Session 05 − **Pain and Nociception**

05.001

The involvement of TRPA1 receptors in the induction and maintenance of prostaglandin-induced hyperalgesia. Bonet IJM[1], DallAcqua M[2], Zampronio AR[3], Tambeli CH[1], Parada CA[4], Fischer L[2] [1]FOP-UNICAMP − Ciências Fisiológicas, [2]UFPR − Fisiologia, [3]UFPR − Farmacologia, [4]UNICAMP − Farmacologia

Introduction: The ionotropic receptor TRPA1 belongs to the superfamily of TRP channels, is expressed mainly in nociceptive C fibers and has received increased attention due to its central role in inflammatory nociceptive mechanisms. In this study, we asked if TRPA1 mediates the induction and/or maintenance of mechanical hyperalgesia induced by an intraplantar injection of prostaglandin in rats. **Methods:** Male wistar rats (200-280g) were used. Prostaglandin (100ng) or its vehicle (0.9%NaCl) was injected in the plantar surface of the rat's hindpaw. The TRPA1 receptor antagonist (HC030031, 300 and 1200 µg) or its vehicle (DMSO) was co-administered with prostaglandin (co-treatment) to verify its role in the induction of hyperalgesia or was injected 2:55 minutes after prostaglandin (post-treatment) to verify its role in the maintenance of installed hyperalgesia. ODN antisense for TRPA1 receptors or mismatch was administered in the subarachnoid lumbar space during four days. In the fourth day, prostaglandin or its vehicle was injected in the paw. The mechanical threshold was measured using an electronic von Frey algometer, 3, 6 and 24 hours after prostaglandin injection. A two-way repeated-measure ANOVA with one between-subjects factor (i.e. treatment) and one within-subjects factor (i.e. time) was used to determine if there were significant ($p \leq 0.05$) differences in the hyperalgesic response among the groups. Data are present as mean + E.P.M. **Results:** The pharmacological blockade of TRPA1 receptor by co (15.8 ± 1.87) and post-treatment (28.3 ± 0.41) with the selective TRPA1 receptor antagonist HC030031 significantly decreased hyperalgesia 3 hours after prostaglandin injection (35.5 ± 1.26). ODN antisense for TRPA1 receptors blocked prostaglandin-induced hyperalgesia 3 (24.8 ± 1.19 vs 4.79 ± 0.32), 6 (17.7 ± 1.03 vs. 1.48 ± 0.51) and 24 (2.4 ± 0.41vs. 0.72 ± 0.28) hours after prostaglandin injection. **Conclusion:** These findings indicate that TRPA1 receptor contributes to the induction and maintenance of the hyperalgesia induced by prostaglandin and suggest that future therapeutic strategies based onTRPA1 blockade may be useful in the treatment of inflammatory pain.

05.002

Evaluation of the involvement of kinin receptors in the nociceptive behavior of mice submitted to the brachial plexus avulsion. Jorge IP[1], Quintão NLM[2] [1]CCS-UNIVALI, [2]UNIVALI − Ciências Farmacêuticas

Introduction: The mechanisms responsible for neuropathic pain are not fully understood. The brachial plexus avulsion (BPA) has been described for mice as a model of long-lasting neuropathic pain (Quintão, *Neuropharmacol*, 50, 614, 2006). During the first week of peripheral nerve injury, dynamic reorganization within the damaged sensory neurons causes a pronounced increase in the retrograde axonal transport of small proteins, such as neurotrophins and cytokines. This study has the aim of evaluating the involvement of kinin receptor (B1 and B2 receptors) in the nociceptive response induced by bradykinin (BK) or formalin in mice submitted to BPA. **Methods:** Male Swiss mice were used in this study (25-35g, N=6-8). Mice were firstly anesthetized with chloral hydrate (7 %; 8 mg/kg; i.p.) and were submitted to the BPA as describe by Quintão et al. (2006). Right brachial plexus was approached and the lower trunk was extorted by traction. A sham-operated group was used as negative control. In the 6th day after the surgery, the animals were submitted to the nociception models induced by BK (0.1; 1; 10 nmoL/paw), DABK (1, 3 or 10 nmoL/paw) or formalin (2.5%). In other set of experiment, the animals were pre-treated with HOE-140 (50 nmoL/kg, s.c.), DALBK (150 nmoL/kg, s.c.) or saline (10 mL/kg) 30 min before the BK (10 nmoL/paw) or formalin (2.5%) injection. The time spent licking the injected paw was timed in all set of experiments and considered as indicative of pain. All the procedures used in the present study were approved by the Animal Ethics Committee of UNIVALI (Protocol numbers 363/2007). **Results:** Operated mice that received i.pl. injection of BK (10 nmoL/paw), into the left or right hindpaw, had the nociceptive response reduced in 75 % when compared with the sham operated group. However, when the mice submitted to the BPA and received i.pl. injection of BK 0.1 or 1 nmoL/paw into the right hindpaw, it was observed an increase of the nociceptive response (204% and 236%, respectively). When BK was injected into the left hindpaw, contralateral side of the BPA, it was observed reduction of the nociception (39.4%) only with the dose of 10 nmoL/paw. The pre-treated with HOE-140 (B2R antagonist) was capable of abolishing the nociceptive response induced by BK (10 nmoL/paw). However, when HOE-140 or DALBK were administered before the formalin injection into the operated mice hindpaw, it was observed an increase of the nociception in the 2nd phase of the test (139% and 170%, respectively) when compared with operated mice pre-treated with saline. Otherwise, the i.pl. injection of DABK into the right or left hindpaw was not able to produce nociceptive response in mice submitted to de BPA. **Discussion:** This data demonstrates that mice submitted to the BPA presented reduction of the nociceptive behavior induced by formalin or BK, probably due to the overexpression of B2Rs in the peripheral endings of the ipsilateral and contralateral hindpaw of the BPA, resulting in a freezing state. This conclusion was supported by the increase of the nociceptive response obtained with lower doses of BK and formalin, and the ability of B2 antagonist in reversing this state. It is also believed that the results obtained with B1 antagonist is a consequence of its action on spinal cord receptors, since no nociceptive response was observed when the B1 agonist was injected into the operated mice hindpaw. **Financial Support:** CNPq; FAPESC-SC; ProPPEC/ ProBIC/UNIVALI

