01. Cellular and Molecular Pharmacology

01.001 Mechanism of action of LASSBio-579, an N-Phenylpiperazine Compound Elected as an atypical antipsychotic drug candidate. Pompeu TET[1], do Monte FM[1], Hermans E[2], Menegatti R[3], Fraga CAM[4], Barreiro EJ[5], Noël F[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]Université Catholique de Louvain – Neurociências, [3]UFG – Farmácia, [4]UFRJ – Farmacologia e Química Medicinal, [5]UFRJ – Ciências Biomédicas

Introduction: We previously elected the *N*-phenylpiperazine LASSBio-579 as atypical antipsychotic lead compound based on extensive in vitro and in vivo assays and defined as multi-target ligand of D_2-*like*/D_4/5-HT_{1A} receptors. We also identified its main active metabolite (*p*-hydroxylated derivative LQFM 037). **Aims:** In an attempt to better understand the molecular mechanism of action of these compounds, we evaluated their intrinsic activity and binding kinetics at the dopamine D_2 receptor, and also their putative effect on glutamatergic neurotransmission. **Methods:** HeLa cells overexpressing D_{2L} receptor were maintained in a complete medium until confluence. After cell lysis, binding experiments were carried out with [^{35}S]-GTPγS for 40 min at 30°C using NaCl or NMDG (*N*-methyl-D-glucamine) buffer. The kinetic of [^{3}H]-YM09151-2 binding to rat striatal D_2 receptors was determined in the absence and presence of a single concentration of the unlabeled competitors. The binding experiments to mGluR2 were carried out with [^{3}H]-glutamate for 30 min at 30°C using human mGLR2 transfected cells and the experiments to AMPA, Kainato and NMDA were custom by CEREP. The inhibition of the [^{3}H]-D-aspartate uptake was evaluated in different cell cultures transfected with glutamate transporters GLAST, GLT-1a/b, EAAC1 and also in astrocytes culture. **Results and Conclusions:** In the NaCl buffer, LASSBio-579 and LQFM 037 elicited a modest increase in the specific [^{35}S]-GTPγS binding. When NaCl was replaced by NMDG, our compounds stimulated [^{35}S]-GTPγS binding in a concentration-dependent manner, with E_{max} of 34 ± 2.1% and 19.4 ± 2.1%, respectively, whether clozapine was without effect. To confirm the partial agonist effect of our compounds, we used the full D_2 receptor agonist, dopamine. In the NaCl buffer, LASSBio-579 and LQFM 037 antagonized the stimulation of [^{35}S]-GTPγS binding promoted by 1 µM dopamine, with IC_{50} of 1.8 and 0.8 µM, respectively. In the NMDG buffer, the antagonism exerted was less obvious, with a shift to the right of the inhibition curves, confirming that LASSBio-579 and LQFM 037 behave as weak partial agonists at the D_{2L} receptor, as aripiprazole but unlike clozapine. In the competition association assay, the time for [^{3}H]-YM09151-2 to reach 50% equilibrium ($t_{1/2}$ = 5.1 ± 0.27 min) was significantly increased in the presence of clozapine (12.6 ± 0.96) but not of haloperidol (7.70 ± 0.63). The effect of LASSBio-579 and LQFM 037 was similar to clozapine, increasing the radioligand $t_{1/2}$ (16.2 ± 2.3 and 17.9 ± 1.8, respectively) indicating a rapid dissociation from the D_2 receptor. These two characteristics could contribute to the atypical-like profile observed after administration of LASSBio-579 to rodents (Pompeu, Prog Neuropsychopharmacol Biol Psychiatry 62:1, 2015), since neither LASSBio-579 and LQFM 037 nor clozapine were able to significantly inhibit [^{3}H]-D-aspartate uptake in any of the cell clones used (GLT-1a/b, GLAST or EAAC1) or in astrocytes, and LASSBio-579 had no affinity for any glutamatergic receptors tested in the binding experiments. **Financial support:** CAPES, INCT- INOFAR. **Ethics Committee:** license DFBC-ICB-011

01.002 Age-related adaptive effects of intermittent fasting during neuroinflammation. Vasconcelos AR[1], Yshii LM[1], Kinoshita PF[1], Böhmer AE[1], Orellana AMM[1], de Sá Lima L[1], Alves R[1], Andreotti DZ[1], Marcourakis T[1], Viel TA[1], Buck HS[2], Mattson MP[3], Scavone C[1], Kawamoto EM[1] [1]USP, [2]Santa Casa de São Paulo, [3]NIH

Introduction: Neuroinflammation is a common characteristic of neurodegenerative disorders that may contribute to loss of function and cell death. Sepsis induced by lipopolysaccharide (LPS) causes neuroinflammation and **Results** in enduring cognitive impairment which is a risk factor for dementia. There are currently no effective treatments for infection-induced cognitive impairment. Previous studies showed intermittent fasting (IF) can increase the resistance of neurons to disease by stimulating adaptive cellular stress responses. However, the impact of IF on the cognitive sequelae of inflammation is unknown. Moreover, LPS signaling is linked to nitric oxide (NO)-Na,K-ATPase pathway in the central nervous system (CNS). IF can suppress inflammation, but the age-related effects of IF on LPS modulatory influence on NO-Na,K-ATPase pathway are unknown. **Aims:** This work evaluated whether IF might modify the adverse effects of systemic inflammation induced by LPS on cognitive function. Also, it was investigated the effects of IF on age-related changes on the activity of α_1,$\alpha_{2,3}$-Na,K-ATPase and oxidative status induced by LPS in rat hippocampus. **Methods**: 4 month-old (mo) Wistar rats on IF for 30 days received LPS (1mg/kg) or saline intravenously. Half of the rats were subjected to behavioral tests(open field, rotarod, Barnes maze and inhibitory avoidance) and the other half were euthanized two hours after LPS administration and the hippocampus was dissected and frozen for analyses of inflammatory parameters(cytokines and brain-derived neurotrophic factor (BDNF) levels, RelA nuclear protein expression, nuclear factor κB (NF-κB) binding activity, toll-like receptor (Tlr)-4 (LPS receptor) and inducible NO synthase (iNos) mRNA levels. Moreover, young (4-mo), adult (12-mo), and aged (24-mo) rats were submitted to the same protocol and the hippocampus was dissected for measurements of α1,α2,3-Na,K-ATPase activity, NOS gene expression and/or activity, cyclic GMP, 3-nitrotyrosine (3-NT)-containing proteins, and thiobarbituric acid reactive substances (TBARS). **Results**: LPS treatment resulted in cognitive deficits in the Barnes maze and inhibitory avoidance task which were ameliorated in rats that were subjected to the IF protocol, with no changes in locomotor activity. IF also: prevented or attenuated LPS-induced elevation of cytokines, iNos and Tlr4 levels in hippocampus and/or serum; prevented LPS-induced BDNF reduction in hippocampus; blocked or mitigated the age-related decline of cGMP and Na,K-ATPase activity, and the increase of iNos gene expression, TBARS, and 3-NT proteins in the presence or absence of LPS. **Conclusion:** Taken together, our results suggest that IF induced adaptive responses in the brain and periphery that can suppress inflammation and preserve cognitive function in an animal model of sepsis. Therefore, IF could reduce the risk for brain function deficits and neurodegenerative disorders linked to inflammatory response in the CNS during aging. **Financial support**: FAPESP, CNPq. All procedures were approved by the Biomedical College of Animal Experimentation and the Ethical Committee for Animal Research ICB/USP (89 fls. 60, book 02).

01.003 Lipid rafts disruption and effects on the migration of tumour cells line MDA-MB 231. Guerra FS[1], Costa ML[2], Fernandes PD[1], Mermelstein C[2] [1]UFRJ – Farmacologia e Química Medicinal, [2]UFRJ – Biologia Celular e Molecular

Introduction: Lipid rafts are cholesterol-rich microdomains and play critical roles in the regulation of several membrane receptors, cell adhesion, migration, proliferation and protein sorting during endocytosis and exocytosis. Cholesterol depletion **Results** in the disorganisation of lipid raft microdomains and also the dissociation of proteins that are bound to the lipid raft. Methyl beta cyclodextrin (MCD) is an agent that can selectively remove cholesterol from the plasma membrane, and has been widely used in studying the effects of cholesterol depletion on lipid raft assembly. Therefore, the study of the participation of rafts in events such as migration, differentiation and cell proliferation may contribute to the discovery of new drugs for inhibiting tumour metastasis. **Aims:** The aim is to elucidate the effects of disruption of lipid rafts through the removal of cholesterol by the action of MCD and the effects on cell migration in tumour cell line MDA-MB 231(ATCC®HTB-26™). **Methods**: For the tests, MCD concentration was 2 mM. The migration was evaluated using wound healing assay in which a scraping is made in the cell monolayer and subject to the closing of the shaved area by the migration of the cells for 24 hours. Morphological analysis was carried out by phase-contrast micrograph, and analysed using Image J software. The tracking of the cells was also done by Image J software after the timelapse of the cells for 24 hours. The dosage of IL-6 and IL-10 was performed by ELISA using reagents kit. Immunofluorescence assays were performed with anti-vimentin antibody. Statistical analyses were performed by ANOVA with Newman-Keuls- post-test (*$p<0.05$). **Results**: The effect of removal of the membrane cholesterol decreased migration of MDA-MB 231 cells by 63.9% when compared to control. Through the morphological analysis, cells with disorganised rafts presented area, height and width reduced by 77%, fusiform and protrusions membrane with filopodia, different from control cells that had sprawling, with cytoplasm extended and forming lamelipódios. Tracking of the cells monitored for 24 hours showed that the total distance travelled by the cells with disorganised rafts was reduced by 42.3% and the speed was reduced by 36.1% compared to control. The concentration of IL-6 secreted by the cells with disrupted rafts was 25% greater than the control cells and the concentration of IL-10 was 47.9% higher when compared to control. In the immunofluorescence assay of vimentin, a predominantly perinuclear marking was observed with less pronounced marking in the cytoplasm of cells with disorganised rafts when compared to the control. **Conclusions**: The **Results** showed that the removal of the membrane cholesterol reduces migration of MDA-MB 231 cells, alters cell morphology and disrupts the intermediate filaments, which are essential for cell movement during migration. Secretion of cytokines such as IL-6 and IL-10 are also increased by disruption of lipid rafts. These **Results** add to a better understanding of the process of cell migration and contribute to the discovery of drugs that act to reduce tumour metastasis processes. **Financial support**: CAPES, CNPq, FAPERJ

01.004 Modulation of lipopolysaccharide-induced immune response in raw 267.4 macrophages: role of insulin and cholecalciferol. Bella LM[1], Tessaro FHG[1], Nolasco EL[1], Ayala TS[1], Azevedo CB[2], Martins JO[1] [1]FCF-USP – Análises Clínicas, [2]Unifesp-EPM – Disciplina de Reumatologia

Introduction: Macrophages are key cells in the immune response against pathogens. Cytokines are soluble molecules important in this process, that can be modulated by several hormones, including insulin and vitamin D. The immunomodulating activity of vitamin D has been described by calcitriol activity. However, there are no studies that show the effect of cholecalciferol (also known as previtamin D) and insulin on cytokine release. **Aims:** This study evaluated the effects of cholecalciferol and insulin in the release of interleukin (IL) 1β, IL-10 and nitric oxide (NO) production by RAW 264.7 macrophages. **Methods:** Murine RAW 264.7 macrophages (between 8^{th} and 9^{th} passages) were cultured in hormone-free medium (DMEM) supplemented with penicillin (40 U/mL) and streptomycin (50 µg/mL). Cells ($2x10^5$) were plated in 12-well plates and maintained at 37°C with 5% CO_2 in a humidified atmosphere for 24h. Cells were divided into twelve groups (n= 4): control; insulin (1mU/mL); LPS (100ng/mL); vitamin D (1nM); vitamin D (10nM); vitamin D (100nM); LPS + vitamin D (1nM); LPS + vitamin D (10nM); LPS + vitamin D (100nM); LPS + insulin + vitamin D (1nM); LPS + insulin + vitamin D (10nM); LPS + insulin + vitamin D (100nM). IL 1β, IL-10 were measured by ELISA and NO by Griess reaction. Data are expressed mean± SEM. Statistical comparisons were made using Student's t- test. Values of $p<0.05$ were considered statistically significant. **Results:** Cells threated with 1nM of cholecalciferol (131,92±6,53 pg/mL) released more IL-10 than untreated (93,46±2,05 pg/mL) cells ($p<0,001$). Cells stimulated with LPS and threated with vitamin D (1 nM= 156,44±9,04 pg/mL, $p<0,001$; 10 nM= 172,87±10,18 pg/mL; $p<0,001$; 100 nM= 159,18±8,94 pg/mL; $p<0,001$) and Insulin showed less IL-10 compared with cells stimulated (4327,81± 353,42 pg/mL) with LPS. Cells threated with cholecalciferol (1 nM = 7,80±0,27 µM; 10 µM= 7,97±0,30 µM) released less NO than untreated cells (9,45±0,30 µM; $p<0,01$ and $p<0,05$, respectively). Cells stimulated with LPS and threated with vitamin D (1 nM= 16,95±0,46 µM, $p<0,001$; 10 nM= 19,37±0,23 µM, $p<0,01$; 100 nM= 16,19±0,41 µM, $p<0,001$) released less NO compared with cells stimulated with LPS (21,84±0,61 µM). Cells stimulated with LPS and threated with vitamin D (1 nM= 13.27±0.28 µM, $p<0,001$; 10 nM= 14,88±0,37 µM, $p<0,001$; 100 nM= 13,53±0,24 µM, $p<0,001$) and Insulin showed less NO compared with cells stimulated with LPS (21.84±0.61 µM). No differences were observed in IL-1β release between treated groups with vitamin D and their respective controls. **Conclusions:** Taking together, these **Results** suggest that insulin and cholecalciferol might decrease the release of IL-10 and NO by LPS-induced RAW 267.4 cell. **Financial support:** FAPESP (2010/02272-0; 2014/05214-1), CAPES, CNPq (Universal), Projeto1 (PRP/USP).

01.005 LDT5 Prevents the increase of rat intra-urethral pressure without causing a hypotensive effect. Nascimento-Viana JB[1], Romeiro LAS[2], Noël F[1], Silva CLM[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]UnB – Lab. Desenvolvimento de Estratégias Terapêuticas

Introduction: Prostate contraction increases the intraurethral pressure (IUP) which reduces bladder empty, and therefore it is partly responsible for the lower urinary tract symptoms (LUTS). LUTS are the major complaint among aging men and they are usually caused by benign prostatic hyperplasia (BPH). The usual pharmacological treatment of BPH is the use of the α_{1A}-AR antagonist tamsulosin. Previous data of our group showed that 1-(2-methoxyphenyl)-4-[2-(3,4-dimethoxyphenyl)ethyl]piperazine (LDT5) has high affinity for α_{1A}-adrenoceptor (AR) (K_B =0.18 nM, n=13) and α_{1D}-AR (0.59 nM, n=8). LDT5 has also high affinity for the 5-HT_{1A} receptors (K_i =2.54 nM) acting as a multi-target antagonist with low affinity for others receptors analyzed, such as α_{1B}-AR. **Aims:** To evaluate the effect of LDT5 on the increase of IUP induced by phenylephrine (PHE) and to estimate the higher dose of LDT5 without significant effect on normotensive rat blood pressure. **Methods**: For these assays, male Wistar rats were anesthetized with pentobarbital sodium (60 mg/Kg, i.p.), each animal was used only once, and all drugs were diluted in isotonic saline. The jugular vein were cannulated for administration of drugs. Intraurethral pressure (IUP): The prostate and bladder were exposed through a midline incision in the lower abdomen. The fluid filled pressure transducer was placed into the prostatic urethra through the bladder and fixed with a suture at the vesical-urethral junction and the distal side of the urethra. The IUP was equilibrated at 20 mmHg by injecting a small volume of saline. After approximately 30 min, IUP was increased by an i.v. administration of 1-100 µg/kg PHE every 10 min (PHE dose-effect curve). Alternatively, a single i.v. (in bolus) dose of 30 µg/kg PHE was injected 10 min before, and 10 min after, the administration of 0.01 - 3 µg/kg LDT5, or 100 µl vehicle (saline), for determination of the LDT5 dose-effect curve (inhibition of PHE-induced increase of IUP). Blood pressure assay: carotid artery was cannulated for connection of the pressure transducer. IUP and blood pressure (mmHg) were monitored continuously (PowerLab, ADInstruments). Data were analyzed by nonlinear regression (GraphPad Prism 5.0) and expressed as mean and SEM. **Results and Conclusions**: The animals pretreated with LDT5 were fully protected (101.7 ± 11.4 % maximal inhibition) from PHE-induced increase of IUP, with a DE_{50} of 0.092 µg/Kg (95% C.I.: 0.044 - 0.191). The administration of LDT5 until the dose of 10 ug/Kg did not alter the basal arterial blood pressure of normotensive rats when compared to saline. This dose is 100 times higher than the DE_{50} for the inhibitory effect of LDT5 on the PHE-induced increase of IUP. Therefore, in terms of blood pressure-related adverse effects, it is plausible to suggest that LDT5 is a safe candidate for the treatment of BPH and associated disorders. These **Results** support the status of LDT5 as our lead compound and allow to move it forward into the drug development process aiming the treatment of BPH. **Financial support**: CNPq, CAPES and FAPERJ. All protocols were approved by the Ethics Committee of UFRJ (DFBC/ICB011).

01.006 Extracellular cyclic AMP: "third messenger" activity in vas deferens contraction? Moro RP, Pacini ESA, Godinho RO Unifesp-EPM – Farmacologia

Introduction: The vas deferens is a muscular tube in the male reproductive system responsible for transporting sperm cells from the epididymis to the urethra. During ejaculation, the vas deferens smooth muscle from both the prostatic and the epididymal portions contracts, and thus propels the sperm forward. Among the molecular mechanisms which regulate smooth muscle contraction, those that increase the intracellular concentration of the second messenger cyclic AMP (cAMP) are distinguished. The activation of receptors coupled to stimulatory G protein (GsPCR) leads to the stimulation of the enzyme adenylyl cyclase (AC), which in turn increases intracellular cAMP concentration. Moreover, cAMP may have an extracellular signaling function (Chiavegatti *et al.,* Br J Pharmacol, 153: 1331, 2008). This property is enabled by the transport of this signaling molecule across the plasma membrane and its conversion into adenosine by ecto-enzymes, in a process called "extracellular cAMP-adenosine pathway" (Jackson *et al.,* Am J Physiol Renal Physiol 281: 597, 2001). Some studies have shown a relevant feedback function of "extracellular cAMP-adenosine pathway" in different tissues and systems (Godinho *et al.,* Front Pharmacol 6: 58, 2015), but there is no evidence of its possible role in modulating contractile responses of the vas deferens. **Aims:** Herein, we evaluated the potential influence of extracellular cAMP in vas deferens contraction. **Methods**: The effects of cAMP, 8-Br-cAMP and adenosine in isometric contractions of rat vas deferens were investigated. Briefly, 1 cm of the epididymal portion of the vas deferens from male adult Wistar rats were isolated and placed in an organ bath filled with Tyrode´s solution. After a 30 min stabilization period, isometric contraction was elicited by 30 mM KCl and 15 minutes later, the effects of 300 µM adenosine, 1 mM cAMP or 100 µM 8-Br-cAMP were studied. The contractions (grams of tension, g) were expressed as mean ± s.e.m. **Results**: KCl produced a biphasic contraction in rat vas deferens, including an initial phasic component that reached 0.45 ± 0.1g and a secondary tonic component of 0.26 ± 0.04 g (n=6). Adenosine increased by up to 81 ± 10% the KCl-induced contraction (n=6). Surprisingly, although cAMP also enhanced the contraction up to 47 ± 11% (n=4), its cell permeable hydrolysis-resistant analog 8-Br-AMPc induced a relaxation that reached 38 ± 11% of basal KCl contraction (n=4). **Conclusion:** Considering that cAMP is not able to move across the cell membrane (Robison e*t al.,* Mol Pharmacol, 1: 168, 1965), this nucleotide would be acting as an extracellular signaling molecule in the vas deferens smooth muscle. Thus, our data indicate that (1) the extracellular cAMP is relevant in the contractile response of the vas deferens and (2) the final cAMP inotropic effect depends on the site of action, i.e., either the intracellular or extracellular compartments. Evaluating the possible contribution of the extracellular cAMP-adenosine pathway in the contractile response of the vas deferens, thus, becomes relevant and may provide new insights regarding how changes in vas deferens contraction might influence male fertility. **Financial support**: Fapesp, CAPES e CNPq
Animal Ethical Committee: CEUA #8359060315

01.007 Changes of heart, kidney and brain Na/K-ATPase in rats with ouabain-induced hypertension. Feijó PRO[1], Neto A[2], Rossoni LV[2], Noël F[1], Quintas LEM[1]
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Introduction: Na^+/K^+-ATPase is an integral membrane protein that transports Na^+ and K^+ against their electrochemical gradients through the hydrolysis of ATP and is involved in diverse physiological functions. It presents a heteromeric structure composed by α and β subunits, whose isoforms are expressed according to the tissue and species. The α-subunit has binding sites for cardiotonic steroids (CTS), such as ouabain. These compounds act by inhibiting Na^+/K^+-ATPase activity. However, more recently it was discovered that binding of CTS to Na^+/K^+-ATPase leads to the activation of intracellular signaling cascades by protein tyrosine kinase Src. Ouabain, which is an endogenous mammalian CTS, induces hypertension in rats when administered chronically, but the effect on the Na^+/K^+-ATPase of tissues involved in controlling blood pressure are poorly understood. **Purpose**: Our goal was to evaluate the effect of chronic treatment with ouabain on the activity and expression of Na^+/K^+-ATPase from rat heart, kidney and brain. **Methods**: Male Wistar rats weighing between 320-450g (6 weeks, n=6) were treated with ouabain (OUA, 8 mg/day subcutaneously) or vehicle (VEH) for 20 weeks. Kidneys, brain and both heart ventricles were dissected, weighed and stored at -80°C. Then, they were homogenized and the pellets from ultracentrifugation were resuspended and used for enzyme activity experiments (Na^+/K^+-ATPase), and protein expression determined by Western blot technique. All animal procedures were approved by the Ethics Committee on Animal Experiments of ICB/USP (protocol: 034/2012). Values are expressed as mean ± SEM and statistical analysis was performed by Student t test ($p < 0.05$ was considered statistically significant). **Results**: There was no cardiac and renal hypertrophy in this model. We observed an increased activity of kidney Na^+/K^+-ATPase (VEH: 9.1 ± 0.5 vs OUA: 11.8 ± 1.1 µmol Pi/mg/h, n=6), but there was no significant changes in enzyme activity in the other tissues. We also detected an increased expression of renal Na^+/K^+-ATPase α1 isoform (VEH: 100 ± 21.6 vs OUA: 187 ± 16%, n=5-6) and α2 in the left ventricle (VEH: 100 ± 8.3 vs OUA: 165 ± 27.6, n=5-6) and a reduced α3 in the brain (VEH: 100 ± 4.9 vs OUA: 41.8 ± 4.7, n=5-6). **Conclusion:** The increase of kidney Na^+/K^+-ATPase may be involved in enhancing electrolyte reabsorption and blood pressure, while increase of cardiac α2 may be an adaptive mechanism of Ca^{2+} overload restriction. Experiments for the evaluation of intracellular signaling pathways and the specific role of hypertension in this process are ongoing. **Financial support**: CAPES, FAPERJ and CNPQ.

01.008 Fast dissociation of LASSBio-579 and its p-Hydroxylated derivative at the Dopamine D2 receptor. Monte FM[1], Pompeu TET[1], Bosier B, Fraga CAM[2], Menegatti R[3], Noël F[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]UFRJ, [3]UFG

Introduction: Schizophrenia is a severe disorder characterized by profound disruptions in thinking, language, perception and the sense of self. Pharmacological treatment with typical antipsychotics, as haloperidol and chlorpromazine, are effective against positive symptoms but often cause extrapyramidal symptoms (EPS). On the other hand, the atypical antipsychotics, as clozapine and risperidone, are more efficacious for improving negative symptoms and are associated with lower incidence of EPS but present metabolic disturbances. Typical antipsychotics are antagonists of the D_2 receptors, while the atypical are multi-target drugs blocking the D_2, 5-HT_{2A} and others receptors. The "fast-off" theory proposes that atypical drugs are loosely bound and quickly released from D2 receptors in the synapse, as an explanation for their lower propensity to induce EPS and hyperprolactinemia (Ginovart, Handb Exp Pharmacol 212:27, 2012). **Aims:** To better understand the molecular mechanism of action of our atypical antipsychotic lead compound LASSBio-579, and of its main metabolite (LQFM037), by testing the hypothesis of fast-off binding at the dopamine D2 receptor. **Methods**: The binding kinetics was evaluated using the competition association assay (Motulsky Mol Pharmacol 25:1, 1983) with a single concentration of LASSBio-579, LQFM037, clozapine or haloperidol, around their Ki values. The assay was initiated by adding 50 µg of rat striatal membranes in a buffer with 0.2 nM [3H]-YM-09151-2 at 37°C in the absence or presence of our unlabeled compounds. The non-specific binding was estimated using 30 µM sulpiride. At different time points, samples were rapidly filtered under vacuum on glass fiber filters The binding kinetics was estimated through the values of half-time to reach equilibrium (t1/2) and kinetic rate index (KRI= binding at 5 min divided by the value at 60 min, as proposed by Guo et al. (Biomol Screen 18:309, 2013). **Results** and **Conclusion:** The time for [3H]-YM09151-2 to reach equilibrium was much more ($p<0.05$) increased in the presence of clozapine (t1/2= 12.6 min) than haloperidol (t1/2=7.7 min). The KRI of LASSBio-579 (0.230) and LQFM 037 (0.190) were comparable to clozapine (0.250) but significantly lower than of haloperidol (0.360, $p<0.05$ - one way ANOVA followed by Newman–Keuls test) indicating that our compounds have a binding kinetic profile similar to clozapine in vitro, i.e. a rapid dissociation from the target receptor, at variance with haloperidol. This characteristic could contribute to the atypical-like profile observed after administration of LASSBio-579 to rodents, in different models of positive and negative symptoms.

01.009 Evaluation of the bone morphogenetic protein 9 role in neonatal rat islets maturation. Silva PMR[1], Leite AR[2], Santos GJ[3], Lellis-Santos C[4], Boschero AC[3], Caperuto LC[4], Gomes PR[1], Anhê GF[1], Bordin S[2] [1]FCM-Unicamp, [2]ICB-USP, [3]IB-UNICAMP, [4]Unifesp

Introduction: Pancreatic β cell function during postnatal development is incompletely understood. It has been proposed that Bone Morphogenetic Proteins (BMPs) should be considered a secreted factor that stimulates insulin production and secretion. BMPs trigger the phosphorylation of transcriptional regulators Smads (Mothers Against Decapentaplegic Homolog), particularly the BMP specific receptor-regulated Smads (BR-Smads) Smad1, Smad5 and Smad8. To date, there are few but strong evidences that BMP-Smads pathway triggers a signaling response that leads to improved cell function. A high-throughput functional genomics screening has revealed that BMP9 improves the glycemic control by the regulation of insulin-mediated glucose uptake, hepatic gluconeogeneis and glucose-stimulated insulin secretion (GSIS). **Aims:** In this study, we investigate how BMP9/BR-Smad pathway may control the maturation of the secretory response in neonatal rat islets. **Methods**: Islets from male and female newborn (up to 2 days-old) Wistar rats were isolated and maintained in culture for 1 and 3 days in RPMI-1640 media supplemented with 11.1 mMglucose, 5%fetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin. The culture medium was daily replaced by a fresh medium, containing or not BMP9 (10ng/ml) or anti-BMP9 antibody (20ng/ml). At the end of the culture period, the islets were collected and pooled for quantitative PCR (qPCR), immunoblotting, chromatin immunoprecipitation (ChIP) assay, and insulin secretion. **Results**: The expression of the BMP-signaling protein Smad1 – but not Smad5 and Smad8 – paralleled the expression of several genes related to the maturation of cell function, as well as the glucose responsiveness. ChIP assay analysis showed an increase in the association of Smad1 to the Glut2 gene promoter during islet maturation. BMP9-treated islets showed a further increase in Glut2 expression, which was abrogated by anti-BMP9 antibody treatment. Glucose-stimulated insulin secretion (GSIS) was abolished by anti-BMP9 antibody, and was enhanced by recombinant BMP9 treatment. **Conclusions**: Our results indicate a unique function of BMP-Smad1 in the regulation of Glut2 expression and in the maturation of GSIS. **Financial support**: FAPESP. Animal Research Ethical Committee Number: 3570-1.

01.010 Effects of the anti-aging hormone Klotho on AKT/FoxO signaling in the central nervous system. Mazucanti C, Cararo M, Sala T, Yshii LM, Scavone C USP – Ciências Biomédicas

Introduction: Klotho gene, a senescence associated gene, was originally identified by insertional mutagenesis in mice and codifies a single pass type I transmembrane protein, known as Klotho. Klotho protein has been considered an aging phenotype repressor, since its suppression in mice precipitates age-related characteristics, such as arteriosclerosis, osteoporosis, skin atrophy, infertility, early thymus involution and pulmonary emphysema, as early as 4 weeks old. Life expectancy of these mice is very low, normally not greater than 8 weeks. Membrane-bound Klotho acts as an obligatory FGF23 co-receptor, hence playing a crucial role on phosphate metabolism. By suffering a shedding process Klotho can be cleaved from its transmembrane domain and act as a regulatory hormone, acting upon ion channels (e.g. TRPV5 and ROMK1) and repressing Wnt and Insulin/IGF signaling. FoxO is a class of transcription factors known to be regulated by insulin signaling and that has been for quite some time related to anti-aging properties. **Objectives**: The objective of this work is to study the influence of Klotho protein upon insulin signaling in the hippocampus of mice lacking Klotho protein, tracking its effects on FoxO phosphorylation and activation state. **Methods**: 2 months old, male klotho knockout mice (and its genotype variants), were euthanized and its hippocampi dissected and collected for western blotting analysis. AKT, mTOR, FoxO1a and FoxO3 phosphorylation states were assessed by calculating the ratio between phosphorylated form and total form of such proteins. For astrocyte-rich cultures, 3 days old pups were decapitated and its cerebral cortices dissected and digested (both enzymatically and mechanically) for primary cell culture. Culture was maintained in a humidified CO2 incubator, in DMEM supplemented with FBS 10% until they reach 100% confluence. At this point, cultures were shaken in an orbital shaker, at 37oC, 180 RPM for 15 hours. Astrocytes, strongly attached to the bottom, were then pre-treated with recombinant Klotho (0,5nM) for 24 hours, and then challenged with H2O2 500µM for 30 minutes. Cell viability was assessed via MTT reduction. **Results**: It is clear that in the hippocampi of Klotho knockout mice, AKT signaling is enhanced and its phosphorylation on Ser473 induces inactivation of both Foxo1a and FoxO3. Klotho-treated astrocytes displayed higher viability when challenged with H2O2, linking FoxO activity with anti-oxidant defense. **Conclusions**: Klotho can modulate insulin signaling and therefore modify FoxO activity. Anti-oxidant properties of this transcription factor may be correlated to its effects as an aging suppressor. **Financial Support**: FAPESP, CAPES, CNPq. This study was approved by the Institute's Animal Research Ethical Committee (CEUA/ICB USP 63, 07, 03).

01.011 Cytotoxicity and chemotactic activity of L-Amino Acid Oxidase from *Bothrops jararaca* snake venom in rat lung macrophages. Fonseca FV[1], Panunto PC[1], Pereira BB[1], Marcelino EP[1], Torres-Huaco FD[1], da Silva IRF[1], Hyslop S[1] – [1]FCM-Unicamp – Bioquímica e Farmacologia

Introduction: Snake venom L-amino acid oxidases (LAAO) catalyze the oxidative deamination of stereospecific L-amino acids. LAAOs have various biological activities, e.g., cytotoxicity, induction of apoptosis, inhibition of platelet aggregation, etc., but their role in venom-induced inflammation remains poorly understood. In this work, we examined the cytotoxicity and chemotactic activity of LAAO from *Bothrops jararaca* venom in rat peritoneal macrophages. **Methods**: LAAO was purified by a combination of size-exclusion (Superdex 75) and ion-exchange (Q-Sepharose) chromatography; purity was assessed by SDS-PAGE and RP-HPLC. Cytotoxicity was examined in freshly isolated rat lung macrophages based on the neutral red reduction assay and release of lactate dehydrogenase (LDH). Macrophages were isolated from male Wistar rats (~300 g) using standard procedures and allowed to adhere to 96-well plates (12 h at 37 °C). The cells were then incubated with venom or LAAO(2-24 h) prior to the neutral red assay and quantification of lactate dehydrogenase release. When required, superoxide dismutase (SOD; 40 U/ml), catalase (CAT; 100 U/ml) and N^{w}-L-nitroarginine methyl ester (L-NAME; 1 mM)were included in the incubation.Chemotactic activity in vivo was assessed24 h after the intraperitoneal injection of LAAO(9 U/cavity) in the absence or presence of SOD (400 U/cavity), CAT (500 U/cavity) or L-NAME (13.5 mg/cavity).The experiments were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 2694-1). **Results:** Incubation with venom (10-1000mg/ml) reduced macrophage viability to62.8±4.6% and 6.2±2.2% for 10 and 1000mg/ml,respectively, after 2 h (mean±SEM; n=5; $p<0.05$ vs. control cells). LAAO (2.5-250U/ml)was also cytotoxic, with the two highest concentrations, 75 and 250 U/ml, reducing cell viability to56.9±7.1% and 28.4±2.6%, respectively (n=6; $p<0.05$ vs. control cells). LDH release by LAAO (75 U/ml) was maximal after 6 h (66.0±7.9vs. control cells, 30.7±6.0 U/l; $p<0.05$;n=5). Co-incubation of macrophages with LAAO (75 U/ml)plus SOD, catalase or L-NAME for 2 h significantly attenuated cytotoxicity by57.9±1.1%,95.9±2.4% and 94.7±0.6%, respectively (n=5; $p<0.05$ vs. LAAO alone). Chemotactic activity 24 h after i.p. injection of LAAO (9 U/cavity) resulted in the migration of$31.9\pm5.9\times10^{6}$ cells/cavity ($p<0.05$ vs. control, $5.8\pm1.5\times10^{6}$ cells/cavity; n=5). Differential counts in control rats showed 22.5±3.1% polymorphonuclear cells and 72.7±5.4% mononuclear cells compared to 33.5±4.5% and 56±2.7%, respectively, after incubation with LAAO (n=4 each).Pretreatment of rats with SOD, catalase or L-NAME prior to injection of LAAO (9 U/cavity) significantly reduced the number of peritoneal cavity cells from $31.9\pm5.9\times10^{6}$ to $3.4\pm1.8\times10^{6}$, $3.6\pm3.0\times10^{6}$ and $8.7\pm2.1\times10^{6}$ (n=5 each; $p<0.05$), respectively. **Conclusion:** *Bothrops jararaca* LAAO is cytotoxic to macrophages and causes the migration of inflammatory cells in vivo that is mediated by H_2O_2, superoxide anion and nitric oxide. This activity may contribute to the local inflammatory response following envenoming by *B. jararaca*. **Financial support:** CAPES, CNPq,FAPESP.

01.012 Heterogeneous population of alpha-1 adrenoceptors in abdominal aorta of male and female rats. Silva KP, Pupo AS IBB-Unesp – Farmacologia

Introduction: **A**lpha-1 adrenoceptors (ARs) are Gq-protein coupled receptors activated by the cathecholamines noradrenaline (NA) and adrenaline. Alpha-1 ARs are comprised by three subtypes named alpha-1A, alpha- 1B, alpha- 1D and are all involved in vascular smooth muscle contraction. Previous studies with abdominal aorta of male rats showed the involvement of alpha-1D in contractions of this vessel in response to adrenergic agonists (Asbún-Bojalil *et al*., Vascular Pharmacology, 38: 169, 2002). **Aims:** This present study advanced the pharmacological characterization of different populations of alpha-1 ARs in abdominal aorta of both male and female rats. **Methods:** All experimental procedures were approved by the local ethics committee for the use of experimental animals (CEUA, Protocol #634/2014). Male and female Wistar rats (90-120 days old) were killed by decapitation and rings (0.5cm length) of abdominal aorta were isolated to record of in vitro isometric contractions to NA or A61603 (an alpha-1A selective agonist). The alpha-1 AR subtypes mediating contractions to NA were identified by Schild analysis of the antagonism displayed by subtype selective antagonists. Antagonist affinities (pK_B) or potencies (pA_2) were evaluated. **Results:** Contractions of abdominal aorta from male and female rats to NA were competitively antagonized with high affinity by prazosin (male: pK_B=9.48±0.05; female: pK_B=9.22±0.22; n=4). The alpha-1D AR selective antagonist BMY7378 displayed complex antagonism against NA-induced contractions of both male (Schild slope: 0.73±0.1; pA_2: 8.25±0.07; n=4) and female abdominal aortae (Schild slope: 0.71±0.13; pA_2: 8.22 ±0.07; n=5). The alpha-1A AR selective antagonist RS100329 displayed high potency, but insurmountable antagonism against NA-induced contractions of aortae from both male and female rats. Contractions of abdominal aortae from male and female rats to A61603 were antagonized by RS100329 (male: pA_2= 9.9±0.08; female: pA_2= 9.48±0.04 n=3 each) but not by BMY7378. The alpha-1B AR selective antagonist L765,314 displayed low affinity competitive antagonism against NA-induced contractions of male (pA_2= 6.2±0.41; n=3) and female (pA_2= 6.9±0.27; n=3) abdominal aortae. **Discussion:** In abdominal aorta of male and female rats, the rightward shifts produced by prazosin in the concentration-response curves for NA indicate that the contractions in response to this agonist result solely from the activation of alpha-1 ARs. The pA_2 values for BMY 7378 in both male and female aortae are consistent activation of alpha-1D AR, but the slope in the Schild plots indicates presence of other alpha-1 AR subtypes. The contraction induced by A61603 was antagonized by the RS100329 with pA_2 values consistent with presence of alpha-1A AR in male and female abdominal aortae, whereas the low potency for L765,314 indicate that alpha-1B ARs are not involved. **Conclusion:** there are functional alpha-1D and alpha-1A AR populations in abdominal aorta of both male and female rats and this tissue is an interesting model for the study os subtype selective ligands. **Financial support:** CAPES, FAPESP 08/50423-7 (to A.S.P).

01.013 Which are the histamine receptors involved in the regulation atrial in Wistar-EPM1 rats? Nascimento SR, Musial DC, Miranda-Ferreira R, de Souza BP, Jurkiewicz A, Jurkiewicz NH Unifesp-EPM – Farmacologia

Introduction: Histamine is a biogenic amine which is able to modulate the sympathetic neurotransmission in the heart. However, the histamine effects in the inotropism and chronotropism atrial are contradictory and also need of additional investigations. **Aims:** Because of it, we decided to study the effects of histamine receptors (H1 and H2) on isolated rat atria. **Methods:** We used 4-month-old male Wistar rats weighing approximately 300 g. The left (LA) and right atria (RA) were isolated and mounted in isolated organ chambers bathed with Krebs-Henseleit solution (95% O_2, 5% CO_2 - 36.5°C). The LA was electrically stimulated (2 Hz; 5 ms, 20-40 V) and after 40 minutes of stabilization, cumulative concentration-response curves for histamine (10-5 to 10-2 M) in the absence or presence of H1-antagonist (Ketotifen 10^{-6}M) or H2-antagonist (Cimetidine 10^{-5}M) receptors were performed. All experimental procedures were approved by Ethical Committee of Universidade Federal de São Paulo - Brazil (protocol: 5784280814). **Results and Conclusions:** The histamine produce a biphasic response characterized by an initial negative inotropic effect (0.5-1 min of duration) followed by a positive inotropic effect which reaches the plateau after 4-5 minutes, in the LA and RA. In the maximal concentration of histamine, the negative inotropic effect was reduced by about 42% and 70% in LA and RA, respectively, compared to basal. Further, the positive inotropic effect induced by histamine was increased about 69% in LA and 63% in RA in relation to basal. We also found a reduced chronotropism of RA by about 30 % in the maximal concentration of histamine. When we use the H1 receptor antagonist, we did not observe any change in the RA contraction force when stimulated with histamine. It means that the H1-receptor is not responsible by the histamine effect in the RA. However, there was a blockade of chronotropic effect in 53% in RA. In the LA, the H1 receptor antagonist reduced in 50% the positive inotropic effect developed by histamine (33%). In the presence of H2-antagonist - cimetidine, the blocked of positive inotropic effect in LA was 40% and RA was 44%, without change the negative inotropic effect and the chronotropism. Our data present important points demonstrating that high concentrations of histamine can produce significant effects on the atrial modulation, which may occur during a septic shock. Apparently both the H1 and H2 receptors are involved in the positive inotropic effect, but only the H1 receptor changes the chronotropism indicating greater participation of this receptor in control of the sinoatrial node. One future perspective for this study is the investigation of the other receptors. **Financial support:** Fapesp and CAPES.

01.014 Adenosine A_{2A} receptor plays a key role in lung fibroblast proliferation and activation triggered by IL-13 *in vitro*. Sá YAPJ, Ciambarella BT, Martins MA, Silva PMR Fiocruz – Inflamação

Introduction: Fibrosis is a response associated with several chronic diseases, and is characterized by scar formation and accumulation of excess fibrous connective tissue. In the lungs, fibrosis leads to thickening of the walls and causes decrease in total pulmonary capacity. IL-13 is an important mediator of inflammation and remodeling which is hypothesized to act via adenosine production. Adenosine is a nucleoside that signals via G protein–coupled receptors (A_1, A_{2A}, A_{2B} and A_3), which was found in elevated levels in the bronchoalveolar lavage fluid from patients with diseases such as asthma and COPD. **Aims:** Fibroblasts are considered crucial cells involved in fibrogenesis. In this study we investigated which receptors are involved in the profibrogenic effect of adenosine on lung fibroblasts *in vitro*. **Methods**: Fibroblasts were obtained from lungs of normal Swiss-Webster mice by means of enzymatic dissociation with collagenase type 1. Cells were cultivated in DEMEN medium supplemented by SBF 10% until the third passage. The analyses included cellular proliferation and chemokine (MCP-1) generation, which were performed by means of ^{3}H-thymidine incorporation and ELISA, respectively. The cells were treated with adenosine receptor antagonists, at the concentrations of 10 and 30 μM), 1 h before stimulation with adenosine (ADO) (10 - 1000 μM) and rmIL-13 (40 ng/ml). All the analyses were performed 24 h post-provocation. Cell viability was evaluated by means of MTT assay. All experimental procedures were approved by the Committee on Use of Laboratory Animals of Oswaldo Cruz Foundation (license LW 57/14). **Results**: We noted that pulmonary fibroblasts responded with increased proliferation rate and MCP-1 generation after stimulation with ADO and IL-13, being the latter more potent than the former. Incubation of cells with adenosine receptor antagonists revealed that ZM-241,385 and SCH 58281(A_{2A}R) prevented proliferation and MCP-1 production triggered by ADO, under conditions where DPCPx (A_1R) and MRS-1754 (A_{2B}R) had no effect. Similar **Results** were obtained when the cells were stimulated with IL-13. None of the antagonists tested showed any cytotoxicity. **Conclusions**: Our findings show that adenosine A_{2A} receptor is involved in ADO-induced fibroblast proliferation and cytokine generation *in vitro*. In addition, since the proliferative activity of IL13 on fibroblasts was prevented by the A_{2A} receptor antagonist, we can speculate that there may be a pathway via which IL-13 and adenosine may stimulate one another. **Financial support:** PAPES6/FIOCRUZ, CNPq, FAPERJ, CAPES and European Community (UE FP7- 2007-2013 - n°HEALTH-F4-2011-281608).

01.015 Glucocorticoid receptor expression during rat wolffian duct morphogenesis. Thimoteo DS[1], Ribeiro CM[1], Silva EJR[2], Hinton BT[3], Avellar MCW[1]
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Introduction: Practice of prenatal glucocorticoid therapy has improved the outcomes of preterm births by accelerating fetal lung maturation. In rats, in vivo treatment of the pregnant females with glucocorticoids negatively impact several reproductive outcomes along the postnatal life of male offspring, thus raising the question of how prenatal glucocorticoid overexposure and glucocorticoid receptor (GR) signaling would affect the embryonic development of the male reproductive organs. **Aim:** In the present study we aimed to characterize the expression and cellular distribution of GR in the rat Wolffian duct (WD), the anlagen of the epididymis. **Methods:** WDs were collected from male Wistar rats at different embryonic days (e): e12.5, e14.5, e16.5, e18.5 and e20.5. Whole epididymides were collected from rat neonates at postnatal day one (pnd1). RT-qPCR and immunohistochemistry were performed. The GR immunodistribution pattern in WD/epididymis was compared to the well-known androgen receptor (AR) localization. **Results:** Gr mRNA levels were detected in all ages analyzed, increasing in abundance between e17.5 and e20.5 to pnd1, a period when WD elongation and coiling is occurring. At protein level, GR-positive staining was observed in both mesenchymal and epithelial cells from WD at all ages analyzed. WD mesenchymal cells from e14.5 through e16.5 presented predominant GR immunolocalization in the nucleus, in a scattered positive cell pattern. Perinatally, however, most of these cells exhibited GR staining. In the WD epithelium, GR distribution switched from a predominant nuclear localization at early fetal ages to a more abundant cytoplasmic distribution at e18.5 and e20.5. This mesenchyme/epithelium distribution pattern of GR in the WD differed from the expected immunolocalization observed for AR: mostly restricted to the nucleus of mesenchymal cells from e14.5 through e16.5, switching to a more abundant immunoreactivity in the epithelial cells of WD from e20.5 and onward ages. **Conclusions:** Together our data indicate that GR is expressed and modulated during WD embryonic development, raising the hypothesis of the importance of glucocorticoids for WD morphogenesis. **Financial support:** CNPq/CSF (PVE 401932/2013-3), Capes, NIH−NICHD #069654. This study was approved by the Research Ethics Committee from Unifesp/EPM (4637290115).

01.016 L6 myogenic cell line as a skeletal muscle model for analysis of anti-catabolic drugs. Eloi FR, Funke MG, Godinho RO Unifesp-EPM – Farmacologia

Introduction: The screening of drugs with anticatabolic action has great relevance for development of new therapeutic strategies for prevention or reversal of skeletal muscle atrophy associated with many diseases, including myopathies, muscular dystrophies, as well as systemic disorders such as cancer, diabetes and sepsis. The molecular regulation of skeletal muscle proteolysis and the pharmacological screening of anticatabolic drugs have been assessed by measurement of tyrosine release from skeletal muscle using ex vivo protocols. Few studies have been performed in cell culture models, but all of them used radiometric assays for measure ^{3}H-tyrosine release, as an index of overall proteolysis. **Aim**: The aim of the present study was to establish the L6 myogenic cell line as a model for evaluating anticatabolic drugs using a fluorometric method to measure tyrosine release. **Material and Methods**: L6 rat myogenic cell line (ATCC) were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with gentamicin 50 µg/mL and 10% fetal bovine serum (DMEM-10) in 24-well microplates at density of $4x10^5$ cells/ ml. Skeletal myogenic differentiation was induced with DMEM supplemented with 5% horse serum starting at day 3. After differentiation into myotubes, 7-8-day-old cultures were incubated with 0.5 mM cycloheximide in carbogen-saturated Tyrode solution (Tyrode C), pH 7.4, at 37ºC, and 30 min latter, were incubated for 1 to 4 h with 220 µl fresh Tyrode-C containing clenbuterol (β_2 adrenoceptor agonist), which is known to reduce muscle proteolysis. The proteolysis was evaluated by measuring the rate of tyrosine release into the incubation medium, using a modified fluorometric method described by Bergantin et al., (J Appl Physiol 111: 1710, 2011). **Results and Conclusion:** The minimum volume of sample necessary for accurate quantification of tyrosine was 150 µl. In control cultures, the basal release of tyrosine increased in a time-dependent manner, from 1.92 ± 0.19 to 4.41 ± 0.34 nmol/well (n = 6), after 1 to 4 h. Treatment of cultures for 2 h with 10 to 100 nM clenbuterol reduced by 12-15 % the basal rate of tyrosine release (n = 8). The anticatabolic effect of clenbuterol was sustained for up to 4 h. These data show that L6 myogenic cells can be used to evaluate skeletal muscle proteolysis, representing an important tool to allow high-throughput screening of drugs for therapeutic anticatabolic purposes. **Financial support**: Capes, CNPq and FAPESP. Animal Ethics Committee: CEUA nº 5523140415.

01.017 Cyclic AMP released from skeletal muscle fiber modulates muscle contraction through the activation of presynaptic adenosine receptors. Duarte T, Pacini ESA, Godinho RO Unifesp-EPM – Farmacologia

Introduction: We have shown that cAMP efflux from skeletal muscle represents an important source of extracellular adenosine, an endogenous purine nucleoside that acts as a neuromodulator of the central and peripheral synapses. Outside the muscle cell, cAMP is sequentially metabolized by ecto-phosphodiesterases and ecto-nucleotidases into AMP and adenosine. The latter is able to stimulate postsynaptic A_1 adenosine receptors (AR), which are associated to negative inotropic effects. Although, adenosine-dependent activation of pre-synaptic A_{2A} receptor subtypes has been associated with increment of ACh release and increasing the force contraction of skeletal muscle, it is not clear whether the cAMP released from skeletal muscle fiber may exert paracrine action on peripheral nerve via activation of presynaptic adenosine receptor. This hypothesis was evaluated in the present study. **Methods**: To investigate the possible presynaptic influence of the "extracellular cAMP-adenosine pathway" on muscle contraction, we analyzed the effects of drugs on the train-of-four (TOF) ratio. TOF ratio is the quotient between twitch tension of mouse diaphragm muscle produced by the 4^{th} (T4) and the 1^{st} (T1) stimulus within 4 consecutive 2 Hz stimuli of phrenic nerve (n=3-4). In the presence of the neuromuscular blockers, such as d-tubocurarine (an nicotinic acetylcholine receptor antagonist), there is a loss of twitch amplitude, which indicates the degree of blockage. The effect of 100 µM cAMP and 100 nM clenbuterol (β_2-adrenoceptor agonist) was evaluated in the partial neuromuscular block induced by 300 nM d-tubocurarine. In some experiments, in order to evaluate the possible involvement of AR in cAMP effects, phrenic nerve-diaphragm preparation was pre-incubated with CGS 15943 (non-selective AR antagonist) or ZM 241385 (selective A_{2A} antagonist) prior to the addition of cAMP. **Results**: Incubation of phrenic-diaphragm preparation with 300 nM d-tubocurarine causes a 25% reduction in the TOF ratio (TOF fade). The pre-incubation of nerve-muscle preparation with 100 µM cAMP prevented tetanic fade elicited by d-tubocurarine. In order to evaluate whether cAMP efflux triggered by activation of β_2-adrenoceptor-dependent activation of adenylyl cyclase could modify the effect of d-tubocurarine on TOF ratio, nerve-muscle preparation was incubated with 100 nM clenbuterol. Although the β_2-adrenoceptors agonist alone increased the fourth (T4) and the first (T1) stimulus with in a train-of-four stimulation by 20%, it prevented tetanic fade caused by 300 nM d-tubocurarine. Blocking adenosine receptors with either 100 nM CGS 15943 or 10 nM ZM 241385 (selective A_{2A} antagonist) prevented the effect of cAMP and clenbuterol on tetanic fade caused by d-tubocurarine. **Conclusion:** Our results demonstrate that "the extracellular cAMP-adenosine pathway" modulates nerve-evoked skeletal muscle contraction through the activation of pre-synaptic adenosine A_{2A} receptors. This pathway was also elicited by the activation of postsynaptic β_2-adrenoceptors, indicating that it may function as a paracrine signaling that creates a regulatory feedback loop between muscle fibers and motor neurons. **Financial support**: Capes, CNPq and FAPESP. Animal Ethics Committee: CEP n. 0022/12

01.018 P2X7 and vanilloid-associated pores: Common events in murine peritoneal macrophages? Ferreira LGB[1], de Melo Reis RA[2], Henriques-Pons A[1], Alves LA[1], Faria RX[1] [1]Fiocruz, [2]UFRJ

Introduction: The P2X7 receptor (P2X7R) is an ion channel expressed mainly in cells of the immune system and it is involved in cellular processes such as inflammation, cell death and pain. At concentrations ranging from micromolar ATP, P2X7R is permeable to mono- and divalent cations such as sodium and calcium ions, though in the range of millimolar concentrations, it shows a formation of a pore in plasma membrane, which allows the passage of molecules up to 900 Da. Meanwhile, the pore formed from the activation of P2X7R has its identity still unknown. According to P2X7R pore formation, in the main theories about this matter, it would be a distinct protein responsible for pore opening of P2X7R. In this context, we study the involvement of another protein forming pores in the plasma membrane; the transient receptor potential vanilloid (TRPV1). TRPV1 is a cationic channel that just as the P2X7 is also involved in cell death, pain and inflammation. The pungent compound capsaicin is a specific agonist of TRPV1, leading to a state similarly to that found in P2X7R activation, which is the opening of a non-selective pore of the same magnitude of the P2X7R. **Aims:** In this study, we verify if communication between both TRPV1 and P2X7R in the formation of ATP and capsaicin-induced pore process. **Methods:** We performed several assays in order to characterize cellular events following capsaicin treatment and their hypothetical communication with P2X7R-associated pore. We used murine peritoneal macrophage and Müller glial cells in this work according to Brazilian College of Animal Experimentation and approved by the Ethics Commission of the use of animals under license L-0041/08. To assess any channel activity, we performed both electrophysiological and fluorimetric calcium assays. The impermeant dye uptake events were assessed by microscopy and flow cytometry approaches to record the pore activity. **Results:** Our results show that more than 90% of $F4/80^{+}$ macrophage cells were responsive to capsaicin in calcium and in electrophysiological assays. Moreover, pore dilation assessments showed that the impermeant fluorescent dye Ethidium bromide was uptaken following treatment with capsaicin (EC_{50} = 51,06 µM) and ATP. This process showed similarities to TRPV1 activity and it is sensitive to TRPV1 antagonists, capsazepine and Ruthenium red. However, it seems that capsaicin-induced dye uptake is cation-selective, once fluorescein was not uptaken. Surprisingly, the P2X7R antagonists, BBG and oxATP (300 µM), inhibited dye uptake following capsaicin treatment in macrophage and glial cells. Interestingly, our results suggest that both P2X7 and Vanilloid-related large pores communicate and exhibit crossed pharmacology. **Conclusions:** Therefore, our results showed, for the first time, that capsaicin triggered intracellular calcium mobilization, ion currents and dye uptake in macrophage cells. It is noteworthy that there is a putative intracellular signaling between P2X7R and TRPV1 receptors. We appreciate the contribution of development agencies: CNPq, FAPERJ and Oswaldo Cruz Institute for **Financial support**.

01.019 $P2Y_1$ Receptor Activation Upregulates the Endothelial Cell ICAM-1 Membrane Expression and Leukocyte Adhesion. Cardoso TC[1], Pompeu TET[1], Silva CLM[1] UFRJ – Farmacologia Bioquímica e Molecular

Introduction: The activation of endothelial cells in an inflammatory process changes their phenotype increasing the expression of adhesion molecules, such as ICAM-1, leading to the rolling, adhesion and migration of immune cells. Several biological molecules modulate this process, including the endothelial cell-derived ATP which may act as a DAMP. Endothelial cells hydrolyze extracellular ATP generating ADP, a known and potent agonist of P2Y1 receptor (P2Y1R). $P2Y1R^{-/-}$ mice show a reduced TNF-mediated rolling and adhesion of leukocytes to the endothelium (Zerr et al., Circulation 123:2404, 2013), however, so far, no direct effect of endothelial P2Y1R was shown. The objective of this study was to evaluate *in vitro* the direct effect of P2Y1R upon mononuclear cell adhesion to endothelial cells. **Methods**: Rat (male) mesenteric microvessels were isolated and minced in a 24-wells plate. Tissues were maintained in DMEM supplemented with 20% fetal bovine serum, gentamicin, and incubated at 37 °C and 5% CO_2. After 48 h the tissue was removed and the medium was replaced every 48h until the endothelial cells (MEC) reached confluence (Oliveira et al., Plos One 6:e23547, 2011). First passage cells were plated (1E4 cells/well). After 48h, MEC were incubated for 4h with vehicle (basal), 2MeSATP (60 µM, P2Y1R agonist) or 2MeSATP (60 µM) plus MRS2179 (0.3 µM, selective antagonist of P2Y1R). Rat mononuclear cells were isolated using Ficoll-Paque Plus gradient. At the end of the fourth hour, mononuclear cells (1E4 cells/well) were added and incubated for 30 min. Then, non-adherent cells were removed by washing with PBS and four fields per well were randomly chosen and analyzed. The number of adherent mononuclear cells per field in each condition was determined by microcopy (Olympus IX71 microscope, 400X). P2Y1R and ICAM-1 expressions were analyzed by Western blot assay (whole cell lysate) and confocal microscopy, respectively. We also investigated the putative effect of melatonin on MEC P2Y1R signaling. Data were expressed as mean and SEM. The differences between groups were analyzed by one-way ANOVA followed by Newman-Keuls test, considering $P < 0.05$. **Results and Conclusions**: MEC express P2Y1R, and the treatment with 2MeSATP (60 µM) increased the adhesion of mononuclear cells from 15.06 ± 1.1 (basal; n = 33 fields) to 33.13 ± 2.5 (n = 33 fields, $P < 0.001$). The MEC pretreatment with MRS2179 fully blocked the agonist effect confirming the direct role of P2Y1R on leukocyte adhesion. This effect depends on intracellular calcium since MEC treatment wih BAPTA also blocked 2MeSATP effect ($P < 0.01$). Moreover, confocal analysis revealed that 2MeSATP (4h) increased ICAM-1 membrane expression of nonpermeabilized MEC, which corroborates functional data. Melatonin (30 nM, 4h) inhibited 2MeSATP effect ($P < 0.01$) mainly by interfering with ICAM-1 membrane expression. Mononuclear cell adhesion to endothelial cell is a key event in inflammatory conditions such as atherosclerosis (Hopkins P, Physiol Rev. 93: 1317, 2013). These **Results** suggest that short-term activation of P2Y1R upregulates leukocyte adhesion to endothelial cells corroborating previous data obtained from $P2Y1R^{-/-}$ mice (Zerr et al., Circulation 123:2404, 2013). It may also be involved in vascular inflammatory conditions such as atherosclerosis. In addition the hormone melatonin has an anti-inflammatory effect. **Acknowledgements**: CAPES, CNPq, FAPERJ All protocols were approved by the ethics committee of the UFRJ (DFBCICB011)