

05. Pain and Nociception

05.001 Antinociceptive effect of intrathecal bolus injection or continuous infusion of the N-type voltage-sensitive Ca^{2+} channel blocker $\text{Ph}\alpha 1\beta$ on a rat model of neuropathic pain. Rosa F¹, Trevisan G¹, Andrade LE², Tonello R¹, Gomez MV³, Calixto JB², Ferreira J¹ ¹UFMSM – Química, ²UFSC – Farmacologia, ³UFMG – Farmacologia

Objectives: Neuropathic pain is considered a severe painful disease of difficult treatment, since the analgesic drugs commonly used have a relative poorly therapeutic efficacy. A novel option for neuropathic pain management would be drugs blocking N-type voltage-sensitive Ca^{2+} channels (NCCs), as ziconotide. It has been used in clinical by intrathecal (i.t) route, for the treatment of severe chronic pain (including neuropathic), but it also causes serious adverse effects at therapeutic doses. One purified peptide of the venom from spider *Phoneutria nigriventer*, $\text{Ph}\alpha 1\beta$, acts by blocking preferentially the NCCs, and its single i.t. administration has been shown the pronounced antinociceptive effect in inflammatory pain models, with a therapeutic window greater than ziconotide. Thus, the aim of this study was to observe the antinociceptive potential of $\text{Ph}\alpha 1\beta$ in a rat model of neuropathic pain and the possible adverse effects related to single bolus i.t. injection or continuous i.t. infusion. **Methods:** All protocols employed have been approved by Ethics Committee of the Federal University of Santa Maria (process number 086/2011). Neuropathic pain was induced by the chronic constriction injury (CCI) of the sciatic nerve in adult male Wistar rats (200-300 g). Eight days after injury, rats were i.t. bolus injected with $\text{Ph}\alpha 1\beta$ (10, 30 or 100 pmol/site) or PBS (10 $\mu\text{L}/\text{site}$) and the mechanical threshold (analyzed through von Frey filaments, expressed as paw withdrawal threshold; PWT in grams), and also the development of side-effects (through a visual scale, scores from 0-7) were evaluated. These parameters were observed 0.25, 0.5, 1, 2, 4 and 6 hours after i.t. injection, and baselines values were assessed before the induction of neuropathy. In another experiment, the same procedure for the induction of neuropathy was performed and after 8 days, for obtain a sustained drug intrathecal delivery, a soft polyurethane catheter was inserted through a small hole in the dura at T1 vertebra level, next, an osmotic pump operating at a rate of 1 $\mu\text{L}/\text{h}$ for 7 days, it was filled with $\text{Ph}\alpha 1\beta$ (60 pmol/ $\mu\text{L}/\text{hr}$) or PBS (1 $\mu\text{L}/\text{hr}$) and attached to the catheter. The mechanical threshold was observed before the neuropathic pain induction, and 8 days after the procedure, and also for 7 consecutively days after the continuous administration of $\text{Ph}\alpha 1\beta$ or vehicle. In addition, we have evaluated the possible development of side effects in the same time points. **Results:** The single bolus injection with $\text{Ph}\alpha 1\beta$ (30 pmol/site) produced a long-lasting reduction (from 0.25 to 4 hours) of CCI-induced hyperalgesia, with maximum effect (I_{max}) of 100% for the doses of 30 and 100 pmol/site. In addition, the i.t. acute injection of $\text{Ph}\alpha 1\beta$ did not induce any detectable side effect. The continuous i.t. infusion with $\text{Ph}\alpha 1\beta$ by osmotic pump produced a significantly reduction in the neuropathy-induced hyperalgesia from the first to the seventh day of infusion, with 63 \pm 13, 90 or 100% of inhibition observed 1, 2, or 3 to 7 days after i.t. infusion, respectively. Moreover, the i.t. infusion of $\text{Ph}\alpha 1\beta$, for 7 days did not induce any alteration in the toxicity parameters evaluated. **Conclusions:** $\text{Ph}\alpha 1\beta$ produced a remarkable analgesia in a rat model of neuropathic pain without showing apparent toxicity after i.t. bolus injection or continuous infusion for 7 days. Thus, $\text{Ph}\alpha 1\beta$ can be an effective and safer N-type calcium channel blocker for the treatment of neuropathic pain. **Financial support:** Capes, CNPq, Fapergs, Famig.

05.002 Peripheral mechanisms involved in the hyperalgesia induced by the activation of P2X7 receptors in the rat knee joint. Teixeira JM, Parada CA, Tambeli CH IB-Unicamp – Biologia Estrutural e Funcional

Introduction: We have previously demonstrated that P2X7 receptors activation is essential for the development of carrageenan-induced hyperalgesia in the rat knee-joint. In this study, we investigated whether the administration of the P2X7 receptor agonist BzATP in the rat knee-joint induces inflammatory hyperalgesia, and if so, the mechanism by which this hyperalgesic response is developed. **Methods:** Male Wistar rats (2 months old, 200-250g) were used in this study and all experimental procedures were approved by the Ethics Committee in Animal Research at the UNICAMP (protocol number: 2049-1). The hyperalgesic responses (gait disturbance) were quantified using the Knee-joint Incapacitation Test (Tonussi, Pain 48; 421, 1992). The local concentrations of TNF- α , IL-1 β , IL-6 and CINC-1 were measured by Enzyme-linked Immunosorbent Assay (ELISA) 3 hours after the activation of P2X7 receptors by BzATP. **Results:** The knee joint injection of BzATP (225 μ g/knee) induced an articular hyperalgesia 3 hours after its injection (Means \pm SEM(s): 30.00 \pm 1.56; n=6) that was blocked by the selective P2X7 receptor antagonist A-740003 (568 μ g/knee; 15.67 \pm 2.12, p<0.05, T test, n=6). The co-administration of by the selective B1- (DALBK, 3.0 μ g/knee) or B2-bradykinin receptor antagonist (Bradyzide, 0.5 μ g/knee), the selective β 1- (Atenolol, 6.0 μ g/knee) or β 2-adrenoceptor antagonist (ICI 118,551, 1.5 μ g/knee) significantly reduced the articular hyperalgesia induced by BzATP (16.67 \pm 2.33; 16.83 \pm 1.83; 20.00 \pm 1.83; 18.50 \pm 1.02, respectively, p<0.05, Tukey test, n=6 in all groups). The pre-treatment with the cyclo-oxygenase inhibitor Indomethacin (100 μ g/knee, i.a., 30 min before P2X7 receptor activation) but not with the nonspecific selectin inhibitor Fucoidan (25mg/kg, i.v., 20 min before P2X7 receptor activation) significantly reduced the articular hyperalgesia induced by BzATP (17.00 \pm 1.89, p<0.05, and 29.40 \pm 1.76, p>0.05, respectively, Tukey test, n=6 in both groups). DALBK (3.0 μ g/paw), Bradyzide (0.5 μ g/Knee), Atenolol (6.0 μ g/Knee), ICI 118,551 (1.5 μ g/Knee) or Indomethacin (100 μ g/Knee) did not affect the articular hyperalgesia induced by BzATP (31.00 \pm 0.53; 27.50 \pm 3.05; 29.67 \pm 2.97; 29.67 \pm 2.00; 29.83 \pm 2.18, respectively, p>0.05, Tukey test, n=6 in all groups) when administered in the contralateral knee joint. The local concentration of TNF- α (172.51 \pm 10.61pg/mL, n=8), IL-1 β (2030.79 \pm 131.36pg/mL n=8), IL-6 (493.07 \pm 69.34pg/mL n=8) and CINC-1 (244.00 \pm 24.29pg/mL n=8) induced by the administration of BzATP (225 μ g/knee) in the rat knee-joint was significantly reduced by the co-administration of the selective P2X7 receptor antagonist A-740003 (568 μ g/knee) (90.88 \pm 12.34pg/mL, 551.17 \pm 72.00pg/mL, 198.91 \pm 73.49pg/mL and 71.26 \pm 11.93pg/mL, respectively, p<0.05, Tukey test, n=8 in all groups). **Discussion:** These results suggest that the P2X7 receptors activation induces articular hyperalgesia by an indirect action on the primary afferent nociceptors in the articular tissue of the rat knee-joint mediated by bradykinin, sympathomimetic amines, prostaglandins and by the previous release of pro-inflammatory cytokines but not by neutrophils migration. Therefore, P2X7 receptors are important targets to control inflammatory pain in the knee joint. **Financial support:** FAPESP.

05.003 Study of visceral antinociceptive potential of bee *Apis mellifera* venom. Costa MFB, Campos AR¹, Abdon APV¹, Vasconcelos RP¹, Castro CA¹, Lima DB², Torres AFC³, Toyama MH⁴, Martins AMC³ ¹UNIFOR, ²UFC, ³UFC – Análises Clínicas e Toxicológicas, ⁴Unesp-Litoral Paulista

Background: Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates. Bee venom is a rich source of biologically active substances. Hymenoptera venoms are complex mixtures of biochemically and pharmacologically active components such as biogenic amines, peptides and proteins. The aim of our study was to determine whether *Apis mellifera* venom (AMV) mitigates visceral nociception. **Methods:** The acetic acid-induced writhing assay was used in mice to determine the degree of visceral antinociception (KOSTER *et al.*, **Fed. Proc.** V.18, P. 412-417, 1959). Visceral antinociceptive activity was expressed as the reduction in the number of abdominal contractions. Mice received an intraperitoneal injection of acetic acid 30 min after intraperitoneal administration of vehicle or AMV (0.08 or 0.8 mg/kg). In mechanistic studies, separate experiments were realized to examine the role of α_2 -receptors, nitric oxide, calcium channels and K^+_{ATP} channel activation on the visceral antinociceptive effect of AMV (0.8 mg/kg), using appropriate antagonists, yohimbine (2 mg/kg, i.p.), L-NAME (10 mg/kg, i.p.), Verapamil (5mg/kg i.p.) and glibenclamide (5 mg/kg, i.p.). This study was submitted to the ethic's committee for animal research (CEPA) of Federal University of Ceará, under protocol number 90. **Results:** Intraperitoneal application of acetic acid provoked a significant increase in abdominal constrictions when compared with treated normal controls. In groups pretreated with AMV (0.8 and 0.08 mg/kg), nociceptive behaviors were significantly inhibited. The antagonists used failed to revert the antinociceptive effect of AMV. **Discussion:** In the search for novel natural substances, which possess visceral antinociceptive property, we used the acute model of visceral pain induced by intraperitoneal administration of acetic acid to produce spontaneous pain-related behaviors in mice. *Apis mellifera* venom (0.08 and 0.8 mg/kg) could significantly suppress the pain-related behavior against the noxious agent used. The present study therefore verified the possible involvement of noradrenergic, nitergic, calcium channels and K^+_{ATP} mechanisms in the antinociceptive effect of AMV. The results reveal that its antinociception could not be blocked by the specific antagonists. **Conclusions:** Although AMV efficiently diminished the acetic acid-evoked pain-related behaviors, its mechanism is unclear from this study and future studies are needed to verify how the sesquiterpene exerts its antinociceptive action. This study was financed by University of Fortaleza and Federal University of Ceará.

05.004 Mechanisms underlying activation of P2X3 receptors induce mechanical hyperalgesia in the gastrocnemius muscle of rats. Schiavuzzo JG¹, Melo B, Santos DFS, Teixeira JM², Oliveira Fusaro MC¹, Parada CA² ¹Unicamp – Ciências Aplicadas, ²IB-Unicamp – Biologia Estrutural e Funcional

Introduction: Among all kinds of pain that affect people throughout their lives, muscle pain is one of the most prevalent. There are evidences of the involvement of endogenous ATP via activation of P2X3 receptors in muscle pain (Reitz M., Cephalalgia 29:58, 2009), however, the mechanisms underlying activation of P2X3 receptors contributes to development of muscle inflammatory pain are unknown. The aim of this study was to verify whether activation of P2X3 receptors in the gastrocnemius muscle of rats induces mechanical hyperalgesia and, in this case, to analyze whether this hyperalgesia is mediated by bradykinin, prostaglandins and/or sympathetic amines. **Methods:** The mechanical hyperalgesia was quantified 1/2, 1, 2, 3, 6, 24 and 48h post administration of the non-selective P2X3 receptors agonist α,β -meATP in the gastrocnemius muscle of rats by the pressure analgesimeter (Randal Sellito). Local administration of the selective P2X3 and P2X2/3 receptors antagonist A-317491 was used to confirm the involvement of these receptors in the hyperalgesic response induced by α,β -meATP. To investigate whether the mechanical hyperalgesia induced by α,β -meATP was mediated by bradykinin or sympathetic amines, the selective bradykinin B1 receptor antagonist DALBK or the selective β_1 - or β_2 -adrenoceptor antagonist atenolol and ICI 118,551, respectively, was co-administered with α,β -meATP in the gastrocnemius muscle of rats. To investigate the involvement of prostaglandins in the mechanical hyperalgesia induced by α,β -meATP, the cyclooxygenase inhibitor indomethacin was administered (i.m.) 30 min. before α,β -meATP. Total volume administered was 50 μ L. All experimental procedures were previously approved by the Ethics Committee in animal research of the State University of Campinas (license number 2518-1 in 04/21/2011). **Results:** α,β -meATP (1000 μ g but not 100 μ g or 500 μ g) induced mechanical hyperalgesia (n=6, p<0.05, Two Way ANOVA, Bonferroni post test) 1/2, 1, 2, 3 and 6h post its administration in the gastrocnemius muscle. The greatest response was 2h post α,β -meATP administration. The co-administration of A-317491 (120 μ g, n=6), DALBK (3.0 μ g, n=6), atenolol (24 μ g, n=6), ICI 118.551 (1.5 μ g, n=6) or pre-treatment with indomethacin (100 μ g, n=6) blocked (p<0.05, One Way ANOVA, test Tukey) the mechanical hyperalgesia induced by α,β -meATP, but not affected these responses when administered on gastrocnemius muscle of contralateral hind leg (n=6, p>0.05, T test). **Discussion:** The results of this study suggest that activation of P2X3 receptors in gastrocnemius muscle of rats by α,β -meATP induces mechanical hyperalgesia by an indirect sensitization of the primary afferent nociceptor mediated by bradykinin, sympathetic amines and prostaglandins. **Financial support:** FAPESP (2011/13884-9).

05.005 Mediation of the antinociceptive effect of crotoxin in characteristic nociception of experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. Teixeira NB¹, Fonseca LA¹, Sampaio SC¹, Basso AS², Cury Y¹, Picolo G¹ ¹IBu – Dor e Sinalização, ²Unifesp – Imunologia

Introduction: Multiple sclerosis (MS) is a Central Nervous System inflammatory demyelinating disease that has as primary symptoms losses of sensory and motor functions and, as a major concern, chronic pain affecting 50% to 80% of patients (Sloane, E., et al. *Brain, Behavior, and Immunity*. 23: 92-100, 2009). MS has no cure, being the therapeutic approaches focused on stopping disease progression and cumulative neurological disability. To date, however, few studies have investigated the mechanisms of chronic pain in animal models of MS since locomotor impairments difficult the pain evaluation. Recently, it was demonstrated that in the MOG₃₅₋₅₅-induced EAE, an animal model of MS, the hypernociception appears before the onset of motor disability, allowing the study of these two phenomena separately (Olechowski, C.J., et al. *Pain*. 149: 565-572, 2010; Rodrigues, D.H. *Arq Neuropsiquiatr*. 67: 78-81, 2009). In this meaning, we evaluated the effect of crotoxin, a neurotoxin isolated from the *Crotalus durissus terrificus* snake venom that displays, at non-toxic dose, antinociceptive, anti-inflammatory and immunomodulatory effects (Sampaio, S.C., et al. *Toxicon*. 55: 1045-1060, 2010), in the pain and in symptoms progression of EAE. Previous results from our group demonstrated that crotoxin induces a long-lasting analgesic effect (5 days) after one single administration, without interfering with the clinical signs of the disease. On the other hands, when crotoxin was administered for 5 consecutive days, it induced analgesia and also reduced EAE progression. Then, the aim of this work was to evaluate the mediators involved in the antinociceptive effect of crotoxin in EAE model. **Methods:** Procedures were approved by the Institutional Animal Care Committee of the Butantan Institute (protocol number 757/10). EAE was induced by s.c. immunization of C57BL/6J mice (18-20g) with 200 µl of an emulsion containing 150 µg of MOG₃₅₋₅₅ peptide and 400 µg of *Mycobacterium tuberculosis* extract in incomplete Freund's adjuvant oil. In addition, the animals received 150 ng of pertussis toxin i.p. on day 0 and day 2. Pain threshold was determined using an electronic pressure-meter test. Motor activity was assessed using the rota rod test. Clinical signs of EAE were assessed according to the following score: 0, no signs of disease; 1, loss of tone in the tail; 2, hindlimb paresis; 3, hindlimb paralysis; 4, tetraplegia; 5, moribund. **Results:** The pain threshold of the animals decreased at day 4, while the first sign of disease appeared at days 11–12, coinciding with the onset of motor abnormalities. Crotoxin (40µg/kg, s.c.) administered 5 days after imunization (1 day after onset of pain threshold alteration) was able to induce antinociception. The antinociceptive effect of crotoxin was blocked by the pretreatment of the animals with Boc2 (10µg, i.p.), a selective antagonist of formyl peptide receptors, with NDGA (30 µg/kg, i.p.), an antagonist of lipoxin A4 and atropine sulfate (10mg/kg, i.p.), an antagonist of muscarinic receptors. **Conclusion:** These results indicate that the antinociceptive effect of crotoxin involves the release of lipoxin A4, acting on formyl peptide receptors and involves the participation of muscarinic receptors. **Financial support:** FAPESP (2010/12903-7), (2011/17974-2) and INCTTOX program (2008/57898-0).

05.006 Euphol, a tetracyclic triterpene produces antinociceptive effects in inflammatory and neuropathic pain: The involvement of cannabinoid system. Dutra RC¹, Silva KABS¹, Bento AF¹, Marcon R¹, Paszcuk AF¹, Meotti FC¹, Pianowski LF², Calixto JB¹ ¹UFSC – Farmacologia, ²Pianowski & Pianowski Ltda

Persistent pains associated with inflammatory and neuropathic states are prevalent and debilitating diseases, which still remain without a safe and adequate treatment. Euphol, an alcohol tetracyclic triterpene, has a wide range of pharmacological properties and is considered to have anti-inflammatory action. **Aim:** Here, we sought to investigate the antinociceptive and anti-inflammatory effects of euphol against mechanical hyperalgesia associated with inflammatory and neuropathic pain states. **Methods:** Inflammatory and neuropathic pain was induced by carrageenan, complete Freund adjuvant (CFA), surgical procedure of partial sciatic nerve ligation (PSNL) and pro-inflammatory mediators, such as cytokines/chemokines. Pro-inflammatory mediators were measured by immunohistochemistry, enzyme-linked immunosorbent assay (ELISA) and real time-PCR. Antagonist or antisense oligodeoxynucleotides (AS-ODN) were used to assess the participation of the cannabinoid system in the antinociceptive effects of euphol. All the procedures used in the present study were approved by the Institutional Ethics Committee of the Universidade Federal de Santa Catarina (PP00148). **Results and Discussion:** Oral treatment with euphol (30 and 100 mg/kg) reduced carrageenan-induced mechanical hyperalgesia. Likewise, euphol given through the spinal and intracerebroventricular routes prevented mechanical hyperalgesia induced by carrageenan. Euphol consistently blocked the mechanical hyperalgesia induced by CFA, keratinocyte-derived chemokine (CXCL1/KC), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) associated with the suppression of myeloperoxidase (MPO) activity in the mouse paw. Relevantly, oral treatment with euphol was also effective in preventing the mechanical nociceptive response induced by PSNL and also significantly reduced the levels and mRNA of cytokines/chemokines in both paw and spinal cord tissues following i.p. injection of CFA. In addition, the pre-treatment with either CB₁R or CB₂R antagonists, as well as the knockdown gene of the CB₁R and CB₂R, significantly reversed the antinociceptive effect of euphol. Interestingly, even in higher doses, euphol did not cause any relevant action in the central nervous system. Acute toxicological studies carried out in rodents showed that euphol is safe and well tolerate. Considering that few drugs are currently available for the treatment of chronic pain states, the present results provided evidence that euphol constitutes a promising molecule for the management of inflammatory and neuropathic pain states. **Financial Agencies:** CNPq; CAPES; PRONEX; FAPESC.

05.007 Cytotoxicity and histological assessment of rat skin after treatment with elastic and conventional liposomal butamben gel formulations. Cereda CMS¹, Franz-Montan M¹, Brito Junior RB², de Araújo DR³, de Paula E¹ ¹IB-Unicamp – Bioquímica, ²SLMandic – Biologia Molecular, ³UFABC – Ciências Humanas e Naturais

Introduction: Butamben (BTB) is an ester-type local anesthetic that has been applied in topical and dermal formulations. Its anesthetic action is characterized by a rapid but brief effect associated to toxic effects due to its systemic absorption (*Maestrelli et al, Int J Pharm* 395:222, 2010). Several studies have shown that the sustained release of local anesthetics from liposomes decreases their systemic toxicity and improves their bioavailability (*Grant et al, Anesthesiol* 101:133-137, 2004). A new generation of lipid vesicles has been described, in which the membranes are in the fluid state, presenting high elasticity. When topically applied, these deformable vesicles are able to penetrate through the stratum corneum, carrying drugs across the skin (*Bouwstra et al, Cell Mol Biol Let.* 7: 222-223, 2002). **Methods:** Conventional and elastic liposomes were composed of egg phosphatidylcholine:cholesterol:α-tocopherol (4:3:0.07 molar ratio) and egg phosphatidylcholine: octaoxyethylene-laurate-ester (3:2 molar ratio), respectively. 10% BTB encapsulated in elastic (BTBLUV-EL) or conventional (BTBLUV) liposomes was incorporated in Carbopol® gel formulations and compared to a plain 10% BTB Carbopol® gel formulation. Cytotoxicity assays were performed using the MTT test in mouse fibroblasts cultures (3T3 cells) and the gel formulations were tested at concentrations ranging from 0.312 mM to 6.25 mM. The inhibition concentration for a fifty per cent reduction of the cell number per culture (IC₅₀) was calculated for each tested formulation by nonlinear regression analysis. A histopathological analysis of the rat skin was performed in Wistar rats (6 per group). The tested BTB gel formulations were applied topically on the rat dorsal region (2cm²) under general anesthesia (urethane-1g/kg and alfa-chloralose-50mg/kg) in order to evaluate the local toxicity (Protocol #2261-1 CEUA, UNICAMP). The skin sample was collected 6h after the topical administration, fixated (Bouin's solution–24h and 10%formaline–48h), dehydrated and embedded in paraffin. Cross-sections (5µm) were obtained at five different depths, stained with hematoxylin-eosin and evaluated under light microscopy. **Results and Discussion:** The IC₅₀ values (Mean±SD) obtained for tested formulations (BTB, BTBLUV, BTBLUV-EL) were: 1.47±0.22, 2.63±0.64, 1.95±0.42mM, respectively. The IC₅₀ values obtained from the gel BTB encapsulated formulations were significantly higher than the plain BTB gel formulation (BTBLUV=P<0.001 and BTBLUV-EL=P<0.01 – *Unpaired T test*). The images obtained in histological assessment revealed no morphological changes in rat skin after application of the gel tested formulations. These results indicate a potential reduced toxicity of BTB gel formulation encapsulated in elastic and conventional liposomes in topical applications. **Supported by** CAPES-PNPD (Proc. n°2611/09-0) and FAPESP (Proc. n°06/0121-9).