05.003

Mechanisms underlying the scratching behavior induced by the activation of proteinase activated receptor-4 (PAR-4) in mice. Patricio ES, Costa R, Figueiredo CP, Motta EM, Calixto JB UFSC − Farmacologia

Introduction: Pruritus is a common symptom present in several cutaneous and systemic diseases. Recently, it was demonstrated the ability of PAR-4 agonist to induce scratching behavior in mice, suggesting the involvement of PAR-4 in itch (Reddy, J Neurosci, 28: 4331, 2008; Tsujii, J Pharmacol Sci., 108: 385, 2008). The present work aims to investigate the cellular and pharmacological mechanisms underlying the pruriceptive actions of the selective PAR-4 agonist AYPGKF-NH2 (AYP) in mice. **Methods:** Female adult CD-1 mice (25-30 g, n=6) received a dorsal intradermal (i.d.) injection of saline (50 µl) containing the selective PAR-4 activating peptide AYP (30-500 nmol/site) or the inactive control peptide YAPGKF-NH2 (YAP; 200 nmol/site). After the treatments, the frequency of scratching bouts with the hind-paws toward the injected site was quantified during 30 min (Ethics Committee Protocol Number: PP00032). **Results:** The i.d. administration of AYP (30-500 nmol/site) elicited a marked and dose-related scratching behavior response. The effective dose ranged from 200 to 500 nmol/site ($p<0.05$). The inactive sequence YAP (200 nmol/site) did not cause any significant alteration. The pre-treatment with the selective PAR-4 receptor antagonist Pepeducin P4pal-10 (5 mg/Kg, i.p.) or the non-selective µ-opioid receptor antagonist naloxone (1 mg/kg, i.p.) significantly reduced AYP (200 nmol/site)-evoked scratching behavior [66% ($p<0.05$) and 57% ($p<0.05$), respectively]. Histological analysis, assessed by Casson´s staining protocol, demonstrated that the scratching behavior was not associated with mouse skin inflammation. Interestingly, we have found, by immunohistochemical detection, that PAR-4 is expressed in dorsal root ganglion neurons at the cervical level (C2-C6). Likewise, the expression of PAR-4 as well as its colocalization with tryptase, a marker for mast cells, was detected in the dermis of mouse skin. In fact, the i.d. pre-treatment with the mast cell degranulator C48/80 (1-10 µg/site, 4 days, once a day), used to promote mast cell depletion, prevented AYP (200 nmol/site)-induced scratching [68% ($p<0.05$)]. In line with these results, histological analysis of mouse skin sections, stained with toluidine blue dye, revealed an increase in the mast cells degranulation after AYP (200 nmol/site) injection when compared to YAP (200 nmol/site; 1.2-fold)- or saline (1.5-fold)-injected groups ($p<0.05$). The pre-treatments with the selective H1 receptor antagonist pyrilamine (10 mg/Kg, s.c.), the non-selective protease-inhibitor gabexate mesylate (10 mg/Kg, s.c.) or the non-selective serotonergic receptor antagonist methysergide (10 mg/Kg, p.o.) did not interfere with AYP (200 nmol/site)-induced scratching. Conversely, the treatment with the selective TRPV-1 receptor antagonist SB366791 (0.5 mg/Kg, i.p.) inhibited by 61% ($p<0.05$) the AYP (200 nmol/site)-induced response. **Discussion:** These findings provide evidence that PAR-4 activation, probably on mast cells and/or sensory neuron membranes, induces scratching behavior in mice. In spite of mast cell involvement, the itching elicited by PAR-4 activation seems to be independent of histamine, serotonin or mast cell protease release. Studies are in progress to better clarify the mechanisms involved in this process. **Supported by**: CAPES/CNPq/ FAPESC.

05.004

Inflammatory muscle hypernociception depends on activation of ERK and NF-kB signaling pathways. Lima FO[1], Verri Jr WA [2], Ribeiro dos Santos R[3], Soares MBP[3], Villarreal CF[4] [1]UEFS − Biotecnologia, [2]UEL − Ciências Patológicas, [3]CPqGM-FIOCRUZ-Bahia, [4]USP − Farmacologia

Introduction: Several studies have shown that the extracellular signal regulated kinase (ERK) is important to the inflammatory pain. ERK activity is known to induce activation of nuclear factor-κB (NF-κB), a key transcription factor acting in pain and inflammation. Muscle pain, a condition with high prevalence in clinical practice, is different from cutaneous pain in its clinical characteristics as well as in physiopathology process. Therefore, we conducted the present work to establish the role of NFκB and ERK in the early phase of inflammatory muscle hypernociception. **Methods**: Mechanical hypernociception was induced by the injection of 30 µL of complete freund adjuvant (CFA) into the gastrocnemius muscle of male Swiss mice (22-25g, n=5-6), and was measured by using von Frey filaments up-down method. NF-κB activation inhibitor (PDTC; 11-100 mg/kg), ERK activation inhibitor (PD98059; 0.3-3 mg/kg) or vehicle (control group) was administered by intraperitoneal route 30 minutes before the CFA injection. Institutional Animal Care and Use Committee FIOCRUZ 26/2009-1. **Results:** The intramuscular injection of CFA decreased the mechanical threshold of the injected paw during 4, 6, 24, 48 and 72 hours after the injection (67%, 62%, 79%, 70%, 66%, respectively). Pretreatment of mice with PDTC (100 mg/kg) significantly reduced the hypernociceptive response 4, 6, 24 and 48 hours after the CFA injection (96%, 78%, 83%, 82%, respectively). Additionally, intraperitoneal injection of PD98059 (3 mg/kg) significantly reduced CFA-induced mechanical hypernociception 4, 6 and 24 hours after the CFA injection (84%, 82%, 55%, respectively), when compared to the control group. **Conclusion**: The results of the present study demonstrate that the activation of ERK and NF-κB signaling pathways may contribute to the induction of inflammatory muscle pain. Therefore, the suppression of NF-κB and ERK could be a promising strategy for treatment of inflammatory muscle pain. **Financial Support:** This work was supported by CNPq, MCT, FAPESB and CAPES.

05.005

Contribution of vanilloid receptor to the nociception induced by peripheral injection of spermine in mice. Gewehr CCV[1], Silva, MA da[2], Trevisan, G[2], Rossato M[2], Drewes, CC[4], Guerra GP[2], Rubin MA[2], Ferreira J[2] [1]UFSM − Fisiologia e Farmacologia, [2]UFSM − Química, [3]USP − Toxicologia e Análises Toxicológicas

Introduction: Polyamines (spermine, spermidine and putrescine) are important endogenous regulators of ion channels. It has been demonstrated that polyamines can modulate the activity of vanilloid (TRPV1), glutamate (NMDA or AMPA/kainate) and acid sensitive ion channel (ASIC) receptors in vitro. In the present study, we have investigated the possible nociceptive effect induced by polyamines and the mechanisms involved in this nociceptive action in vivo. Material and Method: Male Swiss mice (30-35 g) were used. A volume of twenty µl of polyamines, diluted in phosphate-buffered saline (PBS), were injected subcutaneously (s.c.) under the surface of the right hind paw. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. This study were approved by the Committee on the Use and Care of Laboratory Animals of our university (no. 23081.012331/2009-81). **Results:** Subcutaneous (s.c.) administration under plantar surface of the right hind paw injection of capsaicin, spermine, spermidine or putrescine produced nociception with DE50 of 0.16 (0.07-0.39) nmol/paw, 0.4 (0.2-0.7) µmol/paw, 0.3 (0.1-0.9) µmol/paw and 3.2 (0.9-11.5) µmol/paw, respectively. The antagonists of NMDA (MK801, 1 nmol/paw) or AMPA/kainate receptors (DNQX, 1 nmol/paw) reduced the nociception caused by glutamate (10 µmol/paw), but not the nociception produced by spermine (1000 nmol/paw). Moreover, the s.c. injection of ASIC receptor blocker amiloride (100 nmol/paw) was not capable of reducing the spermine-trigged nociception. However, the TRPV1 antagonists, capsazepine or SB366791 (1 nmol/paw), inhibited spermine- or capsaicin-induced nociception, with inhibition of 81±10 and 68±9 or 81±6 and 72±8%, respectively. The pre-treatment with resiniferatoxin (RTX), a desensitization treatment, largely reduced the spermine-induced nociception. Consequently to TRPV1 desensitization treatment there was a marked decrease of TRPV1 receptor expression in sciatic nerve, with an inhibition of 67±11% compared to control group. Furthermore, the administration of low doses of resinferatoxin (0.005 fmol/paw) did not produce nociception when administered alone, but caused a pronounced effect when administered in association with a subdose of spermine (100 nmol/paw). In addition, different concentrations of spermine (3-300 µM) were capable of enhance the specific binding of [3H]resiniferatoxin to TRPV1 receptor. **Conclusion:** Taken together, exogenous polyamines produce spontaneous nociceptive effect through the stimulation of TRPV1, but not of ionotropic glutamate or ASIC receptors. Thus, polyamines could be important peripheral modulators of pain, especially during inflammatory processes when local polyamine levels are increased. **Acknowledgements**: This study was supported by Conselho Nacional de Desenvolvimento Científico (CNPq, Brazil), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes). J.F. and M.A.R. are recipients of Productivity Fellowships from CNPq.

05.006

Cnidaria venom as pharmacological tool for studying the signaling pathways of pain and its control. Ferreira-Junior WA[1], Zaharenko AJ[2], Fernandes ACO[1], Zambelli VO[1], Gutierrez VP[1], Konno K[3], Tytgat[4], Picolo G[1], Cury Y[1] [1]IBu − Dor e Sinalização, [2]IB-USP − Fisiologia, [3]Universidade de Toyama − Medicina Natural, 4Universidade Católica de Leuven − Toxicologia

Introduction: Animal toxins are directed against a wide variety of pharmacological targets, making them an invaluable source of ligands for studying the signaling pathways of pain and its control. Sea anemone (cnidaria) venoms contain many biologically active compounds such as cytolysins (18-20 kDa) and ion channel modulators (3–5 kDa). In addition, low molecular weight compounds have been isolated and identified in these venoms; however few studies have been carried out in order to determine the biological activity of such compounds. BDS 391 is a low molecular weight (~390 Da) and non-peptidic compound purified from the Brazilian sea anemone Bunodosoma cangicum venom. Studies on the structure of BDS 391 have demonstrated that this compound is composed of a bromoindole group connected to histidine. Our recent data have indicated that BDS 391 administered by intraplantar (i.pl.) route into the rat hind paw induces potent peripheral analgesia in models of acute and chronic pain. Initial results indicate that peripheral 5-HT receptors and KV channels mediate the analgesic action of this compound Objective − The aim of the present work is to further characterize the analgesic action of BDS 391 and its mechanisms, determining the type of 5-HT receptor involved in this effect, the presence of these receptors in the inflamed tissue and the ability of BDS 391to directly activate KV channels. **Methods:** – Male Wistar rats and Swiss mice were used. The effect of BDS 391 was evaluated in the rat paw pressure test, before and 3 h after injection of prostaglandin E2 (PGE2, 100 ng/paw) and against nociception induced by 1% formalin solution in mice. Spiroxatrine, Ketanserin or Ondansetron (6 mM/paw, antagonists of 5-HT1a, 5-HT2 and 5-HT3 receptors, respectively), were used to characterize the type of serotonin receptors involved the analgesic effect. Expression of serotonin receptors in the paw tissue was evaluated by immunoblotting and immunohistochemistry assays. In voltage clamp studies, BDS 391, was screened in 9 cloned voltage gated potassium channels. Results − BDS 391 (0.15 − 1.5 μM) inhibited PGE2-induced hyperalgesia and nociceptive response induced by formalin. The Ondansetron but not Spiroxatrine and Ketanserin was able to totally reverse the antinociceptive effect induced by BDS 391. These pharmacological data indicated that peripheral 5-HT3, but not 5-HT1a and 5-HT2 receptors, mediate the action of BDS 391.The immunoblotting and immunohistochemistry data showed that 5-HT receptors are expressed in nerve paw and that PGE2-induced hyperalgesia increases (15 – 20%) the expression of these receptors. BDS 391 did not modify the peak or shape of ionic potassium current. Conclusion − These data indicate that peripheral 5-HT3 receptors are involved in the analgesic effect of BDS 391 and demonstrate for the first time, that inflammation induces up-regulation of 5-HT receptors. The opening of Kv channels induced by BDS 391does not result from a direct action of the compound, but could be due to activation of 5-HT3 receptors (a channel activated by ligand). These results also contribute to the better characterization of the role of 5-HT3 receptors in pain control. Ethics Committees: Protocol no115 CEEA and no 494/08 CEUAIB Keywords: Sea anemone, Analgesia, Serotonin receptors Acknowledgements: FAPESP (2008/01988-1; 2009/08089-5) and CNPq

05.007

Reduced hyperalgesia and allodynia in neuropathic pain models by intraperitoneal and oral administration of new pirazol pirrol piridine derivative. Mendes TCF[1], Nascimento-Jr NM[2], Antunes F[3], Barreiro EJ[4], Fraga CAM[4], Sudo RT[1], Zapata-Sudo G[4] [1]UFRJ − Farmacologia e Química Medicinal, [2]IQ-UFRJ − Química, [3]CCTA-UENF, [4]UFRJ

Introduction: New pyrazolo[3,4-b]pyrrolo[3,4-d]pyridine derivatives structurally designed by using zolpidem as lead compound and previously designed for the treatment of anxiety were tested, in order to identify novel therapeutics for neuropathic pain. Among the derivatives, LASSBio-981 was the most potent to produce sedative, hypnotic, analgesic and antinociceptive effects mediated by activation of muscarinic system. Based on these, LASSBio-981 was administered in rats submitted to surgery to induce peripheral neuropathy. **Methods:** Neuropathic pain was induced in male Wistar rats (200 – 250 g), anesthetized with the association of ketamine (100 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) which were submitted to a chronic constriction injury (CCI) or spinal nerve ligation (SNL). Four hours after surgery, animals were randomly divided in two groups: 1. intraperitoneal administration of LASSBio-981 (6 mg/kg); 2. oral administration of LASSBio-981 (30 mg/kg) for seven consecutive days. All rats were tested for the presence of heat thermal sensitivity (SNL and CCI) using the plantar analgesia meter and the SNL group was also tested for the presence of mechanical allodynia using digital analgesia meter (Von Frey). Nociceptive threshold was determined before and three and seven days after nerve ligation. Withdrawal of the hind limb was considered a positive response to the mechanical allodynia (in grams) and heat hyperalgesia (in seconds). Data were expressed as mean ± S.E.M of 5 animals. This study was performed in compliance with the Animal Care and Use Committee at Universidade Federal do Rio de Janeiro (UFRJ) under the protocol number DFBCICB017. **Results:** In peripheral neuropathy protocol, injury produced allodynia because it significantly decreased the withdrawal threshold of hind paw from 30.7 ± 1.8 to 18.3 ± 2.3 g seven days after SNL. Heat hyperalgesia was determined by significant decrease in withdrawal threshold of hind paw from 8.8 ± 0.6 (control) to 3.8 ± 0.4 s and 8.7 ± 0.3 (control) to 3.9 ± 0.4 s, seven days after ICC and SNL, respectively. Intraperitoneous (6 mg/kg) or oral (30 mg/kg) administration of LASSBio-981 prevented the development of heat hyperalgesia from 9.1 ± 0.9 to 8.9 ± 1.3 s and 9.3 ± 1.0 to 7.6 ± 0.7 s, respectively in CCI. In SNL group, LASSBio-981 (6 mg/kg) inhibited the development of paw heat hyperalgesia (from 10.1 ± 1.1 to 8.8 ± 0.7 s) and mechanical allodynia (from 35.0 ± 2.5 to 33.3 ± 2.7 g). When orally administered, the derivative also reduced heat hyperalgesia development (from 11.3 ± 0.6 to 8.1 ± 0.6 s) and mechanical allodynia (from 34.9 ± 3.5 to 33.5 ± 1.5 g). **Discussion:** Oral and intraperitoneal administration of LASSBio-981 interfered with the development of allodynia and hyperalgesia in both SNL and ICC. Apoio Financeiro: CNPQ, FAPERJ, FUJB e CAPES

05.008

Interaction between cyclooxygenase-2 and heme oxygenase-1 / biliverdin / carbon monoxide pathways in nociception control in mice. Grangeiro NMCG[1], Silva AAR[2], Chaves HV[2], Val DR[1], Aguiar JA[3], Souza RB[1], Albuquerque RAF[3], Bezerra MM[1] [1]FM-UFC-Sobral − Biotechnology, [2]UFC-Sobral − Dentistry, [3]FM-UFC-Sobral

Introduction: Heme oxygenase-1 (HO-1) is induced in a variety of cells including endothelial cells, monocytes/macrophages and neutrophils by heme, endotoxins, cytokines, nitric oxide and other mediators produced during inflammatory responses. Cyclooxygenase-2 (COX-2) is induced by a variety of noxious stimuli leading to production of great amounts of PG associated with pain development. A possible interplay between HO-1 and COX-2 systems has recently been addressed. The aim of this study was designed to investigate the effect of hemin (substrate of HO-1/Biliverdin/CO pathway), DMDC (CO-releasing molecule) or ZnPP-IX (specific HO-1 inhibitor) on the antinociceptive effect of etoricoxib, a selective COX-2 inhibitor in the acetic acid-induced writhing test. Experiments were approved by the Institutional Animal Care and Use Committee of the Federal University of Ceará (UFC), Fortaleza, Brazil (Protocol Number 26/08). **Methods:** mice (20-25g) were pretreated with etoricoxib (0.1, 1 or 10mg/Kg; i.p) or with HO-1/BVD/CO pathway modulators, knowingly: Hemin (0.3, 1 or 3mg/Kg; s.c), DMDC (0.00025, 0.025 or 2.5µMol/Kg; s.c) or ZnPP-IX (1, 3 or 9mg/Kg; s.c) 30 or 60 min before acetic acid 0.6% injection. Next, the number of writhes was quantified. Non-treated group receive appropriate vehicles before acetic acid 0.6% (NT-0.6). Animals pretreated with ZnPP-IX received acetic acid 0.3% in order to promote a submaximal writhing response. In other series of experiments, animals were pretreated with ineffective doses of hemin or DMDC followed by an ineffective dose of etoricoxib. Also, animals were pretreated with ZnPP-IX followed by an effective dose of etoricoxib. 4h after the acetic acid injection, bilirubin levels (product of Biliverdin conversion) were diagnosed in the peritoneal lavage. Naive group represent animals which received (i.p) only saline. **Results and Discussion:** Etoricoxib 1 or 10 mg/kg (17±3.4 or 2.5±1.3), Hemin 3 mg/kg (10.6±3.8) or DMDC 2.5µMol/Kg (14.2±3.5) reduced (<0.05) the number of writhes, compared to NT-0.6 (35.9±3.2). On the other hand, ZnPP-IX 3 mg/kg (32±3.9) potentiated ($P<0.05$) the effect of acetic acid by increasing ($p<0.05$) the number of writhes, as compared to NT-0.3 (17±2.8). The coadministration of etoricoxib 0.1 mg/kg with hemin 0.3 mg/kg (12±3.1) or DMDC 0.025µMol/Kg (9.8±3) reduced ($P<0.05$) the number of writhes, as compared to NT-0.6. When etoricoxib 1 mg/kg was coadministered with ZnPP-IX 3 mg/kg (30.2±5.1), it was observed that ZnPP-IX reduced the analgesic effects of etoricoxib, as compared to etoricoxib alone (17±3.4). Acetic acid 0.6% promoted an increase in bilirubin levels in peritoneal exudates (0.11±0.006), compared to naïve group (0.01±0.002). Bilirubin levels in peritoneal exudate were reduced by pretreatment with ZnPP-IX (0.01±0.001). The pretreatment of mice with etoricoxib increased ($P<0.05$) the bilirubin concentration in peritoneal exudates (0.37±0.030). The HO-1/BVD/CO pathway is activated in the abdominal writhe model induced by acetic acid. The analgesic effect of etoricoxib at least partially depends on the participation of the HO-1/BVD/CO pathway. Acknowledgments: Funcap and CNPq.

05.009

IL-33 receptor deficiency reduces inflammation in septic arthritis in mice. Staurengo-Ferrari L[1], Cardoso RDR[1], Xu D[2], Liew FY[2], Cunha FQ[3], Pelayo JS[4], Saridakis HO[4], Verri Jr WA[1] [1]UEL − Ciências Patológicas, [2]University of Glasgow − Immunology Infection, Inflammation, [3]FMRP-USP, [4]UEL − Microbiologia, [6]UEL − Ciências Patológicas

Introduction: IL-33 is a cytokine family of IL-1, which signals through its receptor ST2 and induces Th2 responses. Recently, it has been demonstrated that IL-33 also induces Th1/Th17 and innate inflammatory responses. In models of rheumatoid arthritis, IL-33 increases the inflammatory responses by inducing neutrophil recruitment to the knee joints. In a model of sepsis induced by cecal ligation and puncture, IL-33 inhibits LPS-induced down regulation of CXCR2 receptors in neutrophils, therefore, maintaining the neutrophil recruitment to the infection focus. There is a link between rheumatoid arthritis and sepsis since rheumatoid arthritis patients are more susceptible to septic arthritis. Herein, we investigated the involvement of IL-33 in the pathophysiology of septic arthritis. **Methods:** A suspension of culture of *Staphylococcus aureus* ATCC number 6336 was prepared in PBS. The suspension was inoculated into the knee joints of animals Balb / c (WT) and ST2-/- , weighing 20-25g (n = 6). The doses used were 1 x 10 5 CFU, 1 x 106 CFU, 1 x 107 CFU per joint. The negative control received 10 ul of saline (NaCl 0.9%). The mechanical hyperalgesia (pain) and oedema were evaluated before the administration of bacteria (time 0h) and then every other day until the 28th day after inoculation. Mice were sacrificed on day 28 and knee joints were harvested for determination of total number of leukocytes in a Neubauer chamber and differential counts were performed in Rosenfelt method stained slices. Differences between groups were evaluated by analyses of variance (one-way ANOVA) followed by the Tukey's test. Statistical differences were considered to be significant at $p < 0.05$. Animal care and handling procedures were approved by the Ethics Committee of the Universidade Estadual de Londrina – UEL (protocol nº. 29165/2009). **Results and Discussion:** The results showed that administration of S. aureus in the femur-tibial joint in mice caused a dose-dependent increase in the mechanical hyperalgesia, edema and cell migration to the infection site. The 107 CFU/ 10 µL per joint dose reached the highest levels of mechanical hyperalgesia, edema and cell migration, this being chosen for subsequent experiments with ST2-/- mice. The mechanical hyperalgesia, edema and cell migration (total of leucocytes; neutrophils and mononuclear cells) were 31, 92 and 67% lower in ST2-/- mice compared to Balb/c mice, respectively at the point of highest difference during the 28 days. The results suggest that IL-33 participates in the development of pain, edema and leukocyte migration in septic monoarthritis induced by intra-articular administration of S. aureus. Furthermore, differently of other therapies used for rheumatoid arthritis (corticoids and anti-TNF) that impair host defense against infections, anti-IL-33 therapy might reduce the inflammation and infection in septic arthritis rather than increase the susceptibility to infection, suggesting anti-IL-33 therapy is more adequate than anti-TNF therapies. **Financial Support:** CAPES, PPSUS/Fundação Araucária, CNPq

05.010

Antinociception induced by LASSBio-1410 in neuropathic pain model. Leal DM[1], Nascimento-Jr NM[2], Leal, CM[2], Mendes TCF[3], Fraga CAM[4], Barreiro EJ[4], Sudo RT[5], Zapata-Sudo G[3] [1]UFRJ − Farmacologia, [2]IQ-UFRJ, [3]UFRJ − Farmacologia Básica e Clínica, [4]FF-UFRJ − LASSBio, [5]UFRJ

Introduction: LASSBio-1410 is a pyrazolo pyrrolo pyridine derivative, which produces antinociception and was evaluated in models of inflammatory and chronic pain. **Methods:** LASSBio-1410 (2, 3 e 4 mg/kg) was administered i.p. in male Swiss mice (20-25 g) to evaluate the antinociceptive activity in the formalin test which consisted in intraplantar administration of formalin 2.5%. Reactivity of animals licking or biting the paw was observed in the control group treated with DMSO and treated with LASSBio-1410. To evaluate possible mechanisms involved in the antinociceptive activity, mice were pretreated with the following blockers: naloxone (2 mg / kg) an opioid receptor antagonist, CTOP (1 mg/kg) a selective μ-opioid receptor antagonist; binaltorphimine (10 mg/kg) a selective κ-opioid receptor antagonist; naltrindole (1 mg/kg) a selective δ-opioid antagonist; flumazenil (20 mg/kg) a benzodiazepine antagonist. Neuropathic pain was induced in male Wistar rats (200 – 250 g), anesthetized with the association of ketamine (100 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) which were submitted to a chronic constriction injury (CCI). Four hours after surgery, animals were randomly divided in two groups: treated with vehicle or LASSBio-1410. The animals were treated daily, once a day. Thermal stimuli (radiant heat) were applied to the plantar surface of the ipsilateral hind paw at 24 hours, 3 and 7 days after surgery, and decreases in the latency to paw withdrawal were considered indicative of thermal hyperalgesia. These experimental protocols had been approved in the animal care and use committee at UFRJ under license DFBCICB012 and DFBCICB017 **Results:** The reactivity of male mice in the neurogenic phase of the formalin test was reduced in a dose-dependent manner, because it reduced from 51.5 ± 5.5 to 49.0 ± 3.5; 38.4 ± 5.5 ($P<0.05$) and 30.8 ± 3.4 s ($P<0.05$) after i.p. administration of 2, 3 and 4 mg/kg of LASSBio-1410. The antinociceptive activity was completely reversed by naloxone (2 mg/kg), flumazenil (20 mg/kg) and naltrindole (1 mg/kg) but not by CTOP (1 mg/kg) or binaltorphimine (10 mg/kg). The reactivity was 55.2 ± 4.4; 63.7 ± 8.1 s and 55.8 ± 5.7 s after pretreatment of naloxone, flumazenil and naltrindole. Reactivity was also reduced in the inflammatory phase by LASSBio-1410. It reduced from 197.5 ± 14.5 (control) to 123.7 ± 16.3; 24.5 ± 10.6; and 15.8 ± 7.5 s at 2, 3 and 4 mg/kg of LASSBio-1410, respectively. Pretreatment with naloxone (45.3 ± 10.4 s, $P<0,05$) and flumazenil (54.3 ± 9.8 s, $P<0,05$) partially reversed the effect of LASSBio-1410. Pretreatment with CTOP increased the inhibitory effect of LASSBio-1410. Seven days after the CCI surgery, the injury produced hyperalgesia in male rats because it significantly decreased the withdrawal of hind paw from 8.7 ± 0.6 s to 5.2 ± 0.6 s which was prevented by LASSBio-1410 (4 mg/kg) (7.9 ± 0.7, $P<0.05$). When treatment was initiated after the establishment of neuropathic pain, LASSBio-1410 not reversed the hyperalgesia with a latency of 6.3 ± 0.5 ($p>0.05$) similar to control latency 5.7 ± 0.1. **Discussion:** LASSBio-1410 promoted antinociception in inflammatory pain model probably mediated by activation of GABAergic and opioid type delta receptors. LASSBio-1410 prevented the hyperalgesia in neuropathic pain model. Financial support: CAPES, FAPERJ, CNPq, FUJB, INCT

05.011

Analysis of the antinociceptive activity of fractions from *Pterodon polygalaeflorus*. Pinto FA, Vigliano MV, Silva GP, Freitas GM, Gayer CRM, Coelho MGP UERJ − Bioquímica

Introduction: Within peripheral damage tissue (such as skin, muscles, joints, viscera), primary afferent neurons transduce noxious mechanical, chemical or heat stimuli into action potentials. After synaptic transmission and modulation within the primary sensory neuron and spinal cord, nociceptive signals reach the brain, where they are finally perceived as “pain” (Kimbery, Brain Research 129:63, 2000). The aim of this work was to fractionate the hexanic extract of Pterodon polygalaeflorus (EHxPpg) and analyze its antinociceptive activity. **Methods:** The hexanic extract obtained from P. polygalaeflorus seeds, was fractionated on silica gel column by step wise elution with hexane giving six fractions. The antinociceptive activities were evaluated by three models. In the acetic acid abdominal constriction test, male SW mice were pre-treated by oral route with fractions or vehicle. One hour later, the acetic acid 0.6% was intraperitoneally injected and the constrictions were counted after 5 minutes during 10 minutes. In the tail immersion test, one third of the tail was immersed into a water bath set at 55°C, with a maximum cut-off time of 10 s to minimize tail skin tissue damage. As positive control, a group of animals received morphine 10 mg/kg, i.p., 45 min before the test. The treatments with fractions occurred one hour before the test. In other model, after injecting 20 µl formalin (s.c.) under the plantar surface of the right hind-paw, the number of licks and bites were measured between 0-5 min (first phase) and between 20 and 50 min. As control, a group of mice was treated with dipyrone (50 mg/kg) 30 minutes before the test. All animal experiments were approved by the ethics committee of IBRAG-UERJ by protocol 05/2009. **Results/Discussion:** In the model of abdominal constrictions Fr IV and Fr VI fractions showed a dose dependent effect showing the highest levels of inhibition in this model. The fractions Fr II and Fr V showed an antinociceptive activity in the highest doses tested, while Fr III exhibited inhibition only at the lowest dose. In the model of formalin, Fr IV and Fr VI fractions exhibited effective antinociceptive activity in both phases with significantly high inhibition than dipyrone ($p<0.0001$). Fr V also showed antinociceptive activity, but mainly in the first phase (neurogenic). In the tail immersion test Fr IV e Fr VI fractions promoted an increase in latency time in the highest doses tested. The results suggest that the fractions Fr IV and Fr VI might be acting on mediators of peripheral action and on opioid receptors. Accordingly, both fractions had presented antinociceptive activity on the two models of central analgesic action (both sensible to opioid drugs like morphine), which action is mediated by receptors coupled to protein G and by receptors of peripheral action (FAINES). Fractions Fr I, Fr II, Fr III, which showed effective action only in the immersion test, may be acting only on mediators of peripheral action, suggesting that they may be inhibiting the synthesis of prostaglandins and other inflammatory mediators. The results observed for Fr V suggest a preferential central action. **Financial Support:** Capes, CNPq and FAPERJ

05.012

Antinociceptive activity of (-)-(2*S*,6*S*)-(6-ethyl-tetrahydropyran-2-yl)-formic acid on acute pain in mice. Marinho BG[1], Miranda, LSM[2], Meireles, BA[2], Vasconcellos, MLAA[3], Pereira, VLP[2], Fernandes PD[4] [1]UFES − Medicina Veterinária, [2]NPPN-UFRJ, [3]UFPB − Química, [4]UFRJ

Introduction: Pain is a major cause of distress, both physical and psychological. There is a continuous search for new pharmacologically active analgesic agents with minor side effects. Recently the synthesis of (-)-(2S,6S)-(6-ethyl-tetrahydropyran-2-yl)-formic acid (tetrahydropyran derivative) was described. The objective of this study was to investigate anti-hyperalgesic effects of the tetrahydropyran derivative. **Methods:** Male Swiss mice (20-22 g) were maintained in a room with controlled temