidemiologic studies lend support to the concept that nutritional deficiencies increase the frequency and severity to infection. Our group has been studying the effects of metabolic programming induced by maternal protein deprivation during lactation on immune function of adult progeny. We are now evaluating the activity of the nitric oxide synthase (NOS) system of peritoneal macrophages isolated from adult rats offspring of dams fed with a diet containing 8% of protein (PD group) or a 22% protein diet (C group) during lactation. For this we evaluated the NO production, iNOS expression and the activity of NF-kB pathway in those cells. Peritoneal macrophages from PD rats incubated *in vitro* in RPMI medium for 24h spontaneously release high amounts of NO. These cells present basal iNOS expression and a decreased expression of IkB and increase of IKK phosphorylation. Contrasting to control cells, the treatment of PD-macrophages with 10 mg/mL LPS and 5ng/mL IFN-γ do not induce a further increase in iNOS expression or NO synthesis in these cells. The data indicate that peritoneal macrophages isolated from adult PD animals present a basal proinflammatory state, suggesting that metabolic programming induced by maternal protein deprivation during lactation affects the profile of innate immune response in adulthood and possibly impairs the mount of inflammatory response. Apoio Financeiro: FAPERJ, CNPq and CAPES

01.052

Interações do íon magnésio com bloqueadores neuromusculares não-despolarizantes: presença de sinergismo de adição ou de potenciação detectados por técnica de análise fracional. Oliveira, A. C. de; Serra, C. S. M. ICB-USP – Farmacologia

Introdução: Na junção neuromuscular, o magnésio diminui a liberação de acetilcolina pela terminação nervosa. Com isso ele pode reforçar a ação de bloqueadores neuromusculares. Teoricamente este reforço pode redundar em sinergismo de adição ou de potenciação. O presente trabalho avalia isto para 3 diferentes bloqueadores neuromusculares, utilizando técnica capaz de discernir entre esses dois tipos de sinergismo. **Métodos**: Os experimentos foram realizados *in vitro* utilizando a preparação nervo ciático-músculo extensor longo dos dedos do rato. Contrações isoladas indiretas foram geradas a 0,1 Hz. Registraram-se os efeitos, sobre essas contrações, do magnésio (Mg^{++}) e dos bloqueadores cisatracúrio (CIS), vecurônio (VEC) e rocurônio (ROC) aplicados isoladamente ou nas associações: Mg^{++}+CIS, Mg^{++}+ROC e Mg^{++}+VEC. Curvas dose-resposta cumulativas forneceram concentrações inibitórias 50 % (CI_{50}) para os compostos estudados isoladamente e para CIS, ROC e VEC estudados na presença de uma fração fixa da CI_{50} do Mg^{++} obtida estudando-o isoladamente. Os dados de associação foram analisados estatisticamente utilizando a análise fracional. Nesta técnica avalia-se se as CI_{50} dos compostos em associação, expressas como frações das CI_{50} obtidas para cada composto estudado isoladamente propiciam soma = 1(sinergismo de adição); < 1 (sinergismo de potenciação) ou > 1 (antagonismo). Com esse intuito, construíram-se, neste trabalho, intervalos de confiança (95%) para as CI_{50} do CIS, ROC e VEC obtidas na presença do Mg^{++} e expressas como frações das CI_{50} desses mesmos compostos obtidas estudando-os isoladamente. **Resultados**: As CI_{50} dos compostos estudados isoladamente foram: Mg^{++} (4,83 ± 0,15 milimolar), CIS (0,37 ± 0,03 micromolar), ROC (1,86 ± 0,06 micromolar) e VEC (0,77 ± 0,08 micromolar). As CI_{50} obtidas na presença do Mg^{++} (3 milimolar) foram: CIS (0,13 ± 0,03 micromolar), ROC (0,47 ± 0,04 micromolar) e VEC (0,32 ± 0,04 micromolar). Em todos os grupos os experimentos foram em número de cinco e os números entre parênteses correspondem a média aritmética ± erro padrão. Os intervalos de confiança obtidos na presença do Mg^{++} foram: CIS (0,87-1,15), ROC (0,86-0,98) e VEC (0,96-1,21). **Discussão e Conclusões**: De acordo com o critério explicitado em Métodos, os intervalos de confiança indicam que a associação com Mg^{++} propiciou um sinergismo de adição nos casos do CIS e do VEC e um sinergismo de potenciação no caso do ROC. Estes resultados não-homogêneos provavelmente decorrem de que os bloqueadores não-despolarizantes utilizados não tem um perfil de alterações celulares exatamente superponíveis, associando ao clássico efeito pós-sináptico competitivo efeitos pré-sinápticos cuja intensidade varia com o bloqueador.

Apoio Financeiro: FAPESP, CNPq, CAPES

01.053

Rhythmic expression of nuclear factor kappa B (NFKB) in Syrian hamster pineal gland – impact of endogenous glucocorticoid regulation. Ferreira, Z. S.[1]; Markus, R. P.[1]; Pevet, P.[2]; Simonneaux, V.[2] [1]IB-USP - Fisiologia; [2]INCI-ULP - Strasbourg - France - Neurobiologie des Rhythmes

Introduction: Recently we reported that NFKB in the rat pineals is constitutively activated and modulates melatonin synthesis, being also a target for cytokines and glucocorticoids (Ferreira *et al*., 2005; J.Pin.Res. 38:182; Fernandes *et al*., 2006; J.Pin.Res. 41:344). Differences in species have been reported for the nighttime regulation of AA-NAT. For *Aa-nat* transcription de novo synthesis of stimulatory transcription factors is required in the Syrian hamster, but not in the rat pineal (Sinitskaya *et al*. 2006; Endocrinol 147:5052). AIM: Considering species diversity and the role for NFKB in the control of *Aa-nat* gene in rat pineals, we addressed the question of NFKB expression and regulation in the Syrian hamster pineals. **Methods**: NFKB was analyzed by EMSA using nuclear extracts from pineals of female Syrian hamster (*Mesodricetus aura*tus) housed under 14:10 h light/dark cycle (LD) and sacrificed every 1-3 h along 24 h (n=3-6 per time point). **Results**: Three clear bands were visualized with nuclear extracts of Syrian hamsters pineals. The specificity of these three different NFKB-DNA complexes detected was confirmed by competition with specific and non-specific unlabeled oligonucleotides. A rhythmic variation in the active form of NFKB was found in nuclear extracts from animals sacrificed at different time points, with a peak of activity at the light phase of LD cycle (ZT10; 5 – 25 times over lowest value depending on the complex) and the lowest levels at ZT21. The effect of light on NFKB translocation was addressed in animals kept in constant light condition (LL) the night before the sacrifice, showing a decrease in the peak of NFKB nuclear translocation observed at ZT10 as compared to animals kept in LD. No shift in the peak translocation was observed, as it was also barely detected at ZT18 (n=6). A parallel increase in plasma levels of corticosterone at ZT10 was observed (1.56 ± 0.20 vs 6.20 ± 2.14 ng/ml; n=3; $p<0.05$). The modulatory effect of endogenous corticosterone on the nuclear translocation of NFKB was evidenced by the blockage of the glucocorticoid receptors. In animals under LL condition, mifepristone (RU486, 50 mg/kg, ip, at the beginning of the dark phase - ZT14) restored the nuclear translocation of NFKB as observed in the pineals of animals under LD. RU486 significantly increased the nuclear translocation of NFKB in all complexes observed as compared with non-treated animals (n=5). *In vivo* administration of PDTC (50-200 mg/kg, ip. ZT9), an antioxidant which has been shown to inhibit NFKB binding activity, significantly inhibited all the three NFKB-DNA binding complexes observed at ZT10 in a concentration-dependent manner. **Conclusion**: The present results show that the NFKB-DNA complexes are rhythmically expressed in Syrian hamster pineal glands being its activity inhibited by PDTC and constant light. Endogenous corticosterone, through GR activation, has an important role in the physiological control of NFKB translocation in Syrian hamster pineals. These data open an additional molecular pathway to investigate the regulation of *Aa-nat* transcription in the Syrian hamster. Apoio Financeiro: CAPES, COFECUB, FAPESP, CNPq.

01.054

Inhibition of NF-kappaB activation promotes resolution of established eosinophilic inflammation via induction of apoptosis. Sousa, L. P.[1]; Pinho, V.[2]; Carmo, A. F.[2]; Vieira A. T.[2]; Alessandri, A. L.[2]; Bonjardim, C. A.[3]; Rossi, A. G.[4]; Teixeira, M. M.[2] [1]UFMG - Patologia Clínica - COLTEC; [2]UFMG - Bioquímica e Imunologia; [3]UFMG - Microbiologia; [4]University of Edinburgh - Queen's Medical Research Institute

Introduction: The family of nuclear transcription factor-kappaB (NF-κB) is a key regulator of eosinophil activation and survival. NF-κB plays a pivotal role in the induction of genes involved in physiological processes as well as in the response to injury and inflammation. However, the intracellular mechanisms involving NF-κB on eosinophil permanence in tissue have not been completely examined. In the present study, we investigated the activation and composition of the NF-κB and the effect of its inhibition by gliotoxin and pyrrolidine dithiocarbamate (PDTC) on the eosinophil inflammatory resolution by accessing the permanence of leukocyte by cell count and apoptosis based assays in the pleural cavity after ovalbumin (OVA) challenge of sensitized mice. **Methods**: C57Bl/6 mice were immunized s.c. (subcutaneous) on days 1 and 8 with 0.2 ml of a solution containing 100 µg of OVA and 70 µg of aluminum hydroxide. Sensitized mice were challenged by i.pl. (intrapleural) administration of OVA or PBS. Gliotoxin (0.8 mg/kg) and PDTC (100 mg/kg) was given locally (i.pl.) 24 h after the administration of OVA. The cells present in the pleural cavity were harvested at different times of antigen challenge or after 24 h of treatment with NF-κB inhibitors and processed for total and differential leukocyte count, western blot and EMSA analysis for NF-κB activation and analysis of apoptosis. **Results**: Administration of antigen to immunized mice induced NF-κB activation, as assessed by DNA-binding activity, p65/p50 nuclear translocation and IkB-α phosphorylation. The kinetics of NF-κB activation temporally correlated with the total leukocyte, eosinophil and mononuclear cell influx into the pleural cavity. Treatment with two structurally different NF-κB inhibitors and the synthetic glucocorticoid dexamethasone at the peak of inflammation, when inflammatory cell influx was established, reduced NF-κB activation and resulted in a profound enhancement in the resolution of eosinophilic inflammation, without a decrease of mononuclear cell numbers. This effect was accompanied by an increase in apoptotic events in the cells into the pleural cavity, as assessed by morphologic criteria, annexin-V binding, DNA fragmentation and caspase-3 activation. **Discussion**: Our results showed that NF-κB is activated in allergic pleurisy in mice and its inhibition, after the inflammatory response induction, reduces the number of eosinophils presents in pleural cavity and increases the number of apoptotic cells, suggesting that treatment with NF-κB inhibitors may represent a novel therapeutic strategy for the treatment of established eosinophilic inflammation. Apoio Financeiro: CNPq

01.055

Identification of inverse agonism of atropine at denervated skeletal muscle is linked to constitutively active muscarinic receptors. Andrade-Lopes, A. L.; Chiavegatti, T.; Pires-Oliveira, M.; Godinho, R. O. UNIFESP – Farmacologia

Introduction: Studies from our group showed that G-protein coupled muscarinic acetylcholine receptors (mAChR) are expressed at noninnervated skeletal muscle, being downregulated by neuromuscular synapse formation and upregulated following adult muscle denervation, which suggests that mAChR could contribute to early development of the neuromuscular synapse and to reinnervation of adult skeletal muscle. Intriguingly, these receptors are abundant in situations where the endogenous agonist acetylcholine is absent. Considering these facts, we studied mAChR functionality at denervated skeletal muscle evaluating receptor coupling to specific Gα isoforms, in the presence or absence of muscarinic agonists. **Methods**: Adult male Wistar rats were phrenicectomized for 7 to 28 days, the hemi-diaphragms were removed and membrane fractions (50 µg/well, n=5) were incubated with 0.1 nM [^{35}S]GTPγS (non-hydrolysable GTP analogue) and 50 µM GDP, in the absence or presence of oxotremorine-M (Oxo M, 0-100 µM) and/or 100 nM atropine. Nonspecific binding was determined with 50 µM unlabeled GTPγS. To discriminate the activated Gα isoforms, [^{35}S]GTPγS functional binding assay using membrane from 14-day-denervated muscles was followed by immunoprecipitation using antibodies against Gαs, Gαq and Gαi. cAMP production was also measured in these membranes after Oxo-M and/or atropine incubation. **Results**: Oxo-M stimulated [^{35}S]GTPγS binding (basal = 32.1 ± 2.4 fmol/ mg protein) increasing by 54% and 46% the activation of Gαs and Gαi, respectively, with no effect on Gαq. Oxo-M (1-100 µM) also reduced by 20% cAMP production, indicating the preferential coupling of mAChR to Gi protein. Interestingly, atropine alone (100 nM) reduced by 50% and 20% the [^{35}S]GTPγS basal binding and cAMP production. The negative efficacy of atropine alone (inverse agonism) indicates that at least part of mAChR expressed at denervated diaphragm is constitutively active and coupled to Gs protein. **Discussion**: Our results show that mAChR expressed at denervated skeletal muscle could promiscuously activate Gi and Gs proteins when occupied by an agonist. Even more relevant is the fact that a fraction of mAChR could activate Gs protein in an agonist-independent manner, which is consistent with the negative efficacy of atropine. This phenomenon may contribute to physiological adaptation of muscle fiber to neurotrophic factor deprivation, including the main neurotransmitter acetylcholine. The ability of receptors to activate G-protein in the absence of an agonist has changed the classical concepts of pharmacology, increasing the complexity of GPCR signaling mechanisms. Apoio Financeiro: FAPESP and CNPq

01.056

PRÊMIO INOVAÇÃO