05.008 Involvement of the NO/cGMP/PKG/KATP pathway and endogenous opioids in the antinociceptive effect of polysaccharide from the red algae, *Gracilaria caudata*. Sales AB¹, Vieira Júnior FC¹, Candeira SJN¹, Medeiros J-VR¹, Souza MHL², Barbosa ALR¹ ¹UFPI-LAFFEX, ²LAFICA-UFC-

Introduction We investigated the antinociceptive activity of a sulphated-polysaccharide (PLS) fraction extracted from the sea algae *Gracilaria caudata* against carrageenan-induced hypernociception in mice and the mechanism underlying the antinociceptive activity. **Methods** Mechanical inflammatory hypernociception was measured with an electronic version of the Von Frey® test. Mice were treated with PLS (2.5, 5.0, and 10 mg/kg, 0.5 mL, p.o.) 1 h before carrageenan treatment. L-Noarg (nonselective inhibitor of NOS, 100 ng/paw; 50 µL) was administered 1 h before carrageenan administration. L-Arginine (L-Arg; substrates for NO, 200 mg/kg; 0.5 mL, p.o.) was administered 10 min before L-Noarg injection. ODQ (inhibitor of soluble guanylate cyclase; 8 µg/paw), KT 5823 (specific inhibitor of protein kinase G; 1.5 µg/paw), glibenclamide (channel antagonist ATP dependent K⁺; 160 µg/paw), and naloxone (opioid antagonist, 1.0 µg/paw) were administered 30 min before carrageenan administration. PLS reduced carrageenan-induced hypernociception (300 µg/paw, 50 µL, intraplantar injection). The project was approved by the Ethics Committee in Research of Universidade Federal do Piauí (Nº. Protocolo: 23111.011989/11-33). **Results and Discussion** PLS-induced analgesia was reversed by L-Noarg, and the effect of L- Noarg was prevented by L-Arg. The soluble guanylyl cyclase inhibitor (ODQ), the protein kinase G inhibitor (KT 5823), the KATP blocker (glibenclamide), and the opioid receptor antagonist (naloxone), significantly reversed (p < 0.05) the antinociceptive effect of PLS. The present study showed an intrinsic peripheral antinociceptive action of PLS administration in mice. This antinociceptive effect seemed to be mediated by activation of the NO/cGMP/PKG pathway followed by the opening of KATP channels, and depended on endogenous opioids. **Financial support:** CNPQ

05.009 The role of substance P and NK₁ receptors in inflammatory and neuropathic orofacial pain. Teodoro FC¹, Martini AC², Rae GA², Zampronio AR¹, Chichorro JG¹ ¹UFPR – Pharmacology, ²UFSC – Pharmacology

Introduction: There is accumulating evidence that substance P released from peripheral sensory neurons participates in inflammatory and neuropathic pain. In this study it was investigated the ability of substance P to induce orofacial thermal and mechanical hyperalgesia, as well as the role of NK₁ receptors on models of orofacial inflammatory and neuropathic pain. In addition, Western blot was used to assess changes in the expression of NK₁ receptors on the trigeminal ganglia of rats. **Methods:** Male *Wistar* rats (200-250 g) were used in all protocols, which were previously approved by the UFPR's Committee on the Ethical Use of Animals (authorization #424). Rats received an injection of substance P (SP, 0.1, 1 and 10 µg/50 µL), carrageenan (50 µg/50 µL) or vehicle (saline, 50 µL) into the upper lip and the thermal or the mechanical hyperalgesia was assessed at 1h-intervals. Other group of animals received an injection of the selective NK₁ receptor antagonist (SR140333B, 1-3 mg/Kg, i.p.) or vehicle (1 mL/Kg) 30 min before the injection of either SP or carrageenan into the upper lip and the thermal hyperalgesia was assessed. In addition, a different group of rats was submitted to the constriction of the infraorbital nerve (CION), a model of orofacial neuropathic pain, and on day 4 and 16 after the surgery, received SR140333B (3 mg/Kg) and thermal and mechanical hyperalgesia was assessed, respectively. The heat stimulus consisted in the approximation of a heat source (~50°C) 1 cm from the vibrissal pad and the cold stimulus was the application of 1-s tetrafluoroethane spray in the orofacial area. Heat and cold hyperalgesia were estimated as a decrease in the latency to display head withdrawal or vigorous snout flicking or an increase in the duration of bilateral facial grooming, respectively. Mechanical hyperalgesia was assessed with von Frey filaments (0.04-8 g) and considered the reduction in the threshold to evoke escape/attack reactions or facial grooming. To analyze changes in NK₁ receptors expression, both in the membrane and the cytosol, 4 hours after carrageenan or saline injection or 4 days after sham or CION surgery, ipsi and contralateral trigeminal ganglia of rats were removed and processed for Western blot. **Results and Discussion:** SP injected into upper lip failed to evoke cold or mechanical hyperalgesia, but induced marked orofacial heat hyperalgesia, which was reduced by the systemic pre-treatment of rats with the NK₁ receptor antagonist SR140333B. Systemic treatment with SR140333B also reduced carrageenan-induced heat hyperalgesia, but did not exert any influence on carrageenan-induced cold hyperalgesia. Moreover, heat, but not cold or mechanical, hyperalgesia induced by CION was abolished by pretreatment with SR140333B. Analysis by Western blot demonstrated increased expression of NK₁ receptors in the cytosol, but not in the membrane, of trigeminal ganglia cells of rats which received carrageenan into the upper lip or submitted to the constriction of the infraorbital nerve. Our results suggest that substance P may have a role in orofacial inflammatory and neuropathic pain. **Financial support:** Fundação Araucária, Sanofi-Aventis, CAPES/PROF

05.010 Synergistic antinociceptive effect of diazoxide, an activator of ATP-sensitive K⁺ channels, and morphine in diabetic neuropathic pain in rats. Neufeld M¹, Pinto RB¹, Schreiber AK¹, Cunha TM², Cunha FQ², Cunha JM¹ ¹UFPR – Farmacologia, ²FMRP-USP – Farmacologia

Introduction: Neuropathic pain is one of the most debilitating symptoms of diabetes and is particularly refractory to opioid analgesics such as morphine (MPH). In experimental models of inflammatory pain, it was established that MPH exerts its antinociceptive effect by activating an intracellular cascade that culminates in the opening of potassium channels (CK⁺), hyperpolarizing the nociceptive neurons. However, the involvement of this pathway in refractory cases of MPH in diabetic neuropathic pain has not yet been characterized, which is the aim of this study.

Methods: Male Wistar rats (180-220 g, n = 4-10) were treated with citrate buffer (CB, 10 mM, pH 4.5, normoglycemic group-NGL) or streptozotocin (STZ, 50 mg / kg diluted in CB; diabetic group -DBT). Three days later, glycemia was determined in a blood sample obtained by tail prick using a strip operated reflectance meter. Only rats with non-fasting blood glucose levels higher than 250 mg/dL were considered DBT and were included in the study. Four weeks later, both NGL and DBT animals received formalin (FML, 0.5%, 50 µL) subcutaneously into the dorsal surface of one of the hind paws, 20 min after ipsilateral administration of saline (30 µL/paw), diazoxide (DZX, 30 or 100 µg/paw), morphine (MPH, 10 µg/paw) or association of DZX plus MPH (at doses indicated). Chemical hyperalgesia was assessed by quantifying the number of flinches after FML injection during 60 minutes, divided into three phases (phase I, IF, 0-10 min; quiescent phase: 10-15 min and phase II, IIF, 15-60 min). All experimental procedures were previously approved by the Committee on the Ethical Use of Animals of the UFPR (authorization # 441). **Results:** DBT rats showed a significant increase in the flinching frequency induced by FML in IF and IIF of the test when compared to NGL rats (71 and 120%, respectively). While the treatment alone with MPH significantly reduced the number of flinches in both phases of the FML test in NGL rats (IF: 49%; IIF: 88%), this treatment does not alter the response in DBT animals. In DBT, but not in NGL animals, the DZX treatment (30 µg) alone significantly reduces (43%) the number of flinches during the IF of FML test. Both doses of DZX (30 and 100 µg) alone were also able to significantly reduce the number of flinches during the IIF of FML test in NGL and DBT animals (dose of 30 µg: NGL, 76%, DBT, 55%; 100 µg: NGL, 61% and DBT, 68%). Interestingly, the treatment with DZX (only at the dose of 30 µg) in combination with MPH reduced the number of flinches during both phases of FML test in DBT rats (IF: 59% and IIF: 84%) and NGL animals (IF: 45% and IIF: 88%). **Conclusions:** Since the maximum reduction of FML-induced nociceptive behaviors was the result of association of DZX plus MPH, our results suggest a synergistic antinociceptive effect of these drugs. Additionally, these results indicate that diabetes seems to impair the opening of CK⁺ mediated by MPH and that the administration of drugs able to open such channels may be an alternative for the treatment of diabetic neuropathic pain. **Financial support:** Fundação Araucária, UFPR/TN).

05.011 Fructose-1,6-bisphosphate reduces neuropathic pain via adenosine activating A1 and A2A receptors: Role of NO/cGMP/PKG/KATP signaling pathway. Milanez PAO, Medeiros DC, Zarpelon AC, Verri Jr WA UEL – Patologia

Introduction: There are several therapies for neuropathic pain control, but current treatments lack expected efficacy and have many side effects, thus, alternative treatments are needed. Fructose-1,6-bisphosphate (FBP) is an intermediate of the glycolytic pathway that has several pharmacological actions, and more recently it was demonstrated its anti-hyperalgesic effect in carrageenin-induced inflammatory pain via production of adenosine. Interestingly, adenosine and adenosine A1 and A2A receptor agonists have anti-hyperalgesic effects in neuropathic pain models. Therefore, we investigated the anti-hyperalgesic effect and mechanism of action of FBP in a model of chronic constriction injury (CCI) of sciatic nerve-induced neuropathic pain and the role of adenosine receptors in its mechanism of action. **Methods:** Adult male Swiss mice (22–28g) were obtained from the Universidade Estadual de Londrina and maintained in a temperature-controlled room with access to water and food *ad libitum* until use. Seven days after CCI surgery, animals were treated with FBP (300 mg/kg, p.o. and 30µg/animal, i.t.) or adenosine (300mg/Kg, p.o. and 30µg/animal, i.t.). Subsequently, the treatment with FBP or adenosine were performed combined with A1 (10µg/animal) or A2A (1µg/animal) receptor antagonists or inhibitors of nitric oxide synthase/guanilate cyclase/Protein Kinase G/ATP-sensitive potassium channel signaling pathway: L-NMMA (100 mg/Kg, i.p.), ODQ (1 mg/Kg, i.p.), KT5823 (0,5 µg/animal, i.p.) and glibenclamide (1 mg/Kg, p.o.), respectively. CCI-induced mechanical hyperalgesia was assessed 7 days after surgery by an electronic version of von Frey filaments at indicated time points. All animal care and experimental procedures were approved by the Ethics Committee of the Universidade Estadual de Londrina (CEUA-UEL/ 07219). **Results:** FBP and adenosine inhibited the mechanical hyperalgesia induced by CCI in a similar profile (FBP treatment: 85% p.o. at 3h; 100% i.t. at 5h. Adenosine treatment: 76% p.o. at 3h; 89% i.t. at 5h). FBP mechanism of action seems to be dependent on adenosine production as its anti-hyperalgesic effect was abolished by adenosine A1 and A2A receptor antagonists, and FBP treatment anti-hyperalgesia was fully dependent on the NO/cGMP/PKG/KATP signaling pathway similarly to adenosine. **Discussion:** The present study demonstrates that: (i) FBP and adenosine inhibited the mechanical hyperalgesia induced by CCI with a similar profile; (ii) the anti-hyperalgesic effect of FBP and adenosine are dependent on activation of adenosine A1 and A2A receptors; (iii) FBP treatment promotes anti-hyperalgesic through NO/cGMP/PKG/KATP signaling pathway similarly to adenosine. Therefore, these results further support the anti-hyperalgesic efficacy of FBP, and that its mechanisms depend on activation of adenosine A1 and A2A receptors and the NO/cGMP/PKG/KATP signaling pathway in neuropathic pain. **Financial support:** CNPq, CAPES and Fundação Araucária.

05.012 Critical role of PAR-2 activation by mast cell tryptase on the development of postoperative pain. Oliveira SM, Silva CR, Ferreira J UFSM – Química

Introduction: Recently, studies have indicated that nearly half of all surgical patients still have inadequate pain relief what become important to understand the mechanisms involved postoperative pain to be better treat it (Weiser et al., 2008; Wu and Raja, 2011). Thus, the aim of this study was to investigate the involvement of mast cell tryptase and its substrate, the protease-activated receptor 2 (PAR-2), in a model of postoperative pain in mice. **Methods:** The experiments were conducted on adult male Swiss mice (25-35 g, n= 6-8). After anesthesia with halothane, a longitudinal surgery was made through the skin and fascia of the plantar foot. The underlying muscle was elevated with a curved forceps, leaving the muscle origin and insertion intact and the skin was sutured. Mice were treated with the tryptase inhibitor (gabexate) or the PAR-2 antagonist (ENMD-1068), anesthetized and submitted to incision or injection of the PAR-2 activator tryptase. After surgery, tryptase activity was evaluated in the tissue perfusates of control, mast cell membrane stabilized (with cromoglycate injection) or depleted (with previous compound 48/80 treatment) animals (Hoffmeister et al., 2011; Kelso et al., 2007; Oliveira et al., 2011; Vergnolle et al., 2001). The experiments were performed with the approval of Ethics Committee of the Universidade Federal de Santa Maria (process number 45/2010). **Results:** The previous treatment (30 min before) with gabexate or ENMD-1068 in the local of the incision prevented postoperative nociception with ID₅₀ value of 0.30 (0.072-1.22) nmol/paw and 59 (27-130) nmol/paw and a maximum inhibition of 76±15% and of 80±13%, respectively, as well as prevent the nociception induced by the PAR-2 activator tryptase (5 ng/paw) with inhibitions at the peak of 100% and 88±14%, respectively. Neither gabexate nor ENMD-1068 was capable of reversing nociception when administered 30 min after incision. Indicating an early tryptase release by mast cells postoperatively, plantar surgery increased activity of tryptase (two-fold higher) in perfusates and produced mast cell degranulation on the incised tissue 10 min after surgery. The local pre-treatment with cromoglycate fully prevented (100% of prevention) the increase in tryptase release and the nociception (inhibition 76±18%) induced by surgery. Accordingly, the depletion of mast cell mediators produced by repeated pre-treatment with compound 48/80 largely reduced tryptase activity (inhibition of 81±14%) in the paw tissue and postoperative nociception (inhibition 74±3%). **Discussion:** In this study, we determined that mast cells contribute to the development of postoperative pain through early tryptase release and subsequent PAR-2 activation. Thus, the mast cell release of tryptase and the further PAR-2 activation are interesting targets for the development of novel therapies to treat postoperative pain. **Financial Agencies:** CAPES; CNPq **Acknowledgments:** This study was supported by the Conselho Nacional de Desenvolvimento Científico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) (Brazil). We also acknowledge the receipt of fellowships from CNPq and CAPES. **References:** - Hoffmeister et al., Pain 152:1777, 2011. - Kelso et al., Arthritis Rheum 56:765, 2007. - Oliveira SM et al., Eur J Pharmacol 672:88, 2011. - Vergnolle et al., Nat Med 7:821, 2001. - Weiser et al., Lancet 372:139, 2008. - Wu and Raja, Lancet 377:2215, 2011.

05.013 The influence of B vitamins on thermal and mechanical hyperalgesia induced by constriction of the infraorbital nerve in rats. Kopruszinski CM, Reis RC, Chichorro JG UFPR – Farmacologia

Introduction: There is a growing body of evidence that the use of B vitamins can help to control neuropathic pain (JOLIVALT, *Eur. J. Pharmacol.* 612; 41, 2009; MEDINA-SANTILLAN, *Proc. West Pharmacol.* 47; 109, 2004). Currently, only the anticonvulsant drug carbamazepine is approved by the FDA for the treatment of trigeminal neuralgia, but its use is associated with numerous adverse effects (KLEEF, *Pain Pract.* 9; 252, 2009). Thus, this study aimed to evaluate the influence of the treatment with different B vitamins (i.e. B1, B6 and B12) on the thermal and mechanical hyperalgesia induced by constriction of the infraorbital nerve (CION) in rats, a model of trigeminal neuropathic pain. In addition, we have investigated a possible synergic effect of Carbamazepine combined with different B vitamins on the thermal hyperalgesia induced by CION.

Methods: Male *Wistar* rats (180-220 g) were used in all protocols, which were previously approved by the UFPR's Committee on the Ethical Use of Animals (authorization # 471). Heat and cold hyperalgesia were estimated as a decrease in the latency to display withdrawal head or snout vigorous flicking, or an increase in the duration of bilateral facial grooming, respectively. Mechanical hyperalgesia was estimated as a decrease on the mechanical threshold by the application of a series of von Frey monofilaments (0.04 – 8 g). Hyperalgesia to heat and cold was measured before and at 2, 4, 6, 9 and 12 days after surgery, while mechanical hyperalgesia was measured before and at 8, 10, 12, 16 and 20 days after the surgical procedure.

Results: CION induced thermal hyperalgesia to heat and cold that peaked on day 4 and mechanical hyperalgesia with a peak on day 20 after the surgery. Repeated subcutaneous treatment (for 5 days, starting on the day of surgery up to day 4 after surgery) of rats with B1, B6 or B12 vitamins (180, 180 and 18 mg/kg, respectively) prevented the development of heat hyperalgesia after CION. On the other hand, only B12 vitamin treatment (18 mg/kg, s.c., for 5 days) was able to reduce the cold hyperalgesia on days 6 and 9 after CION (maximal reduction by 94%). Moreover, only B6 vitamin treatment (180 mg/kg, s.c., for 5 days, starting on day 8 up to day 12 post-surgery) blocked the develop of the mechanical hyperalgesia of rats submitted to CION. Additionally, a single injection of carbamazepine (30 mg/kg, i.p.) was able to reduce heat and cold hyperalgesia on day 4 after nerve injury, but was not able to modify the mechanical hyperalgesia assessed on day 20 post-surgery. The combination of B vitamins (B1, B6 and B12, at 18, 18 e 1.8 mg/kg, s.c., 5 injections) with carbamazepine (at 10 mg/kg, i.p., single injection) caused a marked reduction of heat hyperalgesia, while the combination of B12 with carbamazepine (same doses above) promoted the reversion of cold hyperalgesia, both evaluated on day 4 post-surgery. **Discussion:** This study provides evidence that B1, B6 and B12 vitamins, alone or in combination with carbamazepine, ameliorate heat, cold and mechanical hyperalgesia in a rat model of trigeminal neuropathic pain. Thus, B1, B6 and B12 vitamins could be an alternative or be used as adjuvant to control some aspects of pain in patients suffering from trigeminal neuralgia. **Financial support:** REUNI/CAPES.

05.014 Role of TNF- α in intense acute swimming-induced delayed onset muscle soreness in mice. Borghi SM¹, Zarpelon AC¹, Cardoso RDR¹, Casagrande R², Verri Jr WA¹ ¹UEL – Ciências Patológicas, ²UEL – Ciências Farmacêuticas

Introduction: Aerobic physical training with mild to moderate intensity (20-30 min) reduces inflammatory and neuropathic pain in animal models. However, intense acute physical exercise of moderate intensity and long duration can induce allodynia and hyperalgesia post-exercise. The acute exercise has also been linked to signaling pathways related to cytokines in post-exercise. Intra-muscular injection of TNF- α evokes muscle hyperalgesia within several hours after injection, and strenuous exercise promotes the increase of TNF- α . In the present study, it was evaluated the role of TNF- α in intense acute swimming-induced delayed onset muscle soreness in mice. **Methods:** Animals' care and handling procedures were in accordance with the International Association for Study of Pain (IASP) guidelines and with the approval of the Institutional Ethics Committee for Animal Research of the Universidade Estadual de Londrina, process number 2066.2011. Sham animals swam for just 30 s, and mice in the swimming group were exposed to water for 1 session of 30, 60 or 120 min, or 1 session of 30 min per day during 5 days. The mechanical hyperalgesia was evaluated between 6-48 h after the swimming session. Mechanical hyperalgesia was tested in mice by electronic von Frey anesthesiometer. TNF- α production was evaluated in peripheral and central sites by ELISA, and the leukocyte recruitment and skeletal muscle GSH levels in the soleus and gastrocnemius muscles was analysed by colorimetric **Methods: Results:** Swimming during 30 min did not induced hyperalgesia. On the other hand, 60 and 120 min of swimming induced significant mechanical hyperalgesia at 6, 12 and 24 h after exercise, and the peak of hyperalgesia was observed at 24 h after swimming session. The time of 120 min was chosen for next experiments in which it was observed: 1) reduced mechanical hyperalgesia in TNFR1^{-/-} mice (80%); 2) increased TNF- α production at 2 h in the soleus muscle (50%), but not in the gastrocnemius muscle, and at 4 h in spinal cord samples (L4-L6) (75%); 3) increased neutrophil recruitment (myeloperoxidase activity) to the soleus muscle at 24 h (peak of hyperalgesia) in swim group (80%) compared to sham group with significant inhibition in TNFR1^{-/-} mice (67%); 4) reduction of GSH levels at 2 and 4 h (95%) after exercise session in the soleus muscle, which was prevented in TNFR1^{-/-} mice (67%); 5) there were no changes in the gastrocnemius muscle. **Discussion:** The present model presents characteristics of delayed onset muscle soreness (DOMS) considering the peak of hyperalgesia between 24-48h and that it occurred in response to intense exercise in untrained animals. The present study provides evidence on the role of endogenous TNF- α in intense acute swimming-induced DOMS in mice. There is a muscle specific role of TNF- α in that its production occurs in the soleus, but not in the gastrocnemius muscle. The soleus muscle is also the foci of inflammatory neutrophil recruitment and oxidative stress upon intense exercise. The mechanical hyperalgesia induced by intense acute swimming depends on peripheral (soleus muscle) and central (spinal cord) production of TNF- α . **Financial support:** CAPES, CNPq, SETI/Fundação Araucária and Governo do Estado do Paraná.

05.015 Cetirizine and Immapip potentiated the analgesic effect of spinal morphine. Stein TS, Souza-Silva E¹, Tonussi CR¹ ¹UFSC – Farmacologia

Introduction: Histamine receptors are known to participate in the spinal cord nociceptive transmission and previous studies suggest that histaminergic receptors are involved in the analgesic effects of morphine. Besides nociception, the sensitization status of spinal cord can also modulate the antidromic traffic of primary afferent nociceptor action potentials back to the tissues, which eventually influence an inflammatory site by the local release of vasoactive neuropeptides. Morphine injected in the spinal cord can act either post-synaptically [Yaksh and Rudy, *Science*. 1976, 192:1357-8], causing antinociception, or pre-synaptically, inhibiting inflammatory edema [Brock and Tonussi, *Anesth Analg*. 2008, 106:965-71]. **Aim:** Our aim was to investigate the possible interaction of H1 and H3 receptors with opioid receptors in the anti-arthritic effect of intrathecally administered morphine in rats. **Methods:** Acute arthritis was induced by carrageenan injection into knee-joint (50µg) in male wistar rats (weighing 300 - 400 g). After inflammatory stimulation, articular incapacitation was measured hourly, up to 6 hours, by an automated counting of the paw elevation time (PET, s) during 1-min periods of stimulated walk. The inflammatory edema was also hourly assessed through the micrometric measurement of the articular diameter (AD, mm). After this time, animals were euthanized for collecting synovial fluid for the evaluation of leukocyte infiltration. The experimental groups received intrathecal injection of morphine (0.5; 5 and 45 nmol), the H1 antagonist cetirizine (12.6 pmol) and the H3 agonist immepip (16 pmol). Spinal treatments were made 20 min before knee-joint carrageenan injection, and were compared to saline treated control group. Either PET or AD time-course curves were compared by two-way ANOVA followed by Duncan post-hoc test. **Results:** Morphine (5 and 45 nmol) inhibited either incapacitation or edema ($P < 0.05$). The co-administration of a subeffective dose of morphine (0.5 nmol) either with cetirizine (12.6 nmol) or immepip (16 pmol) decreased incapacitation. None of the treatments modified the synovial leukocyte content. **Conclusion:** Spinal delivered morphine was found to be antinociceptive and anti-edematogenic in the present model of acute arthritis. Cetirizine and immepip were found to potentiate the analgesic effect of morphine, but not the anti-edematogenic effect. These results suggest that the histaminergic and opioid receptors interact in a post-synaptic level, since opioid-induced antinociception was potentiated. However, it seems that there is no pre-synaptic interaction, since edema was not affected. This study was approved by the local ethical committee for animal use (CEUA/PP00368/2009). **Financial support:** CNPq; CAPES;

05.016 Mechanisms involved in the nociception triggered by the venom of the armed spider *Phoneutria nigriventer*. Gewehr CCV¹, Oliveira SM², Rossato MF², Trevisan G², Ferreira J², Gomez MV¹ ¹IEP-Santa Casa BH, ²UFMS – Química

Envenomation with *Phoneutria nigriventer* venom (PNV) is the most important cause of intoxication by spiders in Brazil. Pain is the main local symptom presented by people inoculated with PNV (Bucharetski et al., 2000); however, the mechanisms involved in this nociception are poorly known. Therefore, the aim of the present study was to identify the peripheral mechanisms involved in PNV-induced nociception in mice. A volume of twenty μ l of PNV, diluted in phosphate-buffered saline (PBS), was injected subcutaneously (s.c.) under the surface of the right hind paw (Oliveira et al., 2009). The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. This study was approved by the Committee on the Use and Care of Laboratory Animals of our university (no. 23081.003193/200940). The s.c. injection of PNV (0.3-10 μ g/paw) produced an immediate and short lasting spontaneous nociception. The effective dose to produce 50% effect caused by PNV was 1.6 (0.8-2.4) μ g/paw and the maximal licking responses were 147.5 \pm 17.0 s. This effect was largely prevented by afferent C-fiber desensitization, but not by mast cell degranulation. Notably, the boiling or the dialyzing of PNV reduced the spontaneous nociception induced by the venom, with inhibition of 55 \pm 14% and 55 \pm 18%, respectively. We identified that serotonin (acting on 5-HT₄ receptor), but not glutamate and histamine, presented in PNV are important due to the nociceptive effect of the venom. Moreover, we detected that PNV contained a tissue kallikrein-like activity that is also involved in the venom-induced nociception. The kinetic curve demonstrated that the tissue kallikrein-like enzyme in PNV had a K_M value of 1.47 \pm 0.09 mM and a maximal velocity of 16.13 \pm 0.62 nmol/mim/mg protein. Moreover, the kallikrein-activity of PNV (500 mg/ml) was inhibited by boiling PNV or by the tissue kallikrein inhibitor aprotinin (10 mg/ml), but not by plasma kallikrein inhibitor SBTI (3 mg/ml). Accordingly, the s.c. co-administration of the inhibitor of tissue kallikrein aprotinin (100 μ g/paw), but not of plasma kallikrein soybean trypsin inhibitor (3 μ g/paw), was capable of reducing the PNV-induced nociception, with an inhibition of 70 \pm 12% compared to the control group. PNV kallikrein may produce kinin, since the s.c. co-administration of the selective B₂ receptor antagonist HOE140 (3 nmol/paw) with PNV reduced the nociceptive effect, with an inhibition of 43 \pm 14% compared to the control group. The blockade of tetrodotoxin sensitive Na⁺ channel, acid sensitive ion channel (ASIC) receptor or vanilloid receptor (TRPV1) also reduced PNV-triggered nociception. Collectively, the results of the present study demonstrated that PNV produces spontaneous nociception by the presence of small molecular size mediators, such as 5-HT or by the activation of a kinin generating kallikrein-like enzyme.

Acknowledgment: Conselho Nacional de Desenvolvimento Científico. Bucaretski, F. A clinic-epidemiological study of bites by spiders of the genus *Phoneutria*. Rev. Inst. Med. trop. S. Paulo 42, 17, 2000. Oliveira, S.M. Antinociceptive effect of a novel tosylpyrazole compound in mice. *Basic Clin. Pharmacol. Toxicol.* 104, 122, 2009.

05.017 Changes in cold sensitivity as predictor of diabetic neuropathic pain: Involvement of TRPM8 and TRPA1 receptors. Jesus CHA, da Justa HC, Nones CFM, Cunha JM UFPR – Farmacologia

Introduction: Neuropathic pain is a major complication of diabetes, characterized by spontaneous pain, hyperalgesia and/or allodynia, induced by stimuli of different natures. Thus, it is reported that allodynia to cold is a common complaint of diabetics and that the abnormalities in response to thermal stimuli of cold is the most sensitive test for early detection of diabetic polyneuropathy and seems to precede clinically the development of mechanical allodynia. Despite of the importance of this clinical sign, little has been explored about the mechanisms involved in the establishment of cold allodynia in diabetes as well as the involvement of TRPM8 and TRPA1 receptors, which is the aim of this work. **Methods:** Wistar rats (180-220 g, n = 8-10) were treated intraperitoneally with citrate buffer (10 mM, pH 4.5, NGL, normoglycemic control group) or streptozotocin (STZ, 50mg/kg, diluted in citrate buffer, DBT-diabetic group). For assessment of cold allodynia, both NGL and DBT animals were instilled with 100 µL of acetone (TRPM8 receptor agonist) in the center of one of their hind paw prior to administration of STZ (Baseline 0, B0) and weekly until week 4 after induction of diabetes (Basal 4, B4). The nociceptive behaviors of licking and shaking paw (flinches) were recorded for 2 minutes after instillation. For assessment of mechanical allodynia, mechanical threshold was recorded using an electronic analgesimeter, prior to administration of STZ (B0) and weekly until week 4 (B4). To evaluate the involvement of TRPA1 receptor, NGL and DBT animals received intraplantar injection (i.pl) of mustard oil (MO, 0.5 or 1%, 50 µL/ paw) at 4 weeks after STZ. The direct nociceptive response (flinches) was evaluated for 20 minutes starting from the injection of MO, and then the mechanical withdrawal threshold was reassessed. All experimental procedures were previously approved by the Committee on the Ethical Use of Animals of the UFPR (authorization # 465). **Results:** The number of flinches induced by acetone instillation was significantly higher (37%) in DBT animals only in week 4 after induction of diabetes, while the mechanical withdrawal threshold has been significantly reduced from week 2 (37%), peaking at week 4 (57%). DBT animals showed no significant difference in the number of flinches induced by i.pl. Administration of MO (both doses used) compared to NGL animals. However, administration of MO (1%) induced a reduction of the mechanical threshold withdrawal (about 50% when compared to B0) in both DBT and NGL animals and a reduction of 32% compared to B4 in DBT animals. The dose of 0.5% promoted a reduction of 55% of the mechanical threshold withdrawal in NGL animals and reduction of 71% in DBT animal, when compared to B0. **Conclusion:** While clinically the changes in perception to cold predict the development of mechanical allodynia, this same pattern of sensory changes was not observed in experimental diabetes. Diabetes seems to sensitize TRPM8 receptors (instillation of acetone results) without interfering with the activity mediated by TRPA1 receptor agonists. However, TRPA1 receptor agonist appears to sensitize the diabetic animals for mechanical stimulus. **Financial support:** CNPq (Process # 477452/2011-6).

05.018 Evidence for the involvement of the opioid system in the antinociceptive effect of hecogenin acetate. Gama KB¹, Santana WA¹, Branco A², Quintas-Junior L³, Quintans JSS³, Soares MBP¹, Villarreal CF^{1,4} ¹CPqGM-Fiocruz-BA, ²Uefs, ³UFS, ⁴UFBA – Farmácia

Introduction: Saponins have been thoroughly described for their pharmacological properties. Hecogenin is the steroidal saponin aglycone present in the leaves of species from the *Agave* genus, which has a wide spectrum of pharmacological activities (BOTURA, 2011; SEO, 2010). The present study was undertaken to evaluate whether hecogenin acetate has antinociceptive properties and to determine its mechanism of action. **Methods:** The antinociceptive activity of hecogenin was evaluated using the tail flick test (water bath $48^{\circ} \pm 0.5^{\circ} \text{C}$) in Swiss male mice (25-30 g, n=6). In the tail flick test, the nociceptive threshold was evaluated before (baseline) and at different times after treatment. Mice motor performance was evaluated in rota rod (length in the cylinder) test. The mechanisms involved in the hecogenin antinociceptive activity were investigated through the use of pharmacological antagonists of opioid receptors. Institutional Animal Care and Use Committee FIOCRUZ L-IGM-012/09. Differences between groups were detected by two-way ANOVA followed by Bonferroni post-hoc test using GraphPad Prism version 5.0. $P \leq 0.05$ was considered significant. **Results:** Intraperitoneal administration of hecogenin acetate (5 – 40 mg/kg) produced a dose-dependent increase of the analgesia index (5 mg/kg: 1.3 ± 1.9 ; 10 mg/kg: 6.4 ± 9.6 ; 20 mg/kg: 32.4 ± 2.8 ; 40 mg/kg: 66.7 ± 4.0) compared to vehicle-treated group ($p < 0.01$, 0.3 ± 3.6). The reference drug, morphine (5 mg/kg, s.c.), also produced antinociceptive effect (53.2 ± 6.8). The antinociceptive effect of hecogenin (40 mg/kg) was prevented by the pretreatment with naloxone (4 mg/kg i.p.; 15 minutes before; 1.3 ± 2.6), a non-selective opioid receptor antagonist, suggesting mediation by the opioid system. In addition, the pretreatment with CTOP (m-opioid receptor antagonist; 1 mg/kg i.p.; 30 minutes after hecogenin), nor-BNI (k-opioid receptor antagonist; 0.5 mg/kg s.c.; 15 minutes before hecogenin), or naltrindole (δ -opioid receptor antagonist; 3 mg/kg s.c.; 5 minutes before) also prevented the hecogenin-induced antinociception (CTOP: 1.4 ± 3.7 ; nor-BNI: 1.3 ± 4.3 ; naltrindole: 1.9 ± 2.0). Hecogenin (40 mg/kg)-treated mice did not show any motor performance alterations. These results validate the antinociceptive activity of hecogenin suggested by the nociceptive test. **Conclusion:** The present results show that hecogenin acetate has antinociceptive activity mediated, at least in part, by the opioid system. **Sources of research support:** This work was supported by FIOCRUZ, CNPq and FAPESB. **References:** BOTURA, M.B. et al. In vivo anthelmintic activity of an aqueous extract from sisal waste (*Agave sisalana* Perr.) against gastrointestinal nematodes in goats. *Vet. Parasitol.*, v. 177, p. 104-110, 2011. SEO, K. A. et al. Metabolism of 1'- and 4-Hydroxymidazolam by Glucuronide Conjugation is Largely Mediated by UDP-Glucuronosyltransferases 1A4, 2B4, and 2B7. *Drug Metab. Dispos.*, v.38, p. 2007-2013, 2010.

05.019 Cannabinoid receptors differentially modulate the peripheral antinociceptive effect of cannabinoids in a model of neuropathic pain induced by streptozotocin in rats. Schreiber AK, Neufeld M, Jesus CHA, Cunha JM UFPR – Farmacologia

Introduction: Pain is one of the most common symptoms in patients with diabetic neuropathy. The treatment options to this kind of pain are currently limited to palliative drugs. Based on known antinociceptive properties of cannabinoids in others pain conditions, this study aims to investigate the potential antinociceptive effect of anandamide (AEA) and drugs that affect the endocannabinoid system on chemical hyperalgesia induced by formalin (FML) in normoglycemic (NGL) and diabetic (DBT rats), identifying the cannabinoid receptor subtype involved. **Methods:** For this, male Wistar rats (n= 6-10/group) received intraperitoneal administration of citrate buffer (CB; 10 mM, pH 4.5; NGL group) or streptozotocin (STZ, 50 mg/kg, dissolved in CB, DBT group). Hyperglycemia was confirmed 3 days after STZ administration by a strip operated reflectance meter in a blood sample obtained by tail prick and confirmed again at ending of the study. Only rats with glucose levels higher than 250 µmg/dL were included. Four weeks later, both NGL and DBT animals received FML (0.5%, 50 µL) subcutaneously into the dorsal surface of one of the hind paws, 20 min after ipsilateral administration of vehicle (30 µL/paw), AEA (0.1 µg/paw), ACEA (a selective CB1 receptor agonist; 30 µg/paw) or AM404 (an AEA transport inhibitor, 50 µg/paw). When associated, cannabinoid receptor antagonists AM251 (a CB1 receptor antagonist, 150 µg) or AM630 (a CB2 receptor antagonist, 25 µg) were ipsilaterally injected 10 min before cannabinoid agonists. Chemical hyperalgesia was assessed by quantifying the number of flinches after FML injection during 60 minutes, divided into three phases (phase I, IF, 0-10 min; quiescent period: 10-15 min and phase II, IIF, 15-60 min). All experimental procedures were approved by the Committee on the Ethical Use of Animals of the UFPR (license #423). **Results:** When compared to NGL rats, DBT animals showed a significant ($p<0.05$) increase in the flinching frequency induced by FML in both IF and IIF (60 and 67%, respectively). Pretreatment with AM404 significantly reduced the number of nociceptive behaviors induced by FML in NGL and DBT groups during IF (57 and 40%, respectively) and IIF (64 and 60%, respectively). Pre-treatment with AM251 significantly prevented the antinociceptive effect of AM404 on IF in NGL group and on both phases on DBT group, while AM630 affected only IIF in DBT rats. Treatment with AEA reduced FML-induced nociceptive response in both phases in NGL (43 and 70%) and DBT groups (46 and 78%, respectively). AM 251 prevented AEA antinociception on IF and IIF in both NGL and DBT groups, while AM 630 affected just IIF in both groups. ACEA significantly lowered the number of FML-induced flinches in NGL and DBT groups during IF (48 and 61%) and IIF (50 and 30%, respectively), effect that was completely blocked by pre-treatment with AM251. **Conclusions:** Taken together, our data suggest that cannabinoids could exert antinociceptive effects in a model of painful diabetic neuropathy when administered peripherally, acting on both CB1 and CB2 receptors, possible CB1 receptors expressed on primary afferent nociceptors and CB2 receptors presented on inflammatory cells as mast cells.

05.020 Characterization of cytoskeleton involvement in crotalphine antinociceptive effect. *In vivo* and *in vitro* assays. de Almeida AC¹, Gutierrez VP¹, Sampaio SC¹, Cury Y¹ ¹IBu – Dor e Sinalização

Introduction: Crotalphine, a 14 amino acid peptide first identified in the venom of the South American rattlesnake *Crotalus durissus terrificus*, induces potent and long lasting (2-5 days) antinociceptive effect. Crotalphine mechanism of action is not completely understood, but involves peripheral κ - (PGE2-induced hyperalgesia) or κ - and δ -opioid (neuropathic and cancer pain) receptor activation, followed by activation of the L-arginin/NO/cGMP pathway and activation of ATP-sensitive K^+ channels. *In vitro* studies also showed that crotalphine activates PKC ζ , which in turn, activates MAP kinases. As observed for other opioid-like drugs, the high efficacy and long-lasting peripheral antinociceptive effect of crotalphine is only detected in the presence of inflammation or tissue lesion. The cytoskeleton is a complex and highly dynamic net of proteins that actively participates of several cellular processes, including nociceptive signaling and control. Additionally, the cytoskeleton regulates the organization and expression of membrane proteins such as opioid receptors, and can modulate the intracellular signaling pathways activated by these receptors. The aim of this work is to characterize the role of cytoskeleton proteins in crotalphine antinociceptive effect. **Methods:** Latrunculin B (0.05 μ g/paw) or cytochalasin D (0.001 μ g/paw) was used to disrupt actin microfilaments; nocodazole (1.0 μ g/paw) or colchicine (0.8 μ g/paw) was used to disrupt microtubules; acrylamide (1.0 μ g/paw) was used to disrupt intermediate filaments. Wistar male rats were treated with intraplantar (i.pl.) administration of PGE2 (100 ng/paw), cytoskeleton inhibitors and crotalphine (0.6 μ g/paw) or κ opioid receptor agonist U50,488 (10.0 μ g/paw, positive control). After 3, 4 and 5 hours from the beginning of the experiment, nociceptive threshold was determined by the paw pressure test. All the procedures were conducted in accordance with the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals, and were approved by the Animal Ethics Committee of the Butantan Institute (Protocol number 855/11). **Results:** PGE2 caused hyperalgesia that was blocked by crotalphine. Latrunculin B, cytochalasin D, nocodazole and acrylamide completely blocked crotalphine antinociceptive effect, while colchicine significantly prevented crotalphine effect. All the cytoskeleton inhibitors completely blocked the antinociceptive effect of the U50,488. None of the cytoskeleton inhibitors altered, per se, the nociceptive threshold of naïve animals, or interfere with PGE2 hypernociceptive effect. **Discussion:** Actin microfilaments, microtubules and intermediate filaments are key elements for the antinociceptive effect of crotalphine, as the selective disruption of these elements prevented or blocked crotalphine antinociceptive effect. Considering that crotalphine mechanism in PGE2-stimulated animals occurs via activation of κ opioid receptor, and that cytoskeleton inhibitors also blocked the antinociceptive effect of the κ opioid receptor agonist, we suggest that disruption of cytoskeleton elements interfered with κ opioid receptor signaling activated by crotalphine. **Financial Aid:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes).

05.021 Aerobic exercise produces antinociception in animals submitted to rheumatoid arthritis model. Lotin MC, Hakbarth TO, Quintão NLM UNIVALI

Introduction: Rheumatoid arthritis (RA) is a chronic, destructive polyarticular joint disease, characterized by synovial proliferation and significant inflammatory cells infiltration. Mazzardo-Martins et al. (2010) have demonstrated that aerobic exercise was able to reduce the nociception induced by acetic acid injection in mice. This effect seems to involve the serotonin release. Miller et al. (2008) also observed that physical exercise reduced some inflammatory biomarkers levels, such as IL-6, TNF α and reactive C-protein. The aim of this study was to evaluate the effects of aerobic exercise on the mechanical hypersensitivity induced by Freund complete adjuvant (CFA) injection in rodents. **Methods:** Male Swiss mice (20-30 g; n=6-8) received an intraplantar (i.pl.) injection of CFA (1 mg/mL) suspended in saline (1:1) into the right hindpaw (50 μ L/paw). The control group received sterile saline solution into the right hindpaw. Animals were placed in a glass compartment filled with water at 37°C. Control and CFA (nonexercised) mice were allowed to swim for just 30 seconds each day and were then gently dried. The animals were submitted to two swimming sessions (SW; 5 min each), following six different protocols: 1- SW 15 days before CFA injection; 2- SW 30 days before CFA injection; 3- SW immediately after CFA injection for 15 days; 4- SW initiated 14 days after CFA injection for 7 days; 5- SW 15 days before and after CFA injection; 6- SW 30 days before and after CFA injection. The mechanical hypersensitivity and paw oedema were evaluated using von Frey filament (6.0 g) and the plethysmometer, respectively. The subcutaneous tissue of right hindpaw was collected to verify the myeloperoxidase (MPO) activity. Areas under curve values were used to compare the groups and are expressed as mean \pm mean standard error. The study was approved by the Ethical Committee for Animal Care of UNIVALI (Process No. 016/10). **Results:** The animals submitted to the protocol 1 did not present significant difference when compared with CFA-injected nonexercised mice. However, the protocol 2 was able to reduce the mechanical hypersensitivity induced by CFA injection (1881 \pm 61 and 1298 \pm 57; %I = 31 \pm 3). The animals submitted to the protocol 3 presented significant inhibition (1602 \pm 36 and 1312 \pm 94), although to a lesser extent, with inhibition of 21 \pm 4%. Similar results were obtained with the protocol 5, when the animals initiated the swimming just 14 days after CFA injection (1752 \pm 39 and 1428 \pm 76; %I= 19 \pm 4). Surprisingly, animals that were trained 15 or 30 days before and after the CFA injection presented inhibitions of 20 \pm 4% (1821 \pm 30 and 1449 \pm 69) and 40 \pm 3 % (1732 \pm 45 and 1054 \pm 42), respectively (protocols 5 and 6). The paw oedema of mice submitted to the protocol 6 was significantly reduced (212 \pm 21 and 135 \pm 8; %I= 42 \pm 4 %) at the first two days after CFA injection. However, no-significant interference was observed in MPO activity. **Discussion:** These results, together with literature data, can suggest that the regular aerobic exercise could be used as an alternative non-pharmacological approach for the management of chronic pain, such as rheumatoid arthritis. **Financial support:** PROBIC-ProPPEC UNIVALI. Mazzardo-Martins et al. J. Pain. 11:1384, 2010. Miller et al. J. Am. Geriatr. Soc. 56:644, 2008.

05.022 Antinociceptive and antidiarrheal effect of hydroalcoholic extract from *Machaerium hirtum* (inner barks) and the involvement of opioid system. Lopes JA¹, Nishijima CM¹, Hiruma-Lima CA¹, Rocha LRM¹, Sannomiya M², de Souza-Maria NCV², Tangerina MM³, Vilegas W³ ¹IBB-Unesp-Botucatu – Fisiologia, ²EACH-USP, ³IQAr-Unesp-Araraquara – Química Orgânica

Introduction: The genus *Machaerium* belongs to the Fabaceae family, and these family's species have important pharmacological and therapeutical effects. Some *Machaerium* species has medical indication, like *Machaerium hirtum* (Vell.) Stellfeld, which barks are used against diarrhea, cough and cancer. The phytochemical profile of the hidroalcoholic extract from *Machaerium hirtum* (HEM) (inner barks) has revealed the presence of a mixture of triterpenes, allantoin and C-glycosilated flavones and studies have reported that these three compounds have antinociceptive and anti-inflammatory activities. The aim of the study was to investigate the antinociceptive effect of the HEM, the involvement of opioid system, and the gastrointestinal motility.

Methods: To investigate the antinociceptive activity and the involvement of opioid system, the procedure used was the formalin test, the same as previously described (Hunskaar & Hole, 1987; Santos *et al.*, 1999, Santos *et al.*, 2005). Swiss male mice (25-30g; n=7-10) received an intraplantar injection of 20 µl of formalin (2.7%) and were observed for 0-5min (neurogenic phase) and 15-30min (inflammatory phase). The time they spent licking the injected paw was registered and considered as indicative of nociception. The animals received vehicle (saline 10mL/kg p.o) or HEM (62.5; 125;250mg/kg p.o) to investigate the antinociceptive effect before formalin injection. In a separate series of experiments mice were pre-treated with vehicle (10mL/kg i.p) or naloxone (1mg/kg i.p), and treated with morphine (2.5mg/kg s.c) or EHM (62.5mg/kg p.o) before formalin injection, to investigate the role of opioid system. In gastrointestinal motility Swiss male mice (35-40g; n=7) received vehicle (10mL/kg p.o), morphine (2.5mg/kg s.c), or HEM (62.5,125,250mg/kg p.o). One hour later the groups received 10% activated charcoal (10mL/kg p.o) and were euthanized 30min later (Stickney & Northup, 1959) for further analyses. The results are expressed as the mean ± S.E.M. Statistical significance was determined by ANOVA followed by Dunnett's test; levels of p < 0.05 were considered to be statistically significant. Animal Ethics Committee Protocol Number: 367/2011 UNESP. **Results and Discussion:** The results in formalin test demonstrated that p.o administration of HEM caused a significant inhibition of neurogenic and inflammatory phases. However, the dose of 62.5mg/kg caused a more pronounced effect against both phases (neurogenic phase - 35%; p<0.001); (inflammatory phase - 44%; p<0.01). The present study also revealed that naloxone completely reversed the antinociceptive effect of the HEM. This observation suggests a role of opioid mechanism in the antinociceptive action of HEM. The evaluation of HEM on gastrointestinal motility in mice, showed that only the doses of 62.5 and 125mg/kg (p<0.05) changed the normal intestinal propulsive movement, producing a greater anti-motility effect. **Financial support:** BIOTA/FAPESP; CAPES. **References:** Al Meshal, I. A. et al. *Fitoter.*, v.53, p. 79, 1982. Fernandez, M.A. et al. New insights into the mechanism of action of the anti-inflammatory triterpene lupeol. *J. Pharm. Pharmacol.*, v. 53, p.1533, 2001. Hunskaar, S. et al. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain*, v. 30, n. 1, p. 103, 1987. Pietrovski, E.F. et al. Antinociceptive properties of the ethanolic extract and of the triterpene 3beta,6beta,16betatrihidroxilup-20(29)-ene obtained from the flowers of *Combretum leprosum* in mice. *Pharmacol Biochem. Behav.*, v.83, p.90, 2006. Pott, A. et al. Features and conservation of the Brazilian Pantanal wetland *Wetlands Ecology and Management*, n.12, p. 547–552, 2004. Sannomiya, M. et al. Análise cromatográfica por HPLC-DAD das folhas e cascas de *Machaerium hirtum* (Fabaceae). 2011. Santos A.R.S. et al. Antinociceptive properties of the new alkaloid, cis-8, 10-di-N-propyllobelidiol hydrochloride dihydrate isolated from *Siphocampylus verticillatus*: evidence for the mechanism of action. *J. Pharmacol. Exp. Ther.*, v. 289, p.417, 1999. Santos A.R.S. et al. Mechanisms involved in the antinociception caused by agmatine in

mice. *Neuropharm.*, v. 48, p. 1021, 2005. Stickney, J.C. Effect of gastric emptying upon propulsive motility of small intestine of rat. *Proc. Soc. Exp Biol. Med.*, v.101, p. 582, 1959. Thirugnanasambantham, P. et al. Analgesic activity of certain flavones derivatives. A structure activity study. *J Ethnopharmacol.*, v.28, p.207, 1990.

05.024 Inhibition of gastrin-releasing peptide receptor is able to block acute and chronic scratching behavior in mice. Machado GDB, Pereira PJS, Campos MM PUCRS – Toxicologia e Farmacologia

Introduction: Pruritus is defined as an unpleasant cutaneous sensation that leads to the desire or reflex to scratch (Steinhoff et al., *J Invest Dermatol.* 126, 1705, 2006). It has self-protective functions, acting as a physiological mechanism of defense against harmful external agents (Ständer et al., *Arch Dermatol.*, 139, 1463, 2003). Recent evidence has implicated gastrin-related peptide (GRP) in itching sensation (Patel et al., *Biochim Biophys Acta.* 1766, 23, 2008; Weber et al., *Curr Opin Endocrinol Diabetes Obes.* 16, 66, 2009). This peptide exerts its effects by binding to its receptor, denoted GRP-R (also known as BB2-R) (Patel et al., *Biochim Biophys Acta.* 1766, 23, 2008). The present study evaluated the effects of the GRP-R non-peptide antagonist PD176252 on either acute or chronic protocols of scratching in mice **Methods:** Male Swiss mice (8 per group, 25-30 g) were used. All the experimental protocols were approved by the Local Ethics Committee (09/00101-PUCRS). Animals were treated with the GRP-R antagonist PD176252 (1 mg/kg, i.p.) 30 min before the experiments. The control groups received saline at the same interval of time. The acute scratching behavior was evoked by the intradermal application of trypsin (200 mg/site, 50 μ l) or the mast cell depletor CP48/80 (10 μ g/site, 50 μ l) into the back of the mouse neck. In a separate series of experiments, we have adopted the chronic dry skin model of itching (a five-day-treatment based on application of a solution containing diethylether and acetone 1:1, upon the shaved back of the neck). Scratching behavior was measured for 40 min, as the number of scratches with forepaws and hindpaws close to the injected site and/or behind the ears. **Results:** The systemic administration of PD176252 produced a significant reduction of scratching behavior induced by the pruritogenic agents trypsin and CP48/80, with inhibition percentages of $78 \pm 8\%$ and $75 \pm 6\%$, respectively. Noteworthy, the treatment with PD176252 (1 mg/kg, i.p.) also displayed a marked reduction of dry skin-induced chronic scratching, with an inhibition percentage of $64 \pm 9\%$. **Discussion:** The present results demonstrate that PD176252 effectively inhibited both acute and chronic pruritus in mice. This is the first evidence showing that systemic administration of this compound is able to exert anti-pruritogenic effects. It is tempting to suggest that PD176252 (or related compounds) might well represent promising alternatives for treating itching-related conditions. **Financial support:** CAPES, PIBIC-CNPq, FINEP/PUCRSINFRA #01.11.0014-00.

05.025 Characterization of the effect of crotalphine in a CFA-induced arthritis model in female rats. Lucena F¹, Bressan E², Cury Y², Tonussi CR¹ ¹UFSC – Farmacologia, ²IBu

Crotalphine, a peptide isolated from the *Crotalus durissus terrificus* snake venom, has demonstrated analgesic properties in acute and chronic pain experimental models induced by PGE₂, bradikinin, carrageenan, and nerve lesion. However, it is not known the effect of crotalphine in a subchronic antigen-induced arthritis model.

We aim to evaluate the articular incapacitation, edema and cell migration induced by CFA injection, and characterize the effect of crotalphine administered either orally or directly into the arthritic knee-joint. The experiments were approved by the local committee for the ethical use of animals under the number PP00723. Female Wistar rats (160-190g; 2 months old) were immunized with 50µL of CFA (0.5mg/mL) i.d. injection in the region of tail insertion, and one week after they were re-stimulated with the same dose of CFA, in the right knee joint. Crotalphine was administered by intra-articular (0.0003µg; 0.003µg; 0.3µg; 3µg/50µL) and oral (12.5µg/kg; 25µg/kg; 50µg/kg; 2mL) routes 24hs after the re-stimulation. Articular incapacitation was measured by counting the paw elevation time (PET; s) during 1-min periods of stimulated walk, daily, until the 7th day. Edema was accessed by the articular diameter (mm). After this period, the synovial fluid was sampled for leukocyte content evaluation. Statistical comparison of incapacitation and edema curves were made by one-way Anova for repeated-measures followed by dunnett's post-hoc test. Leukocyte contents were compared by one-way Anova for simple means. Our results showed that Intra-articular crotalphine had no effect on the articular incapacitation and leukocyte infiltration, but the two higher doses increased articular edema (p<0.0001). On the contrary, oral crotalphine doses were able to decrease the articular incapacitation (p<0.0001) and edema (p<0.0001), but only the dose of 12,5µg/kg altered the leukocyte infiltration, decreasing the number of polymorphonuclear cells (p<0.03). Oral crotalphine confirmed its previously shown antinociceptive effect, but also seems to be effective in a subchronic immune arthritis model. The inhibitory effect on leukocyte infiltration in the synovia, may be a relevant feature for the control of chronic inflammatory process. The present findings however, did not confirm the local analgesic effect previously shown. Indeed, local administration of crotalphine may even worse the inflammation. Sources of research support: CNPq; CAPES.

05.026 Quercetin inhibits zymosan-induced articular inflammation in mice: Inhibition of oxidative stress and cytokines production. Zarpelon AC¹, Guazelli CF¹, Staurengo-Ferrari L¹, Casagrande R², Verri Jr WA¹ ¹UEL – Ciências Patológicas, ²UEL – Ciências Farmacêuticas

Introduction: Rheumatoid arthritis (RA) is an autoimmune disease that affects primarily the joints manifesting as pain and stiffness. The intra-articular administration of zymosan is an experimental model that promotes the emergence of inflammatory parameters similar to RA. Recent studies show that zymosan binds to Toll-like receptor 2 (TLR-2) inducing proinflammatory cytokines, chemokines and endothelins that participate in knee-joint inflammation. It has been reported that the flavonoids quercetin has antioxidant properties and anti-inflammatory properties. It is not completely understood, but it is likely that there is a link between the antioxidant and anti-inflammatory effects of quercetin. Therefore, we evaluated the activity of quercetin on arthritis model, focusing on hyperalgesia, cytokines and neutrophil recruitment.

Methods: The experiments were performed on male Swiss mice weighing between 20 and 25 g, and with the approval of the Animal Ethics Committee of the Universidade Estadual de Londrina (07819). Articular mechanical hyperalgesia was evaluated using an electronic version of von Frey filaments test. To evaluate the articular pain, was used a large tip (4.15mm²), to exclude subcutaneous effect. Cytokine production/mRNA expression was evaluated on femur-tibial tissue by ELISA or quantitative PCR, and neutrophil recruitment by Rosenfeld stained slices. Mice received subcutaneous treatment with quercetin (10-100 mg/kg) or vehicle (20% Tween 80 in saline) 30 min before intrarticular injection of zymosan (10-100µg/mice) and the hyperalgesia, oedema was determined 1, 3, 5 and 7h after. Neutrophil recruitment was evaluated at 7h. Cytokine analysis and pre-proET-1 mRNA expression were evaluated at 5 and 2h, respectively. Femur-tibial joints were collected at 3, 5 and 7 hours after intrarticular administration of zymosan or saline, and the GSH levels were determined using a spectrophotometric method. **Results:** Zymosan induced significant mechanical hyperalgesia, edema and neutrophil recruitment in a dose-dependent manner, and the 100µg dose was chosen for next experiments. The treatment with quercetin (10-100mg/Kg) inhibited in a dose-dependent manner the hyperalgesia (21.2, 36.5, 146.4%), oedema (17.2, 56.1, 90.6%) and recruitment of neutrophils to the articular cavity induced by zymosan (20.4, 33.6, 81.5%) at 7h. Furthermore, quercetin (100mg/Kg) also inhibited the cytokines production (IL-1β – 59.2%, and TNFα – 29.1%) and treatment with quercetin prevented the depletion of GSH by zymosan stimulus at 3h. Furthermore, quercetin inhibited zymosan-induced expression of preproET-1 mRNA expression by 79.5%. **Discussion:** These results demonstrate that quercetin inhibits zymosan-induced femur-tibial joint inflammation and hyperalgesia by inhibiting cytokine production, oxidative stress and preproET-1 mRNA expression in mice. Therefore, quercetin represents a possible therapeutic strategy in arthritis. **Financial support:** FAPESP, CNPq and CAPES and SETI/Fundação Araucária and Governo do Estado do Paraná.

05.027 LASSBio-1247 antinociceptive effect: A new prototype drug candidate to treat rheumatoid arthritis. Santos EAP, de Sá Alves FR, Fraga CAM, Barreiro EJ, Miranda ALP UFRJ – FÁRMACOS

Introduction: Rheumatoid arthritis (RA) is characterized by persistent synovitis with cartilage and bone damage at multiple joints. Most of these changes are mediated by cytokines produced by leukocytes, as TNF- α , IL-1 β and IL-6. Besides cell chemotaxis, TNF- α plays crucial role in pathogenesis and associated pain of RA (FELDMAN, Nat Rev Imm,2,364,2002). Thus, the reduction of TNF- α is an important strategy to treat RA. Previous study showed that LASSBio-1247 decreases TNF- α *in vitro*, has a relevant anti-hypernociceptive effect and reduces MPO level in an inflammatory carrageenan model (SANTOS, Dissertação-Ms,84,2009). The present study aimed to deepen the anti-hypernociceptive and anti-inflammatory properties of LASSBio-1247 and to evaluate in RA model. **Methods:** To access cell viability (MTT test) and TNF- α production, macrophages were stimulated by LPS and incubated with LASSBio-1247 at different concentrations to determine the IC₅₀. Cell migration was accessed by neutrophil chemotaxis induced by fMLP in a Boyden chamber (FIERRO, J Immun, 170,2688,2003). The anti-hypernociceptive effect was evaluated on thermal (hot-plate) LPS- and capsaicin-induced hypernociception (VAJJA, Int Imm,4,901,2004; MIZUSHIMA, Pain,113,51,2005) and on mechanical hypernociception in the mBSA-induced delayed-type hypersensitivity RA model. Total leucocytes count of the articular washes was determined by optical microscopy (CUNHA, Eur J Pain, 12,1059,2008). The stomachs were removed in order to evaluate the gastric damage in treated animals. Animal protocols were approved by UFRJ ethical animal care committee (FARMACIA02). Results are expressed in mean \pm standard error and were statistically analyzed using ANOVA-oneway (n=6-8 animals/group; *p<0.05). **RESULTS & DISCUSSION:** LASSBio-1247 showed a IC₅₀=12,9 μ M on the TNF- α production and the inhibition is not associated with cell death, since cell viability was reduced only by 30% at 100 μ M, a concentration 10 times higher than its IC₅₀. LASSBio-1247 inhibited 55%* the LPS-induced thermal hypernociception that is mediated by TNF- α , IL-1 β and IL-6 (VAJJA, Int Imm,4,901,2004). LASSBio-1247 at 100 μ mol/kg orally twice/day (5 days) didn't induce stomach damage in the hypernociceptive test induced by mBSA. Besides, the compound reversed the animal's pain at several points of the acute and chronic phases*, reduced the total number of leukocytes at the joint* and also the neutrophil chemotaxis *in vitro* by 50%*. LASSBio-1247 inhibited by 70%* the hypernociception induced by capsaicin, a response mediated by the p38MAPK and TRPV1 activation (MIZUSHIMA, Pain,113,51,2005). The ensemble of the results suggests that the anti-hypernociceptive and anti-inflammatory effects of LASSBio-1247 could be in part due to the decrease of TNF- α , leading to a decrease of cell migration. Furthermore, it may be acting on the p38MAPK activity/phosphorilation or as TRPV1 antagonist, whose effects are under investigation. These results point out LASSBio-1247 as a promising and interesting prototype of a drug candidate to treat RA. **Financial support-**CAPES INCT-INOVAR FAPERJ

05.028 The contribution of the transient receptor potential A1 (TRPA1) in a mice model of sympathetically maintained neuropathic pain. Pinheiro KV¹, Pinheiro FV¹, Silva CR², Oliveira SM², Villarinho JG¹, André E³, Ferreira J¹ ¹UFMS – Fisiologia e Farmacologia, ²UFMS – Química, ³UFRN – Biofísica e Farmacologia

Introduction: Some forms of neuropathic pain are maintained by sympathetic fibers, but their underlying mechanisms are poorly understood. Thus, the aim of this study was to investigate the involvement of the TRPA1 receptor in a mice model of sympathetically- maintained neuropathic pain. **Methods:** Male Swiss mice (N=6-10, 25-30 g) were used in this study (Ethics Committee:164/2011). Neuropathy was induced by chronic constriction injury (CCI) of the sciatic nerve. Sham surgery was done by exposing the sciatic nerve without performing constriction. The paw withdrawal threshold (PWT) to mechanical stimulus was measured through the application of von Frey filaments to verify the development of mechanical allodynia, characterized by the decrease of PWT. Cold allodynia was verified by application of acetone in the paw. Mice were treated with HC-030031, TRPA1 antagonist, or vehicle. Chemical sympathectomy was produced by the treatment of mice with guanethidine. To investigate the norepinephrine (NE)-induced hypersensitivity, animals were treated subcutaneously with vehicle or NE and the time of paw licking was measured as nociceptive response. Phentolamine, propranolol, desipramine, clorgyline and HC-030031, were all locally co-administered with NE. **Results and Discussion:** Allodynia was induced by CCI in mice compared with sham-surgery (PWT of 0.35 ± 0.06 and 2.89 ± 0.17 g, respectively). Systemic injection of HC-030031 (100 mg/kg) reversed mechanical and cold allodynia produced by CCI (inhibition of $76 \pm 16\%$ and 100% at 1 h). Guanethidine treatment (30 mg/kg) prevented both CCI-induced mechanical and cold allodynia. Intraplantar injection of NE induced a few spontaneous nociceptive behaviors in sham- operated animals (8.0 ± 3.2 s), which were no different than the responses to vehicle in sham or nerve-injured mice (5.1 ± 2.0 and 4.4 ± 2.3 s, respectively). In contrast, the NE (30 ng/paw) produced nociceptive response in neuropathic mice compared with the vehicle (39.3 ± 7.1 and 8.0 ± 3.2 s of response, respectively). The co-administration of the phentolamine (100 ng/paw), but not the propranolol (300 pg/paw), was capable of somewhat, but still significantly, inhibiting ($24\pm 7\%$) NE-induced nociception. Furthermore, co-administration with desipramine (100 ng/paw) or clorgyline (100 ng/paw) reduced (inhibition of $88\pm 3\%$ and $90\pm 7\%$, respectively) the nociception caused by NE (30 ng/paw) in neuropathic mice. Systemic or local injection of HC-030031 also reduced (inhibition of 81 ± 9 and $63\pm 8\%$, respectively) NE-induced nociception (30 ng/paw) in nerve-injured animals. Finally, the peripheral injection of HC-030031 was also able to abolish the mechanical and cold allodynia induced by nerve injury. The present findings reveal the role of TRPA1 in mechanical and cold hypersensitivity as well as in hypersensitivity to NE after nerve injury, suggesting that TRPA1 antagonists may be useful to neuropathic patients that present sympathetically maintained pain. **References:** Baron R: Mechanisms of disease: neuropathic pain - a clinical perspective. *Nat Clin Pract Neurol* 2:95-106, 2006. Sommer C, Schmidt C, George A: Hyperalgesia in experimental neuropathy is dependent on the TNF receptor 1. *Exp Neurol* 151:138-142, 1998. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL: Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 53:55-63, 1994. Bennett GJ & Xie YK: A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 33: 87–107, 1988. Caspani O, Zurborg S, Labuz D, Heppenstall PA. The contribution of TRPM8 and TRPA1 channels to cold allodynia and neuropathic pain. *PLoS One* 4:e7383, 2009. Andersson DA, Gentry C, Moss S, Bevan S: Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. *J Neurosci* 28:2485-2494, 2008. Ferreira J, Trichês KM, Medeiros R, Calixto JB: Mechanisms involved in the nociception produced by peripheral protein kinase c activation in mice. *Pain* 117:171-181, 2005. Da Costa DS, Meotti FC, Andrade EL, Leal PC, Motta EM, Calixto JB: The involvement of the transient receptor potential A1

(TRPA1) in the maintenance of mechanical and cold hyperalgesia in persistent inflammation. *Pain* 148:431-743, 2010. Drummond PD: Sensory disturbances in complex regional pain syndrome: clinical observations, autonomic interactions, and possible mechanisms. *Pain Med* 11:1257-1266, 2010. Xanthos DN, Bennett GJ, Coderre TJ: Norepinephrine-induced nociception and vasoconstrictor hypersensitivity in rats with chronic post-ischemia pain. *Pain* 137:640-651, 2008. **Financial support:** CNPq, CAPES, CCS/UFSM. **Acknowledgments:** We acknowledge the support from CNPq and CAPES.

05.029 Effect of pregabalin in orofacial thermal hyperalgesia associated with experimental diabetes induced by streptozotocin in rats. Nones CFM, Cunha JM, Chichorro JG UFPR – Farmacologia

Introduction: Peripheral neuropathy is one of the most common complications of diabetes and is often accompanied by episodes of pain, which can affect approximately 50% of patients. There is growing evidence that diabetic neuropathy also involves the trigeminal nerve, altering the transmission of orofacial sensory information. This study aimed to compare the pregabalin and morphine effects on orofacial thermal hyperalgesia associated with streptozotocin-induced diabetes in rats. **Methods:** After 12 h of food deprivation, male *Wistar* rats (180-220 g; n= 8-10), received vehicle (citrate buffer, 10 mM, pH 4.5; 1 mL/kg, i.p.; normoglycemic group-Ngl) or streptozotocin (STZ, 50 mg/kg, i.p.; diabetic group-DBT). Three days later, the development of diabetes was confirmed by tail vein blood glucose levels. Only rats with non-fast blood glucose levels higher than 250 mg/dL were included in the study. Cold stimulation consisted in the application of a tetrafluoroethane spray to the center of the vibrissal pad. Time of facial grooming behavior was recorded over the first 2 min as an index of cold-induced nociception. Heat hyperalgesia was estimated as a decrease in the latency to display head withdrawal or snout vigorous flicking after application of radiant heat to the face. Both thermal heat and cold hyperalgesia were assessed before diabetes induction (basal zero; B0). At weeks 4 and 5, both Ngl and DBT groups had thermal hyperalgesia assessed before and at 1-h intervals up to 6 h after following treatments: pregabalin (PGL; 10 or 30 mg/Kg; p.o.) or vehicle (CMC 0.5%, 1 mL/kg, p.o.) and morphine (MPH; 2.5 mg/Kg, s.c.) or vehicle (saline, 1 mL/Kg, s.c.). All experimental procedures were previously approved by the Committee on the Ethical Use of Animals of the UFPR (authorization # 534). **Results and Discussion:** Diabetic animals exhibited significant cold and heat hyperalgesia 4 and 5 weeks after diabetes induction when compared to Ngl. After treatments, Ngl group did not show differences. In diabetic group, heat hyperalgesia was significantly reduced by both PGL (at higher dose) and MPH treatment. PGL at 10 mg/Kg was not effective. On the first hour after PGL treatment the latency time was significantly increased by 86% compared to the vehicle group (9.7 ± 1.7 s and 5.2 ± 0.4 s, respectively, $p < 0.05$) and on the second, third, fourth, fifth and sixth hour after PGL treatment it was observed an increase in the latency time by 85%, 198%, 172%, 173% and 151%, respectively. On the other hand, MPH treatment caused a significant increase in the latency time only at 30 minutes and 1 hour after its administration (increases by 71% and 84% respectively). Cold hyperalgesia was also reduced by PGL (at 30 mg/Kg) and MPH treatments. On the first hour after PGL treatment, the facial grooming elicited by the cold stimulus was reduced by 72% compared to the control group (8.2 ± 2.8 s and 29.3 ± 6.7 s, respectively) and on the second, third and fourth hour, PGL decreased the facial grooming time by 46%, 74% and 84%, respectively. MPH treatment also reduced cold hyperalgesia by 87%, but only at 30 minutes after its administration. Our results suggest that orofacial thermal hyperalgesia associated with experimental diabetes is marked ameliorated by PGL. **Financial support:** CAPES.

05.030 Anti-hyperalgesic effects of two sphingosine derivatives AA 2829 and OA 1028 in different models of hyperalgesia in mice. Cavichioli FJ¹, Bernal GNB¹, Holzmann I², Klein JB², Escarcena R³, Del Olmo E³, San Feliciano A³, Cechinel Filho V², Quintão NLM².

Introduction: Sphingosine is an essential component of sphingolipids and ceramides. In mammals, it is firstly released by ceramide and is bio-synthesized by its precursor dihidroesfingosídeo (DHEF). Recently, Campos-Buzzi et al. (2010) demonstrated that compounds derived from ethylenediamine and β -aminoalcohol significantly inhibited nociception in several models of acute nociception in mice. The aim of this study was to evaluate the effects of two sphingosine derivatives AA 2829 (AA) and OA 1028 (AO) in different models of hyperalgesia in mice. **Methods:** Male Swiss mice (20-30 g; n=6-8) were used throughout this study. The animals were pre-treated with AA or AO (0.3, 1 or 3 mg/kg), intraperitoneally (i.p.). After 30 min, they received an intraplantar (i.pl.) injection of I-carrageenan (300 μ g/paw), lipopolysaccharide (LPS; 100 ng/paw), bradykinin (BK; 500 ng/paw) or Prostaglandin E₂ (PGE₂; 0.1 nmol/paw) and the mechanical withdrawal thresholds were evaluated using von Frey filament (0.6 g) at different time points. This study was approved by the Ethical Committee for Animal Care of UNIVALI (Process No. 025/2011). **Results:** The animals pre-treated with the compounds AA and AO presented reduction in the mechanical hypersensitivity induced by carrageenan with inhibition of 70 ± 6 % and 54 ± 12 %, respectively, for the dose of 3 mg/kg. The same treatment was able to inhibit the hypersensitivity induced by LPS, with inhibition of 30 ± 8 % and 41 ± 7 %, respectively for AA and AO. The compounds also reduced the mechanical sensitization induced by BK (38 ± 2 % and 42 ± 7 %, respectively for AA and AO). However, none of them interfere with the mechanical hypersensitivity induced by i.pl. injection of PGE₂. **Discussion:** These results demonstrated that the sphingosine derivatives AA and AO presented important anti-hypersensitive effects. This data also suggests the interaction with the kinins and/or their receptors, however additional studies are necessary to better delineate the anti-hypersensitive effects of these sphingosine derivatives. **Financial support:** PROBIC-PropPEC UNIVALI. Campos-Buzzi et al. *Pharmacol. Rev.* 62:1734, 2010.

05.031 Pyrrolidine dithiocarbamate inhibits superoxide anion-induced inflammation. Ribeiro FAP, Fattori V, Zarpelon AC, Verri Jr WA UEL – Patologia

Introduction: The superoxide anion, produced by NADPH oxidase during the inflammatory process, can induce the expression of proinflammatory molecules through the activation of the nuclear transcription factor NF- κ B pathway. Pyrrolidine dithiocarbamate (PDTC) is recognized as a NF- κ B inhibitor. In the present study, it was investigated whether PDTC inhibits superoxide anion-induced inflammation. **Methods:** Male Swiss mice, 20-25g from Universidade Estadual de Londrina were used in this study with the approval of the local Ethics Committee (no. 07882). Mice were treated with PDTC (10, 30 and 100 mg/kg, subcutaneous) 1 h before the intraplantar stimulus with KO₂ (superoxide anion donor, 30 μ g/paw). The edema (analog caliper), mechanical (electronic version of von Frey filaments) and thermal (hot plate) hyperalgesia were evaluated between 0.5-7h, and at 7 h animals were euthanized. Statistical analysis were performed using Graph Pad Prism 4.0, One-way ANOVA followed by Tukey's t test and significant differences considered using $p < 0.05$. **Results:** PDTC inhibited in a dose-dependent manner KO₂-induced inflammation, the results are described as following: a) the edema was reduced by PDTC 30 mg/kg treatment up to 77% at 7h and up to 66% at 7h by PDTC 100 mg/kg treatment; b) mechanical hyperalgesia was inhibited up to 71% at 7h and up to 40% at 30 min by PDTC 100 mg/kg and PDTC 10 mg/kg treatments, respectively; c) treatments caused no significant differences in thermal hyperalgesia. **Conclusion:** PDTC showed anti-inflammatory and analgesic effects in different components of superoxide anion-induced paw inflammation. Further studies are necessary to determine whether the effects of PDTC are related to NF κ B inhibition. **Financial support:** CNPq, CAPES, SETI/Fundação Araucária and Governo do Estado do Paraná.

05.032 Involvement peripheral but not central of α 2-adrenoreceptors in the antinociception induced by aerobic exercise. Galdino GS¹, Silva JF², Cruz JS³, Brum PC⁴, Duarte IDG¹, Perez AC¹ ¹UFMG – Farmacologia, ²UFMG – Fisiologia, ³UFMG – Bioquímica e Imunologia, ⁴USP – Educação Física e Esporte

Introduction: Several studies have demonstrated exercise induced-antinociception, however the specific mechanisms for this effect is not well understood¹. Thus, the present study aimed to investigate the involvement of α 2-adrenergic receptors (α 2-ARs) in the antinociceptive effect produced by aerobic exercise in rats and mice.

Methods: Male Wistar rats, male α 2A/ α 2C-ARs knockouts mice (KO) and their wild-type (WT) were submitted to a acute aerobic (AA) protocol². Acute aerobic exercise (AE) was performed in a rodent treadmill. Animals run at 20 m/min and 0% inclination, an average time of 49.06 (\pm 3) min, until fatigue. Fatigue was defined as the point at which the animals were unable to keep pace with treadmill. The back of the treadmill has an electrical stimulator (3 v) in order to encourage the animals to run. To familiarize the rats to exercise, reducing the effects of stress, they run daily on the treadmill at 5 m/min for 5 min/day during 3 consecutive days prior to the experiments. **Results:** After AA the nociceptive threshold of rats and WT was increased, (except in KO). This effect was reversed by yohimbine, a non-selective α 2-ARs antagonist (IOI, 4 mg/kg, s.c.); rauwolscine, a selective α 2C-ARs antagonist (RVN, 4 mg/kg, s.c.); BRL 44408, a selective α 2A-ARs antagonist (BRL, 4 mg/kg, s.c.) and guanethidine, a selective inhibitor of transmission in adrenergic nerves (GNTD, 30 mg/kg, i.p.). Furthermore, when given i.t or i.c.v, yohimbine did not alter antinociception induced by exercise. In addition, α 2-ARs expression in brain of the rats did not change after AA. In addition, the Western blot analysis demonstrated that the AA did not alter the α 2C-adrenergic receptor subtype expression in brain of rats. **Discussion:** Thus, these results suggest a peripheral involvement of α 2-ARs in the antinociception induced by aerobic exercise.

Financial support: CNPq and FAPEMIG Ethics Committee for Animal Experimentation/ Protocol: 185/2007.

05.033 Pharmacological properties of a new series of oxime ethers compounds designed as new anti-inflammatory drugs. Castro JP¹, Motta NAV¹, Fumian MM¹, Veiga F², Veloso MP², Brito FCF¹ ¹UFF – Fisiologia e Farmacologia, ²UNIFAL – Fitoquímica e Química Medicinal

Aim: Searching for new anti-inflammatory drug candidates, inhibitors of the arachidonic acid cascade, a new series of oxime ethers (RC-1, RC-2, RC-3, RC-4, RC-5, RC-6, RC-7), were synthesized. These oxime ethers were synthesized from eugenol (4-Allyl-2-methoxyphenol), an abundant secondary metabolite of plants in Brazilian flora. One of the most important characteristics of these new compounds is the presence of two functionalized aromatic rings in their structural configuration, being the methyl sulfoxide and methyl sulfone pharmacophoric units present in one of the aromatic rings. We have evaluated the antinociceptive properties of the oxime ethers employing the writhing model of nociception and its anti-platelet effects using the lumi-aggregometer.

Methods and Results: The animal protocols were submitted to the Ethics Committee for Experimental Research of the Federal Fluminense University (CEPA/UFF protocol number 251) The analgesic activity was determined *in vivo* by the abdominal constrictions test induced by acetic acid 0.6% (0.1ml/ 10g) in mice (Whittle, BA, Br. J. Pharmacology; 22: 246, 1964). Swiss mice of both sexes (18 – 25g) (n= 10 for each experimental group) were pre-treated orally (*p.o.*) with RC compounds (100 µmol/ kg), diluted in a mixture of tween 80, ethanol and water (1:1:8) (vehicle). Acetic acid (0.1N) was administered *i.p.* one hour after the administration of oxime ethers derivatives. Ten minutes following *i.p.* acetic acid injection, the number of constrictions per animal was recorded for 20 minutes. Control animals received an equal volume of vehicle. Analgesic activity was expressed as a percentage of inhibition of constrictions when compared with the vehicle control group. We performed the analysis of variance (one-way ANOVA) and tested the statistical significance of differences between groups by Dunnett's Multiple Comparison Test. We have also evaluated the anti-platelet properties of these compounds (100 and 300 µM concentration) employing rat platelet rich plasma (PRP) stimulated by collagen 5 µg/ mL and ADP 5 µM. At the screening dose employed (100µmols/ kg), the oxime ethers RC-7 and RC-6 presented a significant inhibition of writhing induced by acetic acid when compared to control group (RC-7, 28.6%; RC-6, 24.7%). At platelet aggregation induced by collagen, the compound RC-7 at 300 µM presented 90% of inhibition. **Conclusion:** The oral treatment of animals with oxime ether compounds induced antinociception when assessed by the acetic acid-induced constrictions, an useful method to screen both peripherally and/or centrally acting analgesic activities. The observed activity in collagen induced platelet aggregation, suggests that these compounds can exert their actions through an action in arachidonic acid cascade. The results obtained so far contribute to elucidate the anti-inflammatory profile of oxime ethers compounds.

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05.034 Interaction between kinin and endothelin systems in a nociception model in mice. Schroeder SD¹, Luiz AP², Rae GA¹ ¹UFSC – Department of Pharmacology, ²UFSC – Physiology

Introduction: Kinins are peptides that act through B₁ and B₂ G protein-coupled receptors. The B₂ receptor is responsible for most effects of kinins, while the B₁ receptor has limited expression under physiological conditions, but is up-regulated in physiopathological conditions. Endothelin-1 (ET-1) is a peptide expressed by several mammalian species which, like other endothelin peptides, also acts through two GPCRs named ET_A and ET_B receptors. Several reports have disclosed possible interactions between these two systems (Momose et al., *Hypertension*, 21: 921,1993; Zeitlin et al., *Immunopharmacology*, 33: 294; Webb et al., *Trends Pharmacol. Sci.*, 19:5, 1998; Tanus-Santos et al., *Eur. J. Pharmacol.*, 397: 367, 2000; Andoh et al., *Peptides*, 31: 238, 2010). The current study aims to investigate interactions between both systems in nociception. **Methods:** Male Swiss mice received an intraplantar (i.pl) 20 mL injection of ET-1 (1-30 pmol), bradykinin (BK) (1-100 nmol) or vehicle into the right hind paw and the time dedicated to licking the paw or lifting it above the floor was recorded over the first 30 min (ET-1) or 10 min (BK) after administration. Immediately after observations, the animals were sacrificed and paw edema was measured as the weight difference between the both hind paws. In a different set of experiments, mice received an i.pl. injection of B₁ or B₂ receptors antagonists (DALBK and HOE 140, respectively) or vehicle 15 min before ET-1 (10 pmol) administration, or of ET_A or ET_B antagonists (BQ-123 or BQ-788, respectively) or vehicle 15 min before BK (10 nmol) administration. Protocols were approved by our institutional ethics committee (23080.016338/2010-16). **Results:** ET-1 induced dose-related licking and edema at 10 and 30 pmol, but failed to evoke paw lifting. In contrast, BK induced both licking and lifting at 10 and 100 nmol and also caused edema at all doses tested, even at 1 nmol which was not nociceptive. Pre-treatment with DALBK (30 and 100 nmol) reduced licking induced by ET-1 without affecting edema, whereas BQ-788 (1 and 10 nmol) reduced only BK-induced lifting and had no action on licking or edema. BQ-123 was unable to affect any behavior induced by BK administration. **Discussion:** The results described herein indicate that both ET-1 and BK are capable of causing nociception and edema *per se*. The hind paw licking response induced by i.pl. ET-1 is partially mediated by the B₁ receptors, despite the very low constitutive expression levels of this receptor, while endothelins acting on ET_B receptors might contribute to BK-induced lifting. **Financial support:** CNPq, CAPES, PRONEX, UFSC.

05.035 Anti-nociceptive effect of citral in acute nociception models: Induced by formalin and plantar incision in mice. Nishijima CM¹, Stramosk J², Mazzardo-Martins L², Martins D², Rocha LRM¹, Santos ARS², Hiruma-Lima CA¹ ¹Unesp-Botucatu – Fisiologia, ²UFSC

Acute postoperative pain is followed by persistent pain in 10–50% of individuals after surgeries. This study aimed to assess the antinociceptive effects of citral in acute nociception model induced by plantar incision or formalin injection and its mechanisms of action. Citral, a terpene found in oil grass citronella, lemon balm, lemongrass and others medicinal species, was evaluated by formalin method as described by Hunskaar and Hole, 1985. Male Swiss mice (n= 7– 8) received by oral route vehicle, citral (25 mg/kg, 100 mg/kg or 300 mg/kg) or morphine (2,5 mg/kg, s.c.). To assess the role of citral in neurogenic inflammation, ear edema induced by xylol was performed according to Swingle e cols., 1981. In order to evaluate the involvement of opioid or serotonin systems in the antinociceptive mechanism of citral, mice were pre-treated with naloxone (1 mg/kg, i.p, 15 min before vehicle, citral or morphine administration), WAY 100635 (0,1 mg/kg, i.p), ketanserin (1 mg/kg , i.p) or ondansetron (0,5 mg/kg, i.p), 30 minutes before vehicle or citral, respectively. To evaluate the effect of citral in the acute post-operative pain, the plantar incision was performed in mice as described by Pogatzki and Raja (2003) followed by prolonged treatment with citral (100 mg/kg, p.o) during 6 days. The frequency of paw withdrawal to 10 applications of the von Frey filament (0.4 g) was considered as nociceptive response. Mechanical hypernociceptive responses were recorded immediately before (0) and after 1, 2, 3, 4 hours treatment to verify the time course effect. Open-field test was performed to exclude effect in locomotor performance. The statistical significance of differences between groups was detected by ANOVA followed by Dunnett test, t-test or Bonferroni test ($p < 0.05$). In the model of formalin test, we founded the most antinociceptive activity in the dose of 300 mg/kg (reduction in 53 %, in neurogenic phase) and effect antinociceptive in inflammatory phase in the doses of 100 and 300 mg/kg (reduction in 36 %, 65 %, respectively) and absence of activity in the dose of 25 mg/kg. The antinociceptive action of citral did not reverse by WAY 100635 and ondansetron (5HT_{-1A} and 5HT_{-3A} antagonist receptor). But citral antinociceptive property was significantly reversed by naloxone and ketanserine (opioid and 5HT_{-2A} antagonist). Citral (300 mg/kg, p.o) showed effect in neurogenic inflammation induced by xylol. In the model of plantar incision, citral has shown the most antinociceptive activity in the dose of 100 mg/kg, 2 hours after oral administration, in the time course. Long-term treatment was effect during 4 days, in the same model, 2 h after oral administration of citral (100 mg/kg). These results suggested that prolonged systemic treatment with citral is effective in preventing neurogenic inflammation and persistent mechanical allodynia caused by plantar incision in mice. Its mechanism of action involves opioid and 5-HT_{2A} receptors. License number of animal ethic committee: 366 CEEA. Financial agencies and acknowledgement: Biota/FAPESP

05.036 Characterization and initial evaluation of a clonidine:hydroxypropyl-beta-cyclodextrin complex. Braga MA¹, Silva CMG¹, Leite MFMB¹, Yokaichiya F², de Menezes M³, de Paula E¹ ¹Unicamp – Bioquímica, ²Síncroton, ³Medley Indústria Farmacêutica

Introduction: Clonidine (CLN) was synthesized in 1960 and it was initially used as a stuffy nose and antihypertensive remedy. After CLN mechanism of action got known new uses were proposed and its interest in anesthesiology increased since it is used as an anesthetic adjuvant in general and local nerve-blockage procedures. Despite of the advantages from such pharmaceutical association, adverse reactions are still reported so that, in this work, we report the preparation of an inclusion complex for CLN in hydroxypropyl- β -cyclodextrin (HP- β -CD), trying to control its release and avoid such side. **Methods:** Clonidine UV/VIS spectroscopic properties, ionization constant (pKa) and partition coefficient (between liposomes or immobilized artificial membrane, IAM, and water, at pH 7.4) were determined. Changes in CLN absorbance upon complexation allowed stoichiometry determination, both by Job plot and modified Benesi-Hildebrand (BH) treatments. X-ray diffraction, calorimetry (DSC) and scanning electron microscopy (SEM) were used to characterize the complex. **Results and Discussion:** CLN exhibited maximum absorption at 271nm, with a bathochromic shift caused by pH increase. The pKa of CLN was determined at pH 8.0 and its partition coefficient between liposomes and water was 83 ± 18 . Partition through IAM ($K_{IAM} = 10.88$) classifies CLN as medium-permeability compound. A 1:1 CLN:HP- β -CD stoichiometry of complexation was determined ($r^2=0.999$ after BH treatment). The loss of the pure compounds' diffraction pattern and lower intensity (75%) in X-ray diffractograms of CLN:HP- β -CD confirmed complexation. Accordingly, DSC runs revealed broader and less intense endothermic peaks for CLN:HP- β -CD and SEM images showed that no crystalline structures prevail in CLN:HP- β -CD samples, compatible with inclusion complex formation. Dialysis experiments revealed a decrease (15-20%) in clonidine release from CLN:HP- β -CD, regarding CLN in solution. Altogether the results shown here point out HP- β -CD as a good carrier, turning CLN more water soluble what can reduce its passage through the blood-brain-barrier avoiding undesirable effects such as rebound hypotension and reinforcing its peripheral, local-anesthetic like, effect. *In vitro* and *in vivo* tests are under way in our lab to evaluate the activity of CLN:HP- β -CD as drug-carrier system. **Financial Support:** FAPESP (# 06/00121-9)

05.037 The role of neurotrophic factors NT-3 and NGF on orofacial thermal hyperalgesia induced by constriction of the infraorbital nerve in rats. Reis RC, Nones CFM, Aguiar DA, Kopruszinski CM, Chichorro JG UFPR – Farmacologia

Introduction: It has been demonstrated that the neurotrophic factors neurotrophin-3 (NT-3) and nerve growth factor (NGF) participate of the nociceptive process, but have opposite roles. The intraplantar administration of NGF in rats was associated to the development of mechanical and thermal hyperalgesia, which could be prevented by the injection of anti-NGF (GOULD, *Brain Res*, 854: 19, 2000), while the local or intrathecal administration of NT-3 inhibited the nociceptive behavior induced by an inflammatory agent or by sciatic nerve constriction (WATANABE, *Neurosci Lett*, 282: 61, 2000; Wilson-Gerwing, *J Neurosci*, 25: 758, 2005). In light of these considerations, the aim of the present study was to investigate the role of these neurotrophic factors in a rat model of orofacial neuropathic pain. For this purpose, it was evaluated the influence of the local treatment with NT-3 and anti-NGF on the thermal hyperalgesia induced by constriction of the infraorbital nerve (CION). **Methods:** Male *Wistar* rats (180-220 g) were used in all protocols, which were previously approved by the UFPR's Committee on the Ethical Use of Animals (authorization # 432). The responsiveness to heat and cold stimulus was assessed before and at 1h-intervals after the injection of NT-3 (0.3 and 1 µg/50 µL) or anti-NGF (1 and 3 µg/50 µL) subcutaneously (s.c.) into the upper right lip on days 4 and 6, respectively, after CION, which was induced by placing two loose silk 4.0 ligatures around the right infraorbital nerve of anesthetized rats. Heat hyperalgesia was estimated as a decrease in the latency to display head withdrawal or snout vigorous flicking after application of radiant heat to the face. Cold hyperalgesia was estimated as an increase in the duration of facial grooming behavior after the application of a tetrafluoroethane spray to the center of the vibrissal pad. **Results:** CION, but not sham surgery, induced thermal hyperalgesia to heat and cold that peaked on days 4 to 6 after the nerve injury. On day 4 after CION, NT-3 (at 1 µg/50 µL) abolished the heat hyperalgesia at the 2nd and 4th hour after its injection, but NT-3 at the lower dose did not modify the heat hyperalgesia. Likewise, anti-NGF treatment reduced the heat hyperalgesia from the 1st up to the 4th hour after its injection, but only at the higher dose (3 µg/50 µL). On the other hand, the same dose of NT-3 that resulted on blockade of heat hyperalgesia, did not influence the cold hyperalgesia induced by CION, and preliminary results suggest that anti-NGF treatment is also ineffective on cold hyperalgesia. **Discussion:** These data indicate that NT-3 and anti-NGF, at doses previously shown to produce antihyperalgesic effects on different pain models, reduced in a dose dependent manner heat, but not cold, orofacial hyperalgesia, suggesting a role for these neurotrophic factors on orofacial nociceptive process. **Financial support:** CNPq, CAPES/DS, CAPES/REUNI.

05.038 Protein fraction of *Calotropis procera* latex reduces inflammatory pain in mice: Inhibition of neutrophil migration and oxidative stress. Luz PB¹, Pinheiro RSP¹, Freitas LBN¹, Aragão KS¹, Bitencourt RS¹, Couto TS¹, Sousa TFG¹, Alencar NMN¹, Ramos MV² ¹UFC – Fisiologia e Farmacologia, ²UFC – Bioquímica e Biologia Molecular

Introduction: *Calotropis procera* is a laticiferous plant widely distributed in Asia, Africa and South America, and abundant in the Northeast of Brazil. Latex of the plant exhibits potent anti-inflammatory, analgesic, antipyretic, anti-diarrhoeal and other activities in animal models. We aimed here to evaluate the effects of a protein fraction isolated from latex of *Calotropis procera* (LP) on inflammatory hypernociception induced by carrageenan (Cg) and complete Freund adjuvant (CFA). **Methods:** Animal handling and experimental protocols were registered on the Institutional Ethics Committee under number 61/11. a) Swiss male mice were used (n=5, 25-30g). b) Mechanical hypernociception (MH) was evaluated by the electronic version of the Von Frey before (T0) and after the injection of Cg (300 µg/paw) or CFA (20 µL/paw) into the mice paw. MH was measured at 1, 3 and 5 hours after Cg and 1-7 days after CFA. c) Mice were treated with LP (5 and 50 mg/kg, i.v.) or indomethacin (5 mg/kg, i.p.) 30 min before Cg. In the model with CFA, mice were treated with LP (5 mg/kg, i.v.), indomethacin (5mg/kg, p.o.) or dexamethasone (2 mg/kg, s.c) during 8 days. d) Recruitment of neutrophils was determined indirectly by the quantification of myeloperoxidase (MPO) activity. e) To assess the involvement of antioxidant system, the glutathione (GSH) tissue's paw levels were determined using a fluorescence assay. e) Regarding statistics we used the ANOVA/Bonferroni's test, p <0.05 was accepted. **Results and Discussion:** Indomethacin, LP5 and LP50 mg/kg inhibited the MH induced by Cg in 44%, 55% and 65%, respectively. LP (5 and 50 mg/kg) decreased MPO activity by 81% and 94%, respectively, when compared to Cg group. Indomethacin, dexamethasone and LP5 inhibited the MH induced by CFA in 63%, 74% and 46%, respectively. LP decreased MPO activity in 55% when compared to CFA group. LP 5 mg/kg prevented carrageenan-induced reduction of GSH levels. Cg group decreased GSH levels by 62% and LP group decreased this one by 18% when compared to saline group. LP has relevant antinociceptive properties for acute and persistent pain-like behavioral animal models. This effect may be associated with the inhibition of neutrophil migration and oxidative stress. **Financial support:** CAPES and CNPq.

05.039 Role of nitric oxide in the abdominal hyperalgesia in secretory phospholipase A₂-induced pancreatitis. Camargo E¹, Danielle DG¹, Silva CI², Teixeira SA², Toyama MH³, Cotrim C³, Landucci ECT⁴, Muscará MN², Antunes E⁴, Costa SKP² ¹UFS – Fisiologia, ²USP – Pharmacology, ³Unesp-São Vicente, ⁴Unicamp – Pharmacology

Introduction: Acute pancreatitis (AP) is a common, painful and inflammatory disease of the pancreas with no effective pharmacological treatment available. Nitric oxide (NO) plays a controversial role in the inflammatory process in AP, however, it is still unknown whether NO is deleterious or protective in the abdominal pain during AP. This study was designed to investigate the role of NO in the abdominal hyperalgesia and inflammation during secretory phospholipase A₂ (sPLA₂)-induced pancreatitis in the rat.

Methods: Acute pancreatitis was induced by injection of sPLA₂ from *Crotalus durissus terrificus* venom (300 µg/kg) into the common bile duct of anaesthetised Wistar male rats, previously treated with L-NAME (20 mg/kg, i.v., -10 min), aminoguanidine (50 mg/kg, i.v., -10 min) or saline. Four hours after induction, the abdominal hyperalgesia and inflammatory parameters were assessed in the pancreas and lung in addition to serum amylase and nitrite/nitrate concentrations. **Results:** Injection of sPLA₂ significantly increased the concentrations of serum amylase and nitrite/nitrate, in addition to abdominal hyperalgesia and inflammatory response in the pancreas and lung, as characterized by increased MPO activity, plasma extravasation and oedema. Pre-treatment of animals with either L-NAME or aminoguanidine almost abolished abdominal hyperalgesia and serum nitrite/nitrate concentrations, but failed to suppress local plasma extravasation (oedema), and serum amylase concentration. Furthermore, L-NAME, but not aminoguanidine treatment, increased MPO activity in the pancreas, without affecting the lung, of rats with AP. **Conclusion:** Inducible NOS-derived NO plays a role in mediating the abdominal hyperalgesia in sPLA₂-induced AP, independently of the inflammatory process. **Financial support:** FAPITEC/SE, FAPESP and CNPq.

05.040 Investigation of the antinociceptive mechanisms of citronellyl acetate. Rios ERV¹, Rocha NFM¹, Carvalho AMR¹, Vasconcelos LF¹, Dias ML¹, de Sousa DP², Sousa FCF¹, Fonteles MMF^{1,3} ¹UFC – Fisiologia e Farmacologia, ²UFS – Fisiologia, ³UFC – Farmácia

Introduction: Many studies suggest that essential oils may present an antinociceptive activity. CAT has already demonstrated many different biological activities, including an antinociceptive behavior when tested in animals. Because of this, we decided to analyze the involvement of different pain pathways at the antinociceptive mechanism of action of CAT. This work was approved by local Ethics Committee on Animal Research, with the number of protocol: 31/12. **Methods:** Male Swiss mice with 25-32g were used in all experiments, and the tests were conducted with 7-8 animals per group. CAT (200 mg/kg, p.o.) was tested at the model of visceral nociception induced by acetic acid 0.6%, where the animals were pretreated with L-arginine (NO donor, 150mg/kg, i.p.), yohimbine (α 2-antagonist, 2.5 mg/kg, p.o.), haloperidol (non-selective dopamine antagonist, 0.2 mg/kg, i.p.) or atropine (non-selective muscarinic antagonist, 1 mg/kg, i.p.) 15 minutes before receiving the CAT. After 30 minutes of the treatment with CAT, animals were submitted to the writhing test induced by acetic acid, and observed for 30 minutes. Finally, the glutamate-induced paw linking test was conducted 30 or 60 minutes after the pre-treatment with CAT (100 or 200 mg/kg, p.o.) or MK-801, NMDA antagonist, (0.5 mg/kg, i.p.), injected 20 μ L of glutamate (20 μ g/paw) into the plantar surface of the right hind paw and observed for 15 minutes. For statistical analysis, we used analysis of variance (ANOVA) and Student-Newman-Keuls as *post hoc* and $p < 0.05$ was accepted. **Results and Discussion:** The systemic increasing of NO promoted by L-arginine or the pre-treatment with yohimbine were not able to change the antinociceptive effect of CAT. Moreover, the pre-treatment with haloperidol or atropine were able to decrease the antinociceptive effect of CAT by 48.02% and 46.68%, respectively, when compared with the group of animals that received CAT only. However, it did not abolish the antinociceptive effect completely. Finally, CAT was able to decrease the pain behavior of the animals by 43.33% (100 mg/kg) and 51.22% (200 mg/kg) in glutamate-induced paw-linking model, and a similar result was found with MK-801 group (93.18% of reduction). **Conclusion:** The results indicate that the modulation of pain caused by CAT might be mediated, at least in part, by dopaminergic and muscarinic receptors, but it is still necessary to conduct more experiments in order to identify a specific receptor, as well as the glutamate pathway, probably peripheral glutamatergic nociceptors. These results are part of the study about this molecule and its antinociceptive activity. **Financial support:** CNPq, CAPES and FUNCAP.

05.041 Evaluation of neuroprotective effect of duloxetine in induced neurotoxicity by the antineoplastic oxaliplatin in mice. Pereira AF, Ribeiro ESSA, Moura CF, Neto CS, Oliveira FFB, Pontes RB, Ribeiro RA, Vale ML UFC – Fisiologia e Farmacologia

Introduction: Oxaliplatin (OXL) is a third-generation platinum agent with potent cytotoxic activity in several types of cancer. Its toxicity differs from other platinum agents. It is less nephrotoxic and haematotoxic, however its neurotoxicity leads to a painful peripheral neuropathy that is difficult to treat and is dose-limiting. Duloxetine (DLX) a selective inhibitor of the reuptake of 5-HT and noradrenaline is used in the treatment of major depression, generalized anxiety disorder, diabetic peripheral neuropathic pain (DPN) and fibromyalgia. DLX has been gaining attention for being a drug with fewer side effects and has analgesic effect in chronic pain from DPN. The objective of this study was to investigate the effect of DLX in the course of peripheral sensory neuropathy induced by OXL in mice. **Methods:** This study was approved by the Ethics Committee on Animal Research of Federal University of Ceará (15/2012). The peripheral neuropathy has been induced, in male Swiss mice (25-30g), by 2 injections/week of OXL (1mg/kg, i.v.) for 4.5 weeks and was analyzed by behavioral nociceptive (mechanical and thermal) tests once every week for 56 days. DLX (5, 10 and 20mg/kg) was given orally, 30 min, before the injection of OXL. Thermal allodynia was evaluated by tail immersion test (TIT) in cold water (10°C) and plantar mechanical hypernociception (MH) by the Electronic Von Frey test. After the 56th day of experiment the spinal cords were harvested and processed for histopathological analysis. **RESULTS:** In the control group (OXL), there was a decrease in nociceptive threshold from 21th day until 56th day, both in TIT(89.2±8.5 versus 47.8±8.8; p<0.05) and in MH(10.6±0.6 versus 5.5±0.3; p<0.05). Pretreatment with DLX significantly prevented (p<0.05) this threshold decrease in all the doses from the 21th day in both MH(11.2±0.4, maximum effect) and TIT(118.4±0.9, maximum effect). From 28th day, only the doses of 5(MH: 11.2±0.4, TIT: 92.2±12.2) and 10 mg/kg(MH: 10.6±0.3, TIT: 118.4±0.9) maintained the antinociceptive effect in both tests. Pretreatment with DLX 20mg/kg did not prevent the lowering of nociceptive threshold (MH: 6.7±0.4, TIT: 54.7±10.1). Instead of this, we observed worsening of this effect, particularly on day 28th in both TIT(54.7±10.1) and MH(7.5±0.3). The histopathological analysis of dorsal horn of spinal cord showed a increase in glial cells and lacunar spaces suggesting edema in the OXL group and a reversion of these in the DLX (5 and 10mg/kg). The DLX 20 mg/kg group showed a worsening of these characteristics, including neuronal death. **Discussion:** Results showed that DLX (5 and 10mg/kg) have antinociceptive effect in both the TIT as MH and may decrease the peripheral neuropathic condition. Our study is in accordance with others which DLX has an antinociceptive activity in clinical studies and in various experimental models of acute and chronic pain, like pain associated with diabetic neuropathy. DLX reversed the histopathological aspect, with exception of the 20 mg/kg dose which worsened the edema and showed neuronal death. We conclude that DLX prevents OXL-induced peripheral neuropathy, depending on the dose. High doses should be avoided, warning for its clinical use. **Financial support:** CAPES and CNPq

05.042 Involvement of alpha1-adrenoceptors in the antinociceptive effect of tricyclic antidepressants in neuropathic pain. Kauchi BAG, Rocha NP, Pupo AS Unesp – Farmacologia

Introduction: Tricyclic antidepressants (TCAs) are inhibitors of serotonin and norepinephrine neuronal reuptake and this action has been implied in changes in pain threshold. However, it is unclear which adrenoceptors are the targets for the increased synaptic levels of norepinephrine which mediate the antinociceptive effect of TCAs. Therefore, the objective of this study is to analyze the involvement of alpha1-adrenergic receptors (ARs) in the antinociceptive effect of TCAs in a model of neuropathic pain in rats. **Material and Methods:** The study was approved by the Ethics Committee on Animal Use – CEUA protocol 377. Pain was induced in male Wistar rats (180-200g) by constriction of the sciatic nerve (CSN) and fifteen days later mechanical hyperalgesia was analyzed in a Basile Analgesy Meter ® by application of force in the rat's paw according to the Randall-Selitto test. Three days before the tests, the right jugular vein was cannulated for the intravenous injection of drugs. Rats were treated with saline (CTL: n= 4, i.p.), or amitriptyline (AMI: 10 mg / kg, i.p., 60 min before the test, n=4). To check for the involvement of alpha-1 adrenoceptors, some rats received the selective alpha1-adrenoceptor antagonist Prazosin (PZS 1mg/kg, i.v., 90 min before the test) or the selective alpha1A-adrenoceptor antagonist 5-methyl-urapidil (5-MU: 50ug/Kg, i.v., 90 min before the test) and then, 30 min later were treated with amitriptyline 10 mg / kg i.p. (n=4). In these rats, mechanical hyperalgesia was evaluated 60 min after the treatment with amitriptyline. The results were analyzed by Analysis of Variance (ANOVA) followed by multiple comparisons with Tukey-Kramer.

Results: Rats submitted to CSN showed mechanical hyperalgesia (CTL: 10.25± 0.629g) and amitriptyline attenuated this effect (1.50± 1.50g, p<0.05 vs CTL). The prazosin administration abolished the antinociceptive effect of amitriptyline (PZS: 11 ± 1.472g; p<0.05 vs AMI; p>0.05 vs CTL). The application of 5-methyl-urapidil also antagonized the antinociceptive effect of amitriptyline (AMI: 2.80 ± 1.828g vs 5-MU: 10.40 ± 0.678g, p<0.05); the pain threshold from 5-methyl-urapidil-treated rats was not different from the control rats (CTL: 11 ± 0.408g vs 5-MU 10.40 ± 0.678g, p>0.05).

Conclusion: These results indicate that alpha1-ARs are involved in the antinociceptive effect of the TCA amitriptyline and suggest that the alpha1A-AR subtype is a target for the increased synaptic levels of norepinephrine resultant from the inhibition of neuronal reuptake by TCAs. **Financial support:** BAAE, IBB-UNESP (to BAGK) and FAPESP (08/50423-7 to ASP)

05.043 Antinociceptive effects of riparin II and its possible action mechanisms. Carvalho AMR¹, Vasconcelos LF¹, Rocha NFM¹, Dias ML¹, Rios ERV¹, Bastos MVR¹, Barbosa Filho JM², Sousa FCF¹ ¹UFC – Fisiologia e Farmacologia, ²UFPB – Tecnologia Farmacêutica

Introduction: Riparin II (riplI) is an alkamide isolated from *Aniba riparia*, collected from the Amazon forest. This substance presents anxiolytic (Sousa et al., 2005) and antidepressant-like effects in different animal models. The antidepressant-like effect of riplI is dependent on its interaction with serotonergic, noradrenergic, and dopaminergic systems (Teixeira et al., 2011). The aim of this work was to evaluate the antinociceptive activity of riparin II in several models of chemical and thermal nociception in mice. Moreover, we investigated the involvement of the opioid system, as well the participation of the nitric oxide pathway in its antinociceptive effect. This work was submitted in local Ethics Committee on Animal Research by protocol number: 40/10.

Materials and Methods: We used Swiss male mice weighing 25-32g and all experiments was conducted with n=6-9. Riparin II was used at the doses at 25 and 50mg/kg, by gavage. Data were analyzed using One-Way ANOVA and Newman-Keuls test *post hoc*. First, riparin II was tested in the antinociceptive models of acetic acid-induced abdominal writhing (Koster et al., 1959) and formalin test (Hunnskaar; Hole, 1987). Indomethacin 10mg/kg (p.o.) or morphine 7.5mg/kg (i.p.) was used as standard drug. In order to verify the mechanisms of action of riplI, we tested the involvement of the nitric oxide pathways in the formalin test, and also the participation of TRPV1 and glutamate receptors, using the tests of capsaicin-induced paw licking time (Sakurada et al., 1992) and nociception induced by glutamate (Beirith et al., 2002), respectively.

Results and Discussion: RiplI, given by gavage, at both doses, decreased significantly the number of writhings (42.02% and 33.32%, respectively) comparing to the animals treated with vehicle, as well as the group of animals treated with indomethacin (61.90%). At the formalin test, the administration of riplI at both doses decreased the paw licking time, but only at the second phase of the test (25mg/kg: 82.11%; 50mg/kg: 86.95%). Morphine reduced the paw licking time at both phases of the test. The pre-treatment with L-ARG (a precursor of NO) decreased significantly the antinociceptive effect of riplI-50 at the second phase of the test, but not at the first phase (reversion of 26.72%). Riparin II, at the doses of 25 and 50 mg/kg, produced significant inhibition of capsaicin-induced licking time in 36.2% and 41.6%, respectively. Ruthenium red was used a standard drug, and suppressed licking time in 96.6%. Riparin II, at both doses, was able to reduce the nociception induced by glutamate, with inhibition of 81.47% and 75.46%, respectively; pre-treatment with MK-801 (standard drug) inhibited the paw licking time in 94.75%. Based on the obtained results, it is suggested that RiplI presents an antinociceptive activity that may be due to peripheral mechanisms (nitric oxide pathway), TRPV1 and glutamate receptors. **Financial support:** CNPq, CAPES and Funcap

05.044 Evaluation of the antinociceptive activity of terpinolene in mice and possible mechanisms of action. Moura JB¹, Freitas FFBP¹, Lima DF¹, Brandão MS¹, De Castro Júnior JR¹, Sousa DP², Almeida FRC¹ ¹UFPI – Plantas Mediciniais, ²UFS – Farmácia

Introduction: The pain is often associated with many diseases. Current therapies are usually insufficient for having severe side effects and limited effectiveness. So, the search for new molecules is continuous and necessary. The essential oils, used in aromatherapy, agricultural and food industries, are products with great pharmacologic potential. The monoterpene terpinolene (TPL) is a chemical constituent of the essential oil of many plant species that exhibit various pharmacological activities, such as analgesic and anti-inflammatory. The objective of this study was to investigate the antinociceptive activity of oral TPL, in chemical nociception models, as well as possible mechanisms involved. **Methods:** Male Swiss mice (n = 6-9, 20-30 g) were treated with TPL (0.78-25 mg/kg, po) 60 min before the intraplantar injection of formalin (2 %), capsaicin (2 µg) or glutamate (10 µmol). Control animals received vehicle (C- 2 % Tween 80 in saline), MK 801 (0.03mg/kg ip) or morphine (5 mg/kg sc). Nociception was evaluated by quantifying paw licking time after formalin (5 min (first phase) and 15–30 min (second phase)), capsaicin (5 min) or glutamate (15 min). To determine the mechanism of action in the glutamate test, the animals were pretreated (20 min before TPL) with naloxone (opioid antagonist, 2 mg/kg sc), glibenclamide (ATP- sensitive K⁺ channels inhibitor, 2 mg/kg sc), L-arginine (NO precursor, 600 mg/kg ip), atropine (muscarinic antagonist, 0.1 mg/kg sc), yohimbine (α₂ adrenergic antagonist, 0.15 mg/kg sc), parachlorophenylalanine (PCPA)(5-HT synthesis inhibitor, 100 mg/kg ip, 4 days) and ketanserin (5-HT_{2A} antagonist, 0.03 mg/kg ip). The locomotor activity was evaluated in the open field task. The protocols were approved by the Animal Ethics Committee/UFPI (n°. 05/2011) and were carried out in accordance with the current ethical guidelines for investigation of experimental pain in conscious animals. **Results.** TPL-6.25 significantly reduced the formalin response in both phases of the test when compared with vehicle, while TPL-12.5 reduced only the second phase. A significant reduction in time length spent on licking the paw was observed with TPL (3.125, 6.25 and 12.5 mg/kg) in the capsaicin test, but it was further found that local TPL (50, 100 and 200 µg ip) did not cause any effect on this response. TPL also reduced the glutamate-induced nociception (C: 75.09 ± 4.62; TPL-1.56: 50.84 ± 6.56; TPL-3.125: 35.55 ± 4.37; TPL-6.25: 52.08 ± 4.93)(*p<0.05). The TPL antinociceptive effect was reversed by PCPA and ketanserin association, but not with the other antagonists evaluated. In the open field test, TPL (3.125 mg/kg po) did not change the frequency of crossings. **Discussion.** These results provided for the first time, convincing evidence that oral administration of the monoterpene TPL exerted pronounced antinociception when assessed in chemical-induced nociception models in mice. The antinociceptive action of TPL possibly involves the serotonergic system via 5HT_{2A} receptor. It is unlikely that the observed effects are due to central depressant activity since it was unable to modify the motor behavior in open field test.

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05.045 Dynamic weight bearing for evaluation of articular pain in mice models.
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Farmacologia

Introduction: Articular pain is a major cause of chronic pain and disability in the world. Evaluation of articular pain, especially in animal models, is complicated, subjective and brings some challenges to researcher. Dynamic Weight Bearing (DWB) is a device recently developed that allows the assessment of both articular pain and mobility of the animal in freely movement, without the interference of the experimenter. The aim of this study was to standard this apparatus for evaluation of articular articular pain in mice.

Methods: The experiments were performed on male Balb/C or C57BL/6 WT mice. Animal care and handling procedures were in accordance with the guidelines of the IASP and with the approval of the Animal Ethics Committee of the University of Sao Paulo with number 115/2011. Articular pain was induced in mice through intrarticular (i.a.) injection of 17, 50 or 150 $\mu\text{g}/10 \mu\text{L}$ of zymosan A (zym) and treated with 5 mg/kg of indomethacin (ind), evaluate until 24 hours, or immunized mice with 500 μg mBSA plus CFA for later challenge with 10, 30, 100 or 300 $\mu\text{g}/10 \mu\text{L}$ mBSA i.a., that was treated with 0,6; 1,8 or 5 mg/kg ind, 0,5; 1 or 2 mg/kg dexametasone (dex), 5, 10 or 20 mg/kg infliximab (inf) and 5, 15 or 45 mg/kg etoricoxib (etx), all evaluate until 72 hours after challenge. DWB was also tested in osteoarthritic model, 50 or 100 $\mu\text{g}/10 \mu\text{L}$ monoiodoacetate (MIA) i.a., analyzed until 28 days or meniscotomy and meniscectomy (surgical models), evaluate until 49 days after surgery. Mice were allowed walk freely in the apparatus for 5 minutes. Sensors in a floor of acrylic box captured dates in pixels and transmit to a software for analyze. The results were expressed in percentage of weight bearing (rate between right hind paw and left). **Results and Discussion:** DWB was showed efficient to evaluate the disability caused by zym, with reduction of weight distribution thresholds after only 3 hours with 150 $\mu\text{g}/10 \mu\text{L}$ and restored after ind administration. The apparatus was also efficient in mBSA model, but only doses i.a. above 100 $\mu\text{g}/10 \mu\text{L}$ 7 hours after challenge and maintained until 72 hours. All the changes in weight distribution was corrected by classical drugs, as ind 5 mg/kg, dex 2 mg/kg, inf 20 mg/kg and etx 45 mg/kg, showed the predictivity of the method. However, DWB is inefficient for show the hypernociception and disability caused by cartilage degeneration, in both chemical and surgical **Methods:** At the day 21, MIA 50 and 100 $\mu\text{g}/10 \mu\text{L}$ showed a same effect and any difference at sham. The same was observed between the surgical animals and sham until 49 days after surgery. **Conclusions:** DWB can be a highlight tool or complementary method for studies of arthritic articular pain and disability in mice models, especially in acute inflammatory process and not so efficient to evaluate osteoarthritic pain parameters. **Financial support:** CNPq

05.046 Involvement of serotonergic receptors in the antinociceptive activity of riparin III. Vasconcelos LF¹, Carvalho AMR¹, Rocha NFM¹, Rios ERV¹, Dias ML¹, Barbosa Filho JM², Sousa FCF¹ ¹UFC – Fisiologia e Farmacologia, ²UFPB – Tecnologia Farmacêutica

Introduction: Riparin III (ripIII) is an alkamid compound that was firstly isolated from unripe fruit of *Aniba riparia*, but now it can be synthesized. This substance presents antimicrobial, anxiolytic (Melo et al., 2005) and antidepressant-like (Sousa et al., 2004) effects in different animal models. The antidepressant-like effect of ripIII is dependent on its interaction with the serotonergic, noradrenergic (α 1 and α 2-receptors), and dopaminergic (dopamine D2 receptors) systems (Melo et al., 2011). This work shows the antinociceptive effect of ripIII and verifies the involvement of the 5HT-1, 5HT-2a and 5HT-3 receptors in the action mechanism. **Materials and Methods:** Were used Swiss male mice weighing 25-32g and all experiments was conducted with n=6-9. Riparin III was used in the doses at 25 and 50mg/kg by gavage (p.o.). This work was submitted in local Ethics Committee on Animal Research by protocol number: 29/12. Data were analyzed using One-Way ANOVA and Student Newman-Keuls test *post hoc*. Riparin III was tested in the antinociceptive models: Acetic acid-induced abdominal writhing (Koster et al., 1959) and formalin test (Hunnskaar; Hole, 1987). Indomethacin 10mg/kg (p.o.) or morphine 7,5mg/kg (i.p.) were used as standard drug. To verify the participation of serotonergic system in the antinociceptive mechanism of ripIII, we induced the depletion of serotonin by pre-treatment of the animals with pCPA 100mg/kg/day for 4 days. Thirty minutes after the last treatment, animals received ripIII-50 mg/kg in the order to submit them for model of visceral nociception induced by acetic acid 0.6%. We also investigated the participation of 5-HT1, 5-HT2a and 5-HT3 receptors in the antinociceptive activity of riparin III. In this case, the various animals groups were pretreated with NAN-190 (1mg/kg), Ritanserin (1mg/kg), Ondansetron (0,5mg/kg). After 15 minutes, the animals received ripIII-50 mg/kg and were submitted at model of visceral nociception induced by acetic acid 0.6%. **Results and Discussion:** RipIII, administrated orally, at both doses, decreased significantly the number of writhings (50.97% and 58.57%, respectively) comparing to vehicle, as well as indomethacin group (69.97%). In the formalin test, the administration of ripIII decreased the paw licking time at both doses only in the late phase (25mg/kg: 87.83%; 50mg/kg: 72.98%). Morphine reduced the paw licking time in the both phases. The pretreatment with pCPA was able to reverse the nociception promoted by ripIII. This result indicates that the modulation of pain caused by ripIII may be mediated, at least in part, by inducing the release of serotonin. The results showed that 5-HT2a and 5-HT3 receptors are able to be involved in the antinociceptive action mechanism. In conclusion, our findings showed the antinociceptive effect of the ripIII, suggesting that this effect may be mediated, at least in part, by modulation of serotonergic pathway by inducing of the release of serotonin and binding in the 5-HT2a and 5-HT3 receptor. **Financial support:** CNPq, CAPES and Funcap

05.047 Synergism between L-tryptophan and dipyrone in animal models of nociception. Rocha NFM, Rios ERV, Carvalho AMR, Dias ML, Vasconcelos LF, Sousa FCF UFC – Fisiologia e Farmacologia

Dipyrone (metamizol) and L-tryptophan are two substances historically associated to pain relief. Dipyrone has a controversial association with serious toxic effects. We utilized the abdominal writhing test induced by acetic acid (modified of Koster, 1959) and hot plate test (Ed & Leimbach, 1953) to evaluate the antinociceptive effect of the Dipyrone, L-tryptophan and the association of both. Were used Swiss mice (male and female) weighing 22-32g divided into groups containing 6-12 animals. The Dipyrone (25, 50, 100 or 200 mg/kg) and L-tryptophan (12.5, 25, 50 or 100 mg/kg) were administered by intraperitoneal route 30 minutes before the injection of 0.6% acetic acid. The dipyrone exhibited an antinociceptive effect at the higher doses 100 and 200 mg/kg (6.55 ± 1.57 and 5.33 ± 0.843 , respectively, $p < 0.05$) compared to mean number of abdominal writhes of control group (24.31 ± 2.88). By the other hand; the L-tryptophan did not exhibit the antinociceptive effect being no different of control group [L-tryptophan 12.5 mg/kg (23.00 ± 3.00), 25 mg/kg (25.50 ± 2.87), 50 mg/kg (22.29 ± 4.04) or 100 mg/kg (18.43 ± 3.84)]. Several combination of treatment were realized demonstrating that L-tryptophan is capable to diminish the doses needed to dipyrone shows antinociceptive effect [Dipyrone 50 mg/kg (28.20 ± 2.75), L-tryptophan 25 mg/kg + Dipyrone 50 mg/kg (4.43 ± 2.51 , $p < 0.05$ vs control group) Dipyrone 25 mg/kg (24.40 ± 2.71), L-tryptophan 50 mg/kg + Dipyrone 25 mg/kg (10.00 ± 2.65 , $p < 0.05$ vs control group)]; the association with L-tryptophan is able to reduce the antinociceptive doses of dipyrone. In the hot plate test the association showed the same capacity to L-tryptophan reduces the antinociceptive dose of dipyrone, thus we show for the first time the dipyrone sparing effect of L-tryptophan. L-tryptophan is a precursor of 5-HT synthesis and it is metabolized to others active compounds, in order to verified the participation of 5-HT synthesis on the effect of the dipyrone and the synergic effect of L-tryptophan, groups of animals were depleted of 5-HT by p-clorophenylanine (inhibitor of 5-HT synthesis, 100 mg/kg) before the abdominal writhing induction. The 5-HT depletion by PCPA blocks partially the dipyrone 100 mg/kg antinociceptive action [PCPA (17.83 ± 3.59 , $p < 0.05$ vs control and dipyrone 100 mg/kg) Dipyrone 100 mg/kg (6.55 ± 1.59), and antagonize the synergic effect of L-tryptophan + Dipyrone (22.75 ± 6.18 , $p < 0.05$ vs L-tryptophan 50 + Dipyrone 50 mg/kg)]. 5-HT influence on the antinociceptive effect of dipyrone was detected by the increase of amount of 5-HT in the brains of animals (quantified in homogenates of whole brain) treated with antinociceptive dose of dipyrone (100 mg/Kg)[Control (0.028 ± 0.003 $\mu\text{M/g}$), dipyrone 100 mg/Kg (0.077 ± 0.022 $\mu\text{M/g}$, $p < 0.05$ vs control) . Non antinociceptive doses of dipyrone were not able to increase central 5-HT amount [25 mg/kg(0.028 ± 0.00581 $\mu\text{M/g}$) 50 mg/kg($0,012 \pm 0,00299$ $\mu\text{M/g}$)]. The treatment with L-tryptophan (25 to 100 mg/kg, $p < 0.05$ vs control) increases the central 5-HT amount [Control (0.0277 ± 0.00296 $\mu\text{M/g}$), L-tryptophan 25 mg/kg (0.0722 ± 0.00457 $\mu\text{M/g}$) and 100 mg/kg (0.0612 ± 0.00738 $\mu\text{M/g}$). We assessed the antinociceptive activity of Kynurenic acid, L-tryptophan metabolite, and this substance exhibited a powerful antinociceptive activity at the doses of 200, 50 and 25 mg/kg (12.20 ± 1.46 ; 7.000 ± 2.74 ; 6.400 ± 1.57 , respectively, $p < 0.05$ vs control); at the dose of 18.75 the effect was intermediary and was not verified the antinociceptive activity when administrated doses of 12.5 or 6.25 mg/kg. However, these lower doses exhibited a synergic effect with dipyrone in reduction of abdominal writhes number (1.40 ± 0.40 or 1.50 ± 0.645 , respectively, $p < 0.05$ vs control). Previous administration of luzindol (melatonin receptor antagonist, 10 mg/kg) blocks partially the effect of dipyrone sparing effect of L-tryptophan in abdominal writhing test [Control: (41.14 ± 2.48), Luzindol 10mg/kg + L-tryptophan 25 mg/kg + Dipyrone 25 mg/kg (30.29 ± 4.83 , $p < 0.05$ vs Control and L-tryptophan 25 + Dipyrone 25 mg/kg)]. Conclusion: L-tryptophan exhibited dipyrone sparing effect in nociceptive tests in mice and this effect looks to be involved with L-tryptophan

biotransformation to 5-HT, Kynurenic acid and Melatonin. **Financial support:** CNPq, CAPES and Funcap.

05.048 Investigation of antinociceptive effect of riparin IV: Role of transient potential receptors (TRP). Dias ML¹, Carvalho AMR¹, Rios ERV¹, Rocha NFM¹, Vasconcelos LF¹, Barbosa Filho JM², Sousa FCF¹ ¹UFC – Fisiologia e Farmacologia, ²UFPB – Tecnologia Farmacêutica

Riparin IV is an alkamide synthesized from *Aniba riparia*. This substance is an analogue of Riparin I, that presents antinociceptive activity (Araújo et al., 2009) in different animal models. The aim of this study was to investigate the antinociceptive effects of Riparin IV and verify the involvement of TRPV1, TRPA1 and TRPM8 ion channels at the mechanism of action of this substance. This work was submitted in local Ethics Committee on Animal Research by protocol number 38/2011. We used Swiss male mice weighing 25-32g, from 6 to 8 animals in each group. Riparin IV was used at the doses of 25 and 50mg/kg, by gavage. Data were analyzed using One-Way ANOVA and Student Newman-Keuls test *post hoc*. First, Riparin IV was tested in three antinociceptive models: Acetic acid-induced abdominal writhing (Koster et al., 1959), formalin test (Hunnskaar; Hole, 1987), hot plate test (Eddy; Leimbach, 1953). Indomethacin 10mg/kg (p.o.) or morphine 7,5mg/kg (i.p.) were used as standard drugs. Rip IV at both doses, decreased significantly the number of writhings (40,46% and 42,84%, respectively), when compared to the control group (vehicle- 3% tween 80 in distilled water), as well as indomethacin (66,69%). At the formalin test, the administration of RipIV decreased paw licking time at both doses, only at the second phase of the test (25mg/kg: 56,03%; 50mg/kg: 53,36%). Morphine reduced paw licking time at both phases. Another test was the hot plate (51°C ± 0.5°C), where the groups receiving RipIV showed no statistical difference when compared to the control group. The animals receiving morphine presented an increase at the response time to heat compared to the control group. To verify the participation of transient potential receptors (TRPV1, TRPA1 and TRPM8) at the antinociceptive effect of RipIV, the animals were pretreated with 20µL of capsaicin 2.2µg/paw (TRPV1 agonist receptor), cinnamaldehyde 10nmol/paw (TRPA1 agonist receptor) or menthol 1.2µmol/paw (TRPM8 agonist receptor) under the ventral surface of the right hind paw. Ruthenium red (3mg/kg, i.p., non-selective TRP antagonist receptor) or camphor (7.6mg/kg, s.c., TRPA1 antagonist receptor) were used as standard drugs. The results show that Riparin IV at the doses of 25mg/kg and 50mg/kg, produced significant inhibition of capsaicin-induced paw licking in 24,64% and 45.12%, respectively, when compared to the control group. Ruthenium red was used as standard drug, and suppressed this parameter in 44,83%. The pretreatment with Rip IV, at both doses, failed to demonstrate any significant influence at the cinnamaldehyde-induced licking time. RipIV at both doses (25 and 50 mg/kg) and camphor were able to significantly reduce the menthol-induced paw licking in 53,89%, 87,11% and 98.88%, respectively. In this context, we have shown for the first time the antinociceptive effect of Riparin IV. Because this substance was able to decrease the nociception induced by formalin, and considering that there are several ion channels involved at the development of nociception at the formalin test, based in our results, we can conclude that TRPV1 and TRPM8 might be involved at the mechanism of action of Riparin IV. **Financial support:** CNPq/CAPES/FUNCAP

05.049 Antyhyperalgesic effect of N-antipirino-3-chloro-4-(4-bromoanilinomaleimida) in persistent models of pain in mice. Silva GF, Buzzi FC, Correa R, Cechinel Filho V, Quintão NLM NIQFAR-CCS-UNIVALI

Introduction: Cyclic imides have attracted the attention of the scientific community, due to their promising therapeutic potential. Studies with the compound N-antipyrine-3,4-dichloromaleimide provided strong evidence that this compound produces antinociception in mice acting at peripheral, spinal and supraspinal sites, and interacts with the group I mGluRs and NMDA receptors contributing to the mechanisms underlying its effect (Quintão et al., 2010). This study aimed to evaluate the anti-hyperalgesic effects of N-antipirino-3-chloro-4-(4-bromoanilinomaleimida) (NA-3Cl-4Br AM) in persistent models of pain in mice. **Methodology:** Female Swiss mice (25-35g, N=6-8) were used. For the induction of inflammatory model of hyperalgesia mice were injected with complete Freund's adjuvant (CFA; 20 µL/paw) and 24 hours before, they were treatment with NA-3Cl-4Br AM (61-610 nmol/kg, i.p. or 85 µmol/kg p.o.) or saline once a day for 4 days and the mechanical withdrawal evaluation were performed 6 h before the daily treatment using von Frey hair (0.6 g). In order to evaluate the effect of the compound in neuropathic pain, the animals were submitted to two different profiles: (1) partial ligation of sciatic nerve model (PLSN; 1/3 to 1/2 of the dorsal portion of the sciatic nerve was tied); (2) brachial plexus avulsion model (BPA; the right brachial plexus was accessed and the lower trunk was clamped and avulsed by traction). For the induction of both models the animals were previously anesthetized with chloral hydrate, 7% (8mL/kg, i.p.). In sham-operated group, the nerve was only exposed without injury. On the fourth day after surgery, the sham-operated and operated animals were treated with NA-3Cl-4Br AM (61-610 nmol/kg, i.p. or 85 µmol/kg, p.o.), saline or gabapentin (400 µmol/kg, p.o.) once a day for 4 days and the mechanical hyperalgesia was evaluated as mentioned above. This study was approved by the Ethical Committee for Animal Care of UNIVALI (Process No. 370/2008). **Results:** Systemic treatment with NA-3Cl-4Br AM was able to reverse the mechanical hyperalgesia induced by CFA with inhibition of $87 \pm 1\%$ (0.61 µmol/kg, i.p.) and $99 \pm 1\%$ (85 µmol/kg, p.o.). The compound also proved to be effective in preventing mechanical sensitization induced by CFA in the contralateral hindpaw with inhibitions of $99 \pm 1\%$ (0.61 µmol/kg, i.p.) and $44 \pm 12\%$ (85 µmol/kg, p.o.). In PLSN NA-3Cl-4Br AM was also effective in reversing hyperalgesia with inhibition of $56 \pm 4\%$ (610 nmol/kg, i.p.) and $70 \pm 1\%$ (85 mmol/kg, p.o.). Finally, animals treated with NA-3Cl-4Br AM had the mechanical hyperalgesia induced by BPA significantly inhibited, with inhibition of $78 \pm 2\%$ (610 nmol/kg, i.p.) and $66 \pm 3\%$ (85µmol/kg, p.o.). **Discussion:** These results demonstrated that NA-3Cl-4Br AM presents important activity against mechanical sensitization induced by both inflammatory and neuropathic pain models in mice. They also allow us to suggest that the long lasting effects of NA-3Cl-4Br AM may be associated to facilitation of inhibitory pathways of pain control and/or the reduction of excitatory pathways of the central nervous system. These findings might have additional therapeutic implications for the development of a new drug to treat chronic pain. **Conclusion:** We have demonstrated that the cyclic imide compound NA-3Cl-4Br AM, administered systemically, reduces the mechanical hypernociception induced by , CFA, PLSN and BPA in mice. These findings might have additional therapeutic implications for the development of a new drug to treat chronic pain. **Financial Support:** CNPq, FAPESC-SC, ProPPEC/UNIVALI. Quintão et al. *Anesth Analg.* 110(3):942, 2010.

05.050 LASSBio-1135: a multi-target compound, orally effective in a model of neuropathic pain, acts as a TRPV1 antagonist, TRPA1 agonist and also reduces cytokine production. Lima CKF¹, Yekkirala AS², Sprague JM², Lacerda RB¹, Barreiro EJ¹, Fraga CAM¹, Cunha TM³, Woolf CJ², Miranda ALP¹ – ¹LASSBio-ICB-UFRJ – Desenvolvimento de Fármacos, ²Harvard Medical School – Neurobiology, Neurobiology, ³FMRP-USP – Farmacologia

Introduction: Treating chronic pain is a great challenge nowadays and multi-target therapies, focusing on neuro-immune interaction, have arisen as an alternative treatment approach. LASSBio-1135 was previously described as an anti-TNF- α , COX-2 inhibitor and TRPV1 antagonist (Lacerda, R.B. Bioorg. Med. Chem. 17: 74, 2009). The aim of this work was to investigate its efficacy in a model of peripheral neuropathic pain, as well as its mechanism of action. **Methods:** HEK-293 cells transfected with human TRPA1 plasmids were submitted to Ca-imaging in order to evaluate the selectivity of the compound. DRG and TG neurons were obtained from C57 mice and cultivated in 384-wells for Ca-imaging assay. Neuropathic pain was induced in Swiss mice using partial sciatic ligation, as described by Seltzer (Seltzer, Z. Pain, 43:245, 1990). 5 days after surgery, mechanical and thermal hypersensitivity were evaluated. After 5 days of treatment samples of DRG and spinal cord were collected and analyzed by RT-PCR for IL-1 β and TNF- α . **Results and Discussion:** In order to characterize the pharmacological selectivity of LASSBio-1135 for TRPV1 we also tested it against hTRPA1 expressed in HEK-293 cells. Intriguingly, LASSBio-1135 activated hTRPA1 (EC_{50} = 11,8 μ M, n=3) which is 20x higher than its potency to block TRPV1. However, it did not affect the activation produced by mustard oil (30 mM), a TRPA1 agonist. The activation produced by LASSbio 1135 was inhibited (50%, n=3) when incubated with TRPA1 antagonist, AP-18 (30 mM). In addition, LASSBio-1135 (10 mM) promoted a small Ca²⁺ influx in DRG and TG neurons, and reduced the activation of TRPV1 by its agonist, capsaicin(1 μ M), by 45% (n=3) in both cultures. In addition to its neuronal activity, this compound also inhibits cytokine production by inhibiting the p38 MAPK signaling pathway. Given this, we investigated its efficacy in vivo in a model of peripheral neuropathic pain induced by partial sciatic ligation. LASSBio-1135, orally administered at 100 μ mol/Kg, showed efficacy through 10 days of treatment, blocking completely the thermal hypersensitivity, although only partially reducing mechanical allodynia (n=10). After 5 days of treatment some of these mice were euthanized and samples of DRG and spinal cord tissues were submitted to RT-PCR for IL-1 β and TNF- α . Treatment with LASSBio-1135 was efficacious in reducing mRNA expression of those cytokines in both samples (TNF- α = 80% DRG, 91% Spinal cord; IL-1 β = 91% DRG, 62% spinal cord). **Conclusion:** LASSBio-1135 is a novel analgesic candidate with a promising mechanism of action, modulating both neuronal and immune response. **Financial support:** FAPERJ, CNPq, CAPES, INCT-INOVAR, NIH.

05.051 Antialgic effect of dipyron metabolites. Assis DCR¹, Malvar DC¹, Vaz ALL², Melo MCC², Clososki GC², Souza GEP² ¹FMRP-USP – Farmacologia, ²FCFRP-USP – Física e Química

Introduction: Dipyron is a pro-drug with potent analgesic and antipyretic effect. After its administration, dipyron is rapidly converted by hydrolysis to 4-methylaminoantipyrine (4-MAA), which is further metabolized in 4-formylaminoantipyrine (4-FAA), 4-aminoantipyrine (4-AA) and 4-acetylaminoantipyrine (4-AAA). (Zylber-Katz. Clin Pharmacol Ther. 58:198, 1995). Different from non steroidal anti-inflammatory drugs, its effect is not only related to the prostanoïd synthesis blockage but also with other pathways not yet entirely understood (Sachs, Proc Natl Acad Sci U S A, 101:3680, 2004). Antialgic determination of the dipyron metabolites may help elucidate the antialgic mechanism of this pro-drug and encourage the development of a safer drug with reduced side effects to treat pain and fever. In view of that, this study aim to investigate the antialgic effect of dipyron metabolites.

Methods: Male, Swiss mice (25g) were pre-treated with saline (0.01ml/g), dipyron or its metabolites 4-AA, 4-MAA, 4-AAA and 4-FAA 30 minutes before acetic acid (0.8%, intraperitoneal) or formalin (2.5%, intra-plantar) injection. Number of writhes and time spent licking the injected paw were measured for the following 30 min after acetic acid injection and at 0-5 min (first phase) and 15-30 min (second phase) after formalin injection and were considered as pain behavior response. Data were expressed as mean± S.E.M. Results were statistically evaluated by ANOVA followed by Tukey test, $p < 0.05$. (Ethic Committee of FMRP/USP, process n° 019/2012). **Results:** Dipyron, 4-MAA, 4-AA and 4-AAA, but not 4-FAA, inhibited the acid acetic-induced writhes. In the formalin-induced pain test, dipyron, 4-MAA, 4-AA inhibited the pain behavior response in both first and second phases, while 4-AAA only inhibited the second phase and 4-FAA did not alters the pain behavior response induced by formalin injection. In both tests, 4-MAA and 4-AA were more potent than dipyron. 4-AAA only produces antialgic effect at high doses. Results are shown in the table below. **Discussion:** The results have confirmed the dipyron antialgic effect on the writhing and formalin tests in mice as already described (*Eur. J. Pharmacol.* 496(1-3); 93, 2004) and suggest that 4-MAA, 4-AA and 4-AAA are responsible for the dipyron antialgic effect in both tests. Additionally, it was showed that 4-AAA was the less potent metabolite and 4-FAA has no significant effect. **Financial support:** CAPES e CNPQ.

Effect of dipyron and its metabolites on behavioral response to formalin and acetic acid-induced writhes in mice.

Treatments	Dose mg/kg	Writhing Test		Formalin Test	
		Writhings number	Time licking		
			Fase 1	Fase 2	
Dipyron	Saline	35.8±5.7	60.3±3.5	104.9±19.5	
	60	17.1±5.0*	42.5±4.2**	61.8±8.5	
	90	10.0±3.6***	16.8±3.7***	22.9±10.8***	
	120	7.2±2.2***	15.2±2.5***	13.9±6.4***	
4-MAA	Saline	42.8±2.5	74.1±4.3	104.9±19.5	
	15	21.9±3.8***	-	-	
	30	22.4±3.1***	51.0±4.5***	69.3±17.5	
	60	5.2±1.3***	21.0±4.0***	38.2±12.8*	
	90	1.3±0.5***	20.1±2.9***	41.0±9.5*	
4-AA	Saline	36.8±4.6	58.9±4.4	118.9±12.3	
	30	23.0±4.5**	55.7±4.7	69.3±4.9*	
	60	11.4±2.0***	18.6±2.6***	37.5±8.3***	
	90	7.5±1.9***	21.5±4.3***	28.9±10.5***	
	120	1.0±0.6***	25.7±4.3***	10.2±5.2***	

4-AAA	Saline	35.0±3.0	64.8±2.1	118.6±16.4
	90	37.4±4.1	63.5±5.0	103.9±14.4
	180	36.0±3.9*	55.3±5.4	54.3±14.2*
	225	9.5±1.8***	-	-
	270	5.2±1.9***	59.8±3.6	21.3±7.1**
4-FAA	Saline	38.4±2.5	79.0±5.3	110.9±24.7
	180	-	64.0±7.0	137.0±14.6
	240	29.4±2.1	-	-
	270	-	67.5±6.5	126.1±11.2
	360	29.2±5.2	-	-

Number of animals per group: 6 to 21

05.052 Investigation of the mechanisms involved in pronociceptive action of spinal activation of NOD2 that account for the genesis of neuropathic pain.

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– ¹FMRP-USP – Farmacologia, ²FMRP-USP – Biologia Celular e Molecular e Bioagentes Patogênicos

Background and Aim: Among PRRs (pattern recognition receptors), NOD-like receptors (NLRs), such as NOD2 are responsible by intracellular detection of MDP (muramyl dipeptide); pathogen-associated molecular pattern (PAMP) found in the peptidoglycan (PGN) from virtually all gram positive and gram negative bacteria. Upon recognition and stimulation by MDP, NOD2 recruits directly RIPK2 (receptor-interacting serine/threonine-protein kinase 2), an adaptor protein important in NOD2-mediated NFκB activation. The expression of NOD2 has been described in macrophages and other cells. Previous work has indicated that PRRs play a crucial role in the activation of spinal cord glial cells in the induction and maintenance of chronic inflammatory and neuropathic pain. Recently, we have shown that NOD2 is also involved in the genesis of neuropathic pain. Therefore, in the present study, we aimed to evaluate the role of NOD2 in the modulation of pain sensitivity, focusing on its importance in the activation of spinal cord glial cells, as well as its signaling pathway (RIPK2) and release of pronociceptive cytokines, such as tumour necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β). **Methods:** The experiments were carried out on male C57BL/6 (WT) mice or male NOD2, RIPK2, TNFR1, TNFR1/2 e MyD88 knockout (^{-/-}) mice. All animal care and experimental procedures were conducted according to the guidelines of the Ethics Committee (106/2011) of the School of Medicine of Ribeirão Preto (University of São Paulo, São Paulo, Brazil) and according to the IASP guidelines on the use of laboratory animals. The mechanical threshold (in grams) was determined by application of von Frey filaments to the hindpaws. Spinal activation of NOD2 were done by intrathecal administration of MDP made in a 5 µl volume. To assess the mechanisms involved in MDP-induced mechanical hypersensitivity, mice were treated with propentofylline 7 nmol, SB 203580 3 µg, IL-1ra 10 µg, fluorocitrate 0,33 nmol (30 minutes) and minocycline 0,15 µmol (60 minutes) before the injection of MDP 10µg. Selected doses were based on dose–response curves. **Results:** Our results demonstrate that WT mice treated with MDP showed a decrease in mechanical nociceptive threshold (peak 3 to 5 hours) compared with the control group (vehicle), returning to the base line after 48 hours. Furthermore, NOD2^{-/-}, RIPK2^{-/-} and TNFR1/2^{-/-} mice treated with MDP did not differ the mechanical nociceptive threshold compared with their respective control groups (vehicle). However, TNFR1^{-/-} and MyD88^{-/-} mice treated MDP, showed a decrease in mechanical nociceptive threshold similar to WT mice treated with MDP. Pretreated of WT mice with IL-1ra, propentofylline, minocycline, and SB 203580 inhibited the development of mechanical hypersensitivity induced by MDP, while the fluorocitrate had little effect. **Discussion:** These data suggest that activation of the intracellular sensor NOD2 present in spinal cord microglial cells stimulates the activation of RIPK2 and p38 MAPK signaling pathways and subsequent production of IL-1β and TNFα, in a MyD88-independent pathways. These mechanisms contribute to the process of mechanical hypersensitivity during peripheral neuropathy and represent a novel approach for elucidating the mechanisms underlying pathophysiology of chronic pain. **Financial support:** CNPq, FAPESP.

05.052 Investigation of the mechanisms involved in pronociceptive action of spinal activation of NOD2 that account for the genesis of neuropathic pain.

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Introduction: Histamine has an important effect on the perception of nociceptive stimulation and an modulatory role in peripheral inflammation through H1 receptors (MOBARAKEH, J. *Eur J Pharmacol*, 391: 81, 2000; SAEKI, K. *Arch Int Pharmacodyn Ther*, 222: 132, 1976). **Aim:** Evaluate the role of the H1 receptor on nociception and inflammation induced by carrageenan injection into rat knee-joints. **Methods:** Male Wistar rats (300-400 g) received intraarticular injection of carrageenin (100 µg). After injection, articular incapacitation was measured hourly by counting the paw elevation time (PET; s) during 1-min periods of stimulated walk. Edema was accessed just after each PET evaluation by measuring the articular diameter (AD; mm). PET and edema were evaluated throughout 6 hours. The H1 antihistamines (loratadine and cetirizine) were injected i.p., 1 hour before carrageenan stimulation. The H1 agonist 2-Pyridylethylamine (2-PEA) was given directly in the knee-joint, 20 min before the third hour measurement, after carrageenan injection. After 6 h, synovial fluid was sampled for the leukocyte count (CEUA/PP00368/2009). **Results:** Cetirizine dose of 10 mg/kg (PET_{3h} = 47.23±5.38 s; p<0.05; control group = 29.62±4.38 s) increased PET. Similary, loratadine doses of 2.5 mg/kg (PET_{3h} = 42,72±6.59 s), 5 mg/kg (PET_{3h} = 55.04±2.18 s) and 10 mg/kg (PET_{3h} = 43.47±4.92 s) increased PET (p<0.01; control group = 36.10±4.37 s). Loratadine 5 mg/kg (AD_{4h} = 0.27±0.02 mm; p<0.01; control group = 0.22±0.02 mm) also increased the articular diameter. None of the antihistamines changed cell migration. However, 2-PEA (0.05 pmol/knee = 95.89±24.84 cell x 10⁵/mL; 50 pmol/knee = 83.67±18.89 cell x 10⁵/mL; control group = 1111.09±294.28 cell x 10⁵/mL) decreased polymorphonuclear cell migration, but did not change PET or articular diameter. **Conclusion:** These results suggest that in this knee-joint inflammation model H1 receptors are tonically stimulated and exerting antihyperalgesic and antiedematogenic effects. A modification in cell content seems to be not related to these effects. **Financial support:** CNPq; CAPES.

05.054 Antinociceptive effect of inosine involves direct interaction with adenosine A1 receptors. Macedo-Junior SJ¹, Nascimento FP¹, Luiz-Cerutti M², Borges FR², Córdova MM², Dutra R¹, Pamplona FA¹, Constantino L³, Tasca CI³, Reid A⁴, Sawynok J⁴, Calixto JB¹, Santos ARS² ¹UFSC – Farmacologia, ²UFSC – Ciências Fisiológicas, ³UFSC – Bioquímica, ⁴Dalhousie University – Pharmacology

Introduction: Inosine is the first metabolite of adenosine. We have been demonstrated that inosine presents antinociception against several models of nociception. This study sought to investigate the involvement of adenosine A1 receptor (A1R) in the antinociceptive effect of inosine, and compare the efficacy and potency of inosine and adenosine to promote this effect, as well as their binding affinity to A1R. **Methods:** Were used male Swiss and *C57/black* 6 mice. It was used the formalin intraplantar (i.pl.) pain test. The nociceptive behavioral was considered as flinching and licking/biting of the injected paw. First, animals received inosine or adenosine intraperitoneally (i.p.) at doses of 1, 10 or 100mg/kg 20 minutes before formalin test. Next, inosine 10 or 20µg/paw was co-administered with formalin. In another approach, animals received inosine and the selective antagonist DPCPX 10nmol/5µl, intratecally (i.t.), 0.1mg/kg, i.p. and 5µg/20µl, i.pl., respectively. Later, the animals were treated with inosine 10mg/kg, i.p. or 20µg/20µl, i.pl. and were submitted to formalin test. Knockout animals were also used. Wild type and knockout animals were treated with inosine 10mg/kg, i.p., 10µg/5µl, i.t. or 20µg/20µl, i.pl. and were submitted to formalin test. Another approach used antisense oligonucleotide (AS-ODN) mismatch or A1R 1µM/site injected twice a day for 5 days, before formalin test. Analysis A1R expression were performed by spinal cord immunohistochemical. To determine direct interaction of inosine with A1R was used specific binding assay performed using membrane fractions of whole brain of rats incubated with 0.5 nM A1R radioactive antagonist [³H]DPCPX. **Results:** Inosine 10 and 100mg/kg, i.p. reduced flinching and licking/biting with I_{max} of 44% and 46%, respectively. Adenosine 10mg/kg, i.p. reduced flinching while 10 and 30mg/kg, i.p. reduced licking/biting with I_{max} of 44% and 47%, respectively. Inosine 20µg i.pl. reduced nociceptive behavioral. DPCPX 10nmol, i.t. and 0,1mg/kg, i.p. prevented inosine antinociceptive effects, while DPCPX 5µg/site, i.pl. prevented inosine effects only in licking/biting behavioral. A1R knockout strategy prevented inosine antinociceptive effects when the nucleoside was administrated by i.t. or i.p. routes, but did not alter the effects of inosine administered by i.pl. route. The AS-ODN approach reduced A1R expression in spinal cord and prevented inosine antinociceptive effects. Inosine and adenosine shifted considerably antagonist [³H]DPCPX binding site, with an IC₅₀ of 36 nM and K_i of 5.9 (1.5-23.7) nM for inosine and IC₅₀ of 113 nM and K_i of 18.9 (2.8–127.2) nM for adenosine. **Discussion:** Inosine exhibited efficacy and potency similar to those of adenosine. The systemic effects of inosine involves central A1R, moreover, the peripherally A1R are not essential for inosine effects. This study showed that inosine binds directly to A1R with similar affinity to adenosine. **Experimental procedures** approved by CEUA/UFSC protocol PP00484, process n° 23080.016339/2010-61 or by UCLA/Dalhousie University, protocol n° 11-021. **Financial support:** Capes and CNPq.

05.055 Effect of LED in the inflammatory hyperalgesia induced by *Bothrops moojeni* snake venom. Nadur Andrade N¹, Toniolo EF², Dale CS², Zamuner SR¹
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Aim: *Bothrops* snake venoms produce marked local effects, including edema, hemorrhage, necrosis and pain. At present, the most effective treatment for *Bothrops* snake-bite accidents is antivenom therapy. However, antivenom treatment has been ineffective in neutralizing the severe local fast-developing tissue damage following snake-bite envenoming. In the present study, we evaluated the effectiveness of light-emitting diode (LED) in reducing pain induced by *Bothrops moojeni* venom (BmV), in mice. **Methodology:** Male Swiss mice (22-25 g) were used. Hyperalgesia and allodynia of the hind paw were assessed as described by Takasaki et al. (2000). Mice were placed individually in a plastic cage and 15 min later injected into the sub-plantar surface of the right hind paw with 1.0 µg of BmV, or with the same volume (50 µl) of sterile saline. Mice were irradiated for 30 min and 3 h after the venom administration with a LED at 945 and 635 nm, power of 110 and 120 mW, dose of 4 J/cm², irradiated area of 1,2 cm², 41s and 38s irradiation time for the red LED (LED_r) and infra-red (LED_{inf}), respectively. von Frey filaments with bending forces of 0.407 g (3.61 filament – allodynia stimulus), 0.692 g; and 1.202 g (3.84 and 4.08 filaments – hyperalgesia stimulus) were pressed perpendicularly against the plantar skin and held for 5 s, at 1, 3, 6 and 24 h after venom injection. The stimulation of the same intensity was applied three times to each hind paw at intervals of five seconds. **Ethics Committee: CEUA2010/01. Results:** Intraplantar injection of BmV induced tactile allodynia and hyperalgesia. BmV caused maximum response observed at 1 h after the venom injection (72%) remaining high until 24 hours. Animals treated with the red LED showed a significant reduction of allodynia and hyperalgesia compared with animals injected with venom alone, producing reduction of 50%, 51% and 61% at 3, 6 and 24 hours respectively. On the other hand, treatment with infrared LED was not effective in reducing hyperalgesia and allodynia caused by BmV. **Conclusions:** In conclusion, the results of this study indicate that *Bothrops moojeni* venom is able to induce paw hyperalgesia in mice. Furthermore, red LED irradiation reduced the hyperalgesia and allodynia induced by BmV even though the irradiation was administered after the symptoms were already present. These results suggest that phototherapy may reduce the local effects induced by *Bothrops* venom and therefore may be clinically relevant. **Sources of research support:** UNINOVE; IEP- Sírío Libanês

05.056 Pharmacotherapy for painful crisis in sickle cell disease in patients admitted to the General Hospital in the north of Espírito Santo State. Sabino MF, Nascimento TD, Nascimento LCN DCS-CEUNES

Sickle cell anemia is considered a public health problem in Brazil, since birth is estimated from 700 to 1000 new cases / year. Sickle cell anemia is due to changes in hemoglobin, which may trigger the clinical manifestations, mainly vaso-occlusive crisis, which leads the patient to seek help and more frequent hospitalization. Thus, this study aimed to characterize children and adolescents with sickle cell disease about the drugs used during hospitalization in a general hospital in the north of Espírito Santo. The population consisted of 09 medical records of hospitalized children and adolescents in the field of pediatrics with a diagnosis of sickle cell anemia in the period July 2011 to February 2012. From the medical records were collected the following information: age, sex, length of stay, race, laboratory results, complications, medications used and whether or not painful crisis and its intensity. From the data analysis it was found that of 09 patients, 06 of them had severe painful crisis at admission, 02 and 01 reported moderate crisis were pain at the time or during hospitalization. The drugs used for pain crisis, it was observed that all patients received dipyrone, 04 used paracetamol, ibuprofen 05, 02 and 01 procaine tramadol. The administration of non-opioid analgesics, these data are in agreement with the literature, whereas non-opioid analgesics may be used in children independent of the intensity of the crisis, respecting the daily doses. However, the use of tramadol is not recommended because it is an opioid, can provide similar side effects to clinical symptoms such as nausea and vomiting. The ideal would be to start trying to control the pain with non-opioid analgesics (such as dipyrone) and identified only after the persistence of pain, start administration of opioid analgesics. The same is indicated for cases of moderate pain unresponsive to opioids and non-severe pain, as prescribed for 03 inpatients. In this context, it is concluded that the data collected in this study were consistent with the findings in the literature in all cases, except in cases that were used in pediatrics tramadol and confirming that the painful crisis is the main factor in the search for hospital and outpatient.

05.057 LASSBio-1473 AND LASSBio-1474: Sulfonyl-hydrazones derivatives with anti-inflammatory activity and effective on neuropathic pain. Santos BLR¹, Lima CKF¹, da Silva LL¹, D'Andrea ED², Lima LM¹, Barreiro EJ¹, Miranda ALP¹ ¹UFRJ – Farmácia, ²UFRJ

Introduction: Neuropathic pain is a disabling condition that usually occurs due to injury in peripheral nerves or neuronal pathways in the central nervous system. The contribution of the inflammatory process in the establishment of neuropathic pain remains unknown; however, there are evidences of the involvement of pro-inflammatory mediators in the chronic stage (Campbell, *Neuron*, 52, 77, 2006). The currently available therapies for the control of neuropathies have low efficacy and high adverse effects, showing the need for elucidation of new targets and new molecules for its control. The study aimed to evaluate the actions of sulfonyl-hydrazones derivatives LASSBio-1473 and LASSBio-1474 on inflammation and neuropathic pain. **Methods:** The *in vitro* TNF- α production was performed on cultures of mice peritoneal macrophages stimulated with LPS (100 ng/ml). The test compounds were incubated for 1 h before stimulation (n = 3-4). The supernatant was collected and stored at -80°C until determination of TNF- α (t = 24 h) by enzyme immunoassay (Gallily, *JPET*, 283, 918, 1997). The *in vivo* anti-inflammatory and anti-hypernociceptive activities were evaluated on the mice carrageenan (1%, 10ul/g, i.p.) induced peritonitis and formalin (2.5%; 20 μ l/paw) induced hypernociception models, respectively (Vinegar, *Proc Soc Exp Bio*, 143, 714, 1973; Tjolsen, *Pain*, 51, 5, 1992). Inflammatory and painful stimuli were administered 1h after administration of test compounds (100 μ mol/kg, p.o.). Neuropathic pain is induced by partial ligation of the sciatic nerve in swiss mice and painful response measured by radiant heat stimulus (Seltzer, *Pain*, 43, 205, 1990). The animals were treated daily (100 μ mol/kg, p.o.) for 10 days. **Results and Discussion:** LASSBio-1473 and LASSBio-1474 inhibited by 83%* the *in vitro* production of TNF- α at 100 μ M concentration, without cytotoxic effect, being more effective than thalidomide (300 μ M; 67%* of inhibition), an anti-TNF- α drug. Both compounds also inhibited *in vivo* TNF-alpha production and the leucocytes recruitment on peritonitis model. The increased cellular infiltration and proinflammatory cytokines play important actions in the pathogenesis of inflammatory and neuropathic pain. LASSBio-1473 and LASSBio-1474 inhibited significantly (57% *) inflammatory pain in a classic test of acute pain: formalin induced nociception. The compounds were still able to reestablish the baseline pain after thermal stimulus in animals subjected to model of neuropathic pain. The anti-hypernociception activity was observed from the second day of treatment on neuropathic pain model of partial sciatic nerve ligation. This study allowed the identification of LASSBio-1473 and LASSBio-1474 as important anti-inflammatory TNF- α inhibitors with promising activity in the control of neuropathic pain. Statistical analysis: one-way ANOVA* p <0.05. CNPq, CAPES, INCT-INOVAR.

05.058 H1 receptor agonist inhibits Mast cell migration and degranulation in the knee-joint of rats. Mascarin LZ, Souza-Silva E, Tonussi CR UFSC – Farmacologia

Introduction: It is suggested that mast cell-released mediators are involved in the second phase (10-60 min) of the nociceptive response induced by formalin. However, we previously observed that histaminergic H1 receptor agonists can inhibit the formalin-induced nociception (p- 05 069, SBFTE 2010). Thus, our aim was to evaluate whether agonist H1 (2-Pyridyletilamine) can modify mast cells degranulation and migration in the knee-joint formalin incapacitation test. **Methods:** Articular incapacitation was measured by counting the paw elevation time (PET; s) during 1 min period of stimulated walk either each 5 min throughout 25 min after 1.5% formalin knee-joint injection. 2-Pyridyletilamine (2-PEA) or sodium chromoglycate were co-injected with formalin into knee-joint. Twenty five minutes after formalin injection, the knee was removed and fixed in formaldehyde solution 4% for 24 hours. The anterior portion of articular capsule was then further processed for histological identification of mast cells by 1% toluidine blue staining. Mast cell degranulation was verified by cell counts per area (mm^2) located in the articular capsule. (CEUA/PP00368/2009).

Results: Formalin induced articular incapacitation ($P < 0.001$) and increased the total mast cell synovial content and degranulation ($P < 0.01$). Sodium chromoglycate (1.6 mg/knee) prevented mast cell degranulation ($P < 0.001$), but did not change PET. 2-PEA (5 nmol/knee) decreased PET ($P < 0.05$), as well as the mast cell content and percentage of degranulation, per tissue area ($P < 0.01$). **Discussion:** These results suggest that H1 receptor activation may reduce synovial mast cell degranulation and migration stimulated by formalin. However, this effect seems to be not involved in the hyponociceptive effect observed, since chromoglycate exerted the same effects on mast cell, but without hyponociception. Further experiments are needed to elucidate this mechanism. **Acknowledgments:** Capes, CNPQ.

05.059 Antinociceptive activity of the monoterpene α -phellandrene in rodents: possible mechanisms of action. Lima DF¹, Brandão MS¹, Moura JB¹, Leitão JMSR¹, Carvalho FAA¹, Miura LMCV², Leite JRSA², Sousa DP³, Almeida FRC¹ – ¹UFPI – Medicinal Plants, ²UFPI – Biodiversity and Biotechnology, ³UFS – Pharmacy

Introduction: Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. The management of pain requires an analgesic drug that is effective and associate with few side effects. The research of analgesic drugs is so important and the natural products seem to be a good source of biomolecules. Essential oils (EO) are an important class that presents several pharmacological properties. Among million of EO constituents we could find the monoterpene α -phellandrene (α -PHE), presents in several species in any varied concentrations. In previous study we have showed antinociceptive activity of α -PHE in chemical and mechanical nociception models. The aims of the present work were to investigate the possible mechanisms of action involved in the antinociceptive activity of α -PHE in the glutamate induced nociception. **Methods:** Mass spectrometry was used to evaluate the purity and exacta molecular mass of α -phellandrene. To evaluate the possible mammalian cells citotoxicity, the MTT (3-[4,5 dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) test was performed. Male Swiss mice (n=6-9, 20-30 g) were used in the glutamate test (Animal Ethics Committee/UFPI, N^o 090/2010). Mice were pre-treated with naloxone (2 mg/kg i.p.), glibenclamide (3 mg/kg i.p.), L-arginine (600 mg/kg i.p.), atropine (0.1 mg/ kg s.c.) and yohimbine (0.15 mg/kg i.p.), 30 min before α -PHE (12.5 mg/kg) and then received intraplantar glutamate (10 μ mol). Control animals received vehicle (C- 2 % Tween 80 in saline) or MK 801 (0.03mg/kg ip). Nociception was evaluated by quantifying paw licking time during 15 min. **Results.** α -PHE purity was 98.2% and molecular mass 136.1 Da. α -PHE did not show cytotoxicity. Naloxone (NAL, opioid antagonist)(C= 101.78 \pm 7.07, α -PHE= 63.62 \pm 6.11, α -PHE+NAL= 125.40 \pm 8.03*) and glibenclamide (GLIB, blocker of K⁺_{ATP} channels) (α -PHE+GLIB= 100.92 \pm 9.82*) significantly reversed the antinociceptive effect of α -PHE. L-arginine (L-Arg, substrate of NO formation)(C= 102.15 \pm 7.39, α -PHE+L-Arg= 93.42 \pm 9.20*), atropine (ATR, muscarinic antagonist) and yohimbine (Y, adrenergic antagonist)(C= 107.20 \pm 8.24, α -PHE+ATR= 91.33 \pm 7.94*, α -PHE+Y= 102.12 \pm 11.82*) also reversed the antinociceptive effect of α -PHE (*p<0.05). **Discussion:** These results are indicating the possible participation of opioid system and K⁺_{ATP} channels in the antinociceptive effect of α -PHE suggesting the possible involvement of nitric oxide, muscarinic cholinergic and adrenergic systems, respectively. **Financial support:** Federal University of Piauí, CNPq, CAPES – Brazil.

05.060 Anti-inflammatory and analgesic effects of hydrogen sulfide donors are not mediated by ATP-sensitive K⁺ (K_{ATP}) channels. Ekundi-Valentim E¹, Mesquita FPN¹, Rodrigues L¹, Santos KT¹, Moreira D¹, Teixeira SA¹, Belizário JE¹, Munhoz CD¹, Wallace JL², Muscará MN¹, Costa SK¹ ¹ICB-USP – Farmacologia, ²McMaster University

Introduction: Hydrogen sulfide (H₂S), previously known as just an environmental pollutant, has an important role in inflammation and pain. H₂S delivered to the rat knee joint results in inhibition of CGN-induced synovitis, thus supporting the use of H₂S donors as potential therapeutic alternatives for arthritis (Br J Pharmacol 2010; 159: 1463). In this study, we characterized some of the mediators affected by exogenous and endogenous H₂S and the mechanism of action of H₂S donors verifying the involvement of K⁺ channels sensitive to ATP in the control of inflammatory and analgesic response in the CGN-induced synovitis. **Methods:** Under approval of the Institute of Biomedical Sciences Ethics Committee of University of São Paulo (no. 171, book 2/2011), male Wistar rats were anesthetized (5% halothane in O₂) and pre-treated with either the CSE inhibitor DL-propargylglycine (PGly, 53 µmol/joint), Glibenclamide an ATP-sensitive K⁺ (K_{ATP}) channel inhibitor (40 mg/k, i.p), or the H₂S donor Lawesson's reagent (LR, 3.6 µmol/joint) before the intra-articular (i.art.) injection of 7.5 mg of CGN. After 4 h, joint swelling and tactile allodynia (Von Frey filaments) were assessed and the number of bone marrow leukocytes was determined. The synovia were collected for analysis of interleukin (IL)-1beta, IL-10, caspase-1, nuclear factor (NF)-kappaB and activator protein (AP)-1. H₂S synthesis by synovia homogenates in vitro was studied, and total sulfide was quantified by the methylene blue method. Neutrophil recruitment (as myeloperoxidase – MPO activity) was also determined. **Results:** CGN induced edema, pain, tactile allodynia, synovitis and increased the number of bone marrow neutrophils, leukocyte infiltration into the joint cavity, synovial IL-1beta concentration, caspase-1 activity, and both NF-kappaB and AP-1 activation. LR treatment led to increased IL-10 (~105%; p<0.05), lowered cell infiltration (~64%; p<0.05) and caspase-1 activity (~38%; p<0.05), and unaffected NF-kappaB activation. PGly treatment only potentiated AP-1 activation (~64%; p<0.05). Glibenclamide alone induced edema and tactile allodynia as much as CGN but, when associated with LR decreased edema (~48%; p<0.05), tactile allodynia (~58%; p<0.05) and neutrophil recruitment (~35%; p<0.05). H₂S synthesis by synovial membrane homogenates was due to both enzymes, as CSE and CBS inhibitors reduced H₂S generation by 50% and 75%, respectively. **Discussion:** It has shown that IL-10 increase and/or caspase-1 decrease may account for the protective effects of LR-derived H₂S on CGN-induced synovitis. In addition, endogenous CSE-derived H₂S limits AP-1 activation, thus showing that, as a whole, H₂S exerts its anti-inflammatory activity through different checkpoints in the CGN-induced synovitis. LR acted on pain and inflammation even in the presence of glibenclamide, demonstrating that its action mechanism is not dependent of K⁺ channels. **Acknowledgements:** Fapesp, Capes, CNPq and University Agostino Neto for **Financial support**.

05.061 Nonpeptidergic C fibers mediate inflammatory hypernociception in mice.
Pinto LG, Souza GR, Lopes AHP, Talbot J, Santos MD, Cunha FQ, Cunha TM, Ferreira SH – FMRP-USP – Farmacologia

Introduction: Sensory information is transmitted from the periphery to the spinal cord by distinct subsets of primary afferent neurons, including small diameter unmyelinated C-fibers, which plays an important role in detecting noxious stimuli. C-fiber nociceptors have been divided into two classes, the peptidergic and nonpeptidergic, based on neuroanatomical and neurochemical criteria. While many of the differences between peptidergic and nonpeptidergic neurons are now appreciated, a possible functional difference between these two classes of C fibers in the genesis of acute nociception as well as inflammatory pain is still unclear. Thus, this study aims to elucidate the role of nonpeptidergic fibers in acute nociception induced by mechanical, thermal and chemical stimuli as well as in inflammatory hypernociception.

Methods: In order to elucidate differences between these two classes of C fibers, a neurotoxin was used to selectively eliminate the nonpeptidergic C fibers: a saporin conjugated to isolectin B4 (IB4). Nociceptive threshold was evaluated through thermal (Hargreaves) and mechanical (filaments and electronic von Frey) tests in C57BL/6 mice. Nociception models were induced by intraplantar (i.pl.) injection of capsaicin and formalin (acute nociception) or by i.pl. administration of prostaglandin E₂, epinephrine and carrageenan (inflammatory hypernociception). P2X₃ expression was analyzed by Western blot of dorsal root ganglion (DRG) and spinal cord. The expression of IB4-labeled in DRG was analyzed by flow cytometry and in spinal cord was determined by immunofluorescence using confocal microscopy (SP5-Laica). This study was approved by Animal Ethics Committee of FMRP/USP (n° 101/2010). **Results:** Firstly, it was observed that the intrathecal administration of IB4-saporin did not change baseline thermal and mechanical nociceptive threshold of the mice paw when compared to saline and saporin-control groups. The intrathecal administration of IB4-saporin reduced mechanical inflammatory hypernociception induced by carrageenan, epinephrine and prostaglandin E₂ in mice. Similarly, the treatment with IB4-saporin inhibited the nociception caused by intraplantar injection of the capsaicin. By contrast, the acute nociception induced by formalin did not change by administration of IB4-saporin. In addition, the expression of ATP receptor P2X₃ in DRG and spinal cord was reduced after treatment with IB4-saporin. Consistent with these findings, we found that IB4-saporin injection decreased the expression of IB4-labeled in spinal cord and DRG neurons. **Conclusion:** These data demonstrated that absence of nonpeptidergic C fibers does not affect basal nociceptive threshold. However, these fibers are essential for the development of nociception in the paw of mice induced by inflammatory stimuli like prostaglandin E₂, epinephrine, carrageenan and capsaicin. **Acknowledgments:** We thank the excellent technical assistance of Ieda Regina dos Santos Schivo, Sérgio Roberto Rosa and Eleni Luiza Tamburus Gomes. **Financial support:** FAPESP and CNPq (Brazil).

05.062 Evaluation of the antihyperalgesic potential of bioactive chalcones on several models of long-lasting pain in mice. Rocha LW¹, Klein JB², Sonza DR², Campos-Buzzi F³, Silva KABS⁴, Quintão NLM³ ¹Unicamp – Fisiologia, ²UNIVALI – Farmacologia, ³UNIVALI, ⁴FURB

Introduction: Chalcones are molecules that have been widely studied by the scientific community, which has already proven its pharmacological activities; such as anti-inflammatory, antitumor, antibacterial, antioxidant activities, among others. The purpose of this study was to evaluate the potential anti-hyperalgesic activity of two chalcones, (2E)-1-(4-aminophenyl)-3-(4-nitrophenyl)prop-2-en-1-one (ANCh) and N-{4-[(2E)-3-(4-nitrophenyl)prop-2-en-1-yl]phenyl}acetamide (AcANCh), using different models of persistent pain in mice. **Methods:** C57BL/6 mice male and female (20-30g, n=6) mice were used. To verify the anti-hyperalgesic activity of the compounds, the following models were used: carrageenan (300 µg/paw), CFA (20 µL/paw), LPS (100 ng/paw), BK (500 ng/paw), PGE₂ (3 nmol/paw), epinephrine (100 ng/paw), TNF-α (40 pg/paw), IL-1β (20 pg/paw), KC (20 pg/paw), partial sciatic nerve ligation (PSNL) and pain induced by tumor cells (B16F10, 2x10⁵ cells). The mechanical hyperalgesia was assessed using Von Frey hairs (0.6g). We evaluated the cytokine IL-1β levels and assessment of neutrophil migration by quantification of the myeloperoxidase enzyme activity. This study was approved by the Ethical Committee for Animal Care of UNIVALI (Process No. 017P1/2010). **Results:** ANCh reduced the mechanical hyperalgesia induced by carrageenan, with inhibition of 40 ± 4%, 43 ± 3% and 36 ± 4% for doses of 1.11, 3.72, 11.18 µmol/kg, respectively. Similar results were found for AcANCh (35 ± 8%, 36 ± 7 and 45 ± 9%) at doses of 0.96, 3.22 and 9.66 µmol/kg, respectively. In hyperalgesia induced by CFA (20 µL/paw), ANCh and AcANCh were also significantly active when administered previously (18 ± 5% and 19 ± 7%, respectively at higher doses) or after (44 ± 9%, 43 ± 2%, 41 ± 1% for ANCh and 13 ± 5%, 24 ± 6% and 36 ± 6% for AcANCh) CFA i.pl. injection. Expressive results were found using the model of neuropathic pain induced by PSNL, presenting inhibitions of 31 ± 7%, 43 ± 7% and 66 ± 8% for ANCh and 25 ± 6%, 39 ± 5% and 60 ± 4% for AcANCh, with the doses mentioned above. In the cancer-induced pain model, the compounds were also effective in inhibiting mechanical hyperalgesia, with inhibitions of 13 ± 8%, 18 ± 5% and 52 ± 6% for ANCh and 8 ± 3%, 25 ± 7% and 28 ± 8% for AcANCh. ANCh and AcANCh were also effective in reducing the frequency of paw withdrawal response in models of pain induced by IL-1β (42 ± 5% and 42 ± 7%), TNF-α (43 ± 11% and 39 ± 11%) and epinephrine (46 ± 8% and 40 ± 7%), at the highest used dose. Although, none of the compounds interfered with hyperalgesia induced by LPS, BK and KC. Both compounds were effective to inhibit the production of IL-1β, (49 ± 4% and 64 ± 2%, respectively). However, only ANCh was effective in inhibiting neutrophil migration, with an inhibition of 67 ± 4% and hyperalgesia induced by PGE₂ (59 ± 4%) at the dose of 11.18 µmol/kg. **Discussion:** In conclusion, the compounds ANCh and AcANCh seems to be promising for the development of novel analgesics for the treatment of long lasting pain. Furthermore, it is important to emphasize the oral bioavailability, which makes them very attractive for the pharmaceutical industry. **Financial support:** CNPq, FAPESC, ProPPEC UNIVALI.

05.063 The blockade of spinal cord receptor Y1 reversed the hyponociception caused by H1 Agonist but not trypsin in the rat knee-joint. Souza-Silva E, Stein TS, Tonussi CR UFSC – Farmacologia

Introduction: Histamine and trypsin are considered as one of the main mediators of pruritus. However the role of these mediators in deep tissue, like articular, remains uncertain. Previous results showed that peripheral antihistamines H1 receptor increased the nociception in the formalin test. The aim of this study was to investigate the role of histamine and trypsin in joint tissue. **Methods:** Articular incapacitation was measured by counting the paw elevation time (PET; s) during 1 min period of forced walk either each 5 min throughout 60 min after formalin knee-joint injection. Formalin 1.5% induced two phases (P1: 0-5 min, and P2: 10- 60 min) of nocifensive behavior. 2-Pyridyletylamine (0.05; 0.5; 5; 500 nmol/knee) was co-injected with formalin. Trypsin (0.001; 0.1 and 10 ng/knee) or antagonist Y1 receptor BIBO 3304 (0.4; 4 and 13 µmol/i.t) were administered 5 and 20 min before formalin, respectively. **Results:** 2-PEA (0.005; 0.5 and 5 nmol/knee P<0.01; P<0.5 and P<0.001) and trypsin (0.1 and 10 ng/knee P<0.05 and P<0.01) was hyponociceptive in 2° phase (5 – 35 min). Unlike, BIBO 3304 (4 µmol/i.t P<0.01) was hypernociceptive in 2° phase (5 – 35 min) and in low dose (0.4 µmol/i.t) none effect was observed in formalin test. BIBO 3304 (0.4 µmol/i.t) reversed the hyponociceptive effect of 2-PEA (5 nmol/knee), but has no changed the hyponociceptive effect of trypsin (10 ng/knee). **Discussion:** These results suggest that histamine and trypsin are hyponociceptive in articular tissue. Furthermore, the Y1 receptor in the spinal cord is only involved with the hyponociceptive effect of intraarticular H1 receptor activation. However, more experiments are needed to elucidate mechanisms by which trypsin promotes hyponociception. This work was approved by the local ethical committee for animal use (CEUA 23080.034306/2009-69). **Financial support:** Capes / CNPQ

05.064 Involvement of transient receptor potential ankirin 1 (TRPA1) in the persistent scratching behavior induced by diphenilciclopropenone (DCP) in mice. Segat GC, Costa R, Manjavachi MN, Calixto JB UFSC – Farmacologia

Introduction: TRPA1 is a cation channel expressed mainly in primary sensory neurons and mediates somatosensation, including nociception. Also, TRPA1 seems to be involved in histamine-independent itching (Wilson *et al.*, Nat. Neurosci. 14: 595, 2011), a common and intractable symptom of skin diseases such as atopic dermatitis and dry skin. Thus, in the present study we sought to investigate the possible role of this cation channel in the chronic scratching behavior associated to the mouse dermatitis induced by diphenilciclopropenone (DCP). **Methods:** Adult female CD-1 mice (~25 g, n= 6-8 per group) had the skin shaved at the back of the neck and received 1% DCP. After 1 week, the animals started receiving a daily topic application of 0.5% DCP at the shaved skin up to 14 days. Control group received only the vehicle (200 μ L of acetone). The scratching behavior of the animals was evaluated every 2-3 days among 1 and 14 days after the first DCP application. The animals were observed during 30 minutes per day, and one scratching behavior was counted when the mouse lifted its hind paw to scratch the inflamed region (Protocol number PP00497). Two independent groups were treated with the selective TRPA1 antagonist HC-030031 (30 mg/kg, i.p.) or its vehicle (9% DMSO + 1% Tween 80 in saline). The treatments started after 3 days of the first DCP application and were given every 12 hours up to 14 days. On day 15, the animals were sacrificed and the skin from the back was collected for histological analysis (H & E staining) and myeloperoxidase (MPO) activity assay. Also, the dorsal root ganglions (DRGs; C₂-T₃) were collected to quantify TRPA1 mRNA by Real-time PCR. **Results:** The daily topic application of DCP elicited a marked scratching behavior in mice when compared to control group ($p < 0.05$), reaching its maximum response after 3 days. Histological analysis of skin sections of DCP-treated mice showed thickening of dermis and epidermis, and polymorphonuclear cells migration. The later event was confirmed by MPO activity assay, an indirect marker of polymorphonuclear cells migration ($p < 0.01$). Importantly, DCP-induced scratching behavior was significantly reduced by the treatment with HC-030031 ($54 \pm 8\%$ of reduction; $p < 0.01$). Nevertheless, the same treatment did not interfere with the skin inflammation induced by DCP, as assessed by MPO activity assay. Also, we did not detect any significant changes in the mouse DRGs level of TRPA1 mRNA when comparing DCP-treated and control animals. Curiously, the group treated with HC-030031 showed an unexpected increase in TRPA1 mRNA level in relation to the vehicle-treated group ($75 \pm 26\%$, $p < 0.05$). **Discussion:** Collectively, the results presented here suggest that TRPA1 exert an important role in the chronic scratching behavior induced by DCP, despite its ineffectiveness on skin inflammation. Therefore, selective TRPA1 antagonists might become potential drugs to treat chronic itching conditions insensitive to current therapy with anti-histamines. Experimental studies are in progress to better clarify the TRPA1 involvement in chronic itching signaling. **Financial support:** CNPq, CAPES, FAPESC. **Key-words:** chronic itching, dermatitis, TRPA1.

05.065 NOD1 and NOD2 contribute to pain hypersensitivity after induction of peripheral neuropathy. Santa-Cecília FV, Ferreira DW, Ferreira SH, Zamboni DS, Cunha TM¹ FMRP-USP – Farmacologia

Introduction: Among pattern recognition receptors (PRRs), the Toll-like receptors (TLRs) and NOD-like receptors (NLRs) are responsible and the most important in recognizing the pathogen-associated molecular patterns (PAMPs). Upon recognition of PAMPs, NLRs, such as NOD1 and NOD2, recruit directly RIPK2 (receptor-interacting serine/threonine-protein kinase 2), an adaptor protein important in NLRs-mediated NFκB activation. A previous work has indicated that TLRs play a crucial role in the activation of spinal cord glial cells in the induction and maintenance of chronic inflammatory and neuropathic pain. Furthermore, in models of inflammation/infection of the central nervous system, the receptors NLRs and TLRs cooperate in the activation of glial cells, which leads us to hypothesize that also in chronic pain models, activation of microglia depends on NLRs. In the present study, we aimed to evaluate the role of receptors NOD1 and NOD2 in the genesis of neuropathic chronic pain, focusing on their signaling pathways (RIPK2). **Methods:** The experiments were carried out on male C57BL/6 mice or NOD1, NOD2 and RIPK2 knockout (KO) mice. All the experimental procedures were conducted according with the guidelines of the Ethics Committee of the FMRP-USP (106/2011) and IASP guidelines. Mice were anesthetized with isoflurane and induced to the Spared Nerve Injury neuropathy model. The common peroneal and the tibial nerves were tight-ligated and sectionated distal to the ligation, removing 2-4mm of the distal nerve stump. The sural nerve was kept intact. The wound was closed in two layers with surgical skin staples. Sham controls involved exposure of the sciatic nerve and its branches without any lesion. After induction of peripheral neuropathy, the mechanical allodynia was assessed for a period of 21 days, by measuring foot withdrawal thresholds in response to mechanical stimuli to the right hind paw using a set of von Frey filaments. **Results:** We tested the susceptibility of the KO mice to nerve injury-induced pain hypersensitivity. The wild-type (WT) mice showed increased sensitivity to mechanical stimuli. The paw withdrawal threshold to the mechanical stimuli decreased at the 3rd day after nerve injury, and reached the lowest threshold on day 14 (0.05 ± 0.01 g), compared with the original threshold before transaction (1.00 ± 0.00 g). The paw withdrawal threshold of the NOD1 KO mice, however, reduced only to 0.53 ± 0.04 g, NOD2 KO to 0.60 ± 0.09 g and RIPK2 KO to 0.49 ± 0.12 g compared with their respective threshold before the surgery (1.00 ± 0.00 g, 1.07 ± 0.07 g, 0.87 ± 0.08 g respectively). These KO mice displayed significantly attenuated mechanical allodynia compared with their respective WT controls for the days 5-21 after the surgery. Pain hypersensitivity was not observed in the sham-operated WT or in contralateral paws. **Discussion:** These data suggest that NOD1 and NOD2 are required for the maximum induction of mechanical allodynia upon nerve injury. These receptors recruit RIPK2 that result in activation of NFκB, leading to transcription of genes for a variety of inflammatory and immunological mediators, contributing to pain hypersensitivity. These mechanisms could be a target for the development of drugs or therapeutic treatment for neuropathic pain syndromes. **SUPPORT:** FAPESP, CNPq, FINEP.

05.066 Antinociceptive mechanism of action of N-acylhydrazone derivative LASSBio-1476. Silva RV¹, Lima CKF^{1,2}, Nogueira MCO¹, Barreiro EJ¹, Miranda ALP¹
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Introduction: The N-acylhydrazone pharmacophore group is present in several bioactive compounds, especially LASSBio 294, a cardiotonic compound with anti-inflammatory and analgesic activity (Barreiro, *Quim. Nova* 25, 1172, 2002). Based on this prototype a new series of N-acylhydrazone derivatives was designed, synthesized and the antinociceptive effect evaluated highlighting the compound LASSBio-1476 (Silva, FESBE, 2011). LASSBio-1476 presented a antinociceptive ED₅₀ of 17.5 µmol/kg in mice writhing test. The study aimed to evaluate the antinociceptive profile and mechanism of action of the N-acylhydrazone derivative LASSBio-1476 (100 µmol/kg, *p.o.*) in different models of nociception. **Methods:** The formalin (2.5%, 20 µl/paw) induced hypernociception test in mice was performed by counting the paw licking and biting time 1 hour after oral administration of the substance or vehicle. Counting began immediately after injection for two periods: 0-5 min (neurogenic phase) and 15-30 min (inflammatory phase) (n = 6-10, *p<0.05, **p<0.01; ***p<0.001, Student *t* test, one-way ANOVA). The involvement of the CB1 cannabinoid receptor was assessed in the formalin test using the CB1 antagonist, AM281 (0.5 mg/kg; *ip*). Central nociceptive activity was investigated using the hot plate apparatus maintained at 55 ± 1 °C. Mice were placed on the heated surface at 0, 30, 60, 90, and 120 min after oral administration of vehicle or test compounds and the time between placement and the first sign of paw licking or jumping was recorded as latency (Brain. Res. 273; 245, 1983). We also investigated the involvement of TRPV-1 receptors by the capsaicin-induced nociception test (1.6 mg / paw) and employing the TRPV1 antagonist capsazepine (10 or 5 µg/paw). The time that mice spent licking paw was recorded 1h after oral administration or 15 min after intra-plantar injection of LASSBio-1476. Animal protocols were approved by UFRJ ethical animal care committee (Farmacia02).

Results and Discussion: LASSBio-1476 significantly inhibited both phases of formalin test, neurogenic and inflammatory, by 36% and 58%, respectively. The administration of the CB1 antagonist, AM281, reversed the inhibition produced by LASSBio-1476, indicating a possible involvement of the cannabinoid system in the mechanism of action. Oral treatment with LASSBio-1476 significantly reduced nociception in capsaicin model, similar to TRPV-1 antagonist, capsazepine, which was not observed when LASSBio-1476 is locally administered. LASSBio-1476 did not present a central effect in hot plate test, unlike the opioid agonist morphine. LASSBio-1476 has an important analgesic potential, interesting for the treatment of pain, with a possible action by modulating cannabinoid system. The perspectives of this work is to characterize the action of LASSBio-1476 on the cannabinoid system and investigate its anti-inflammatory effect *in vitro* and *in vivo*. **Financial support:** CNPq, FAPERJ, PIBIC-UFRJ, INCT-Inofar, PRONEX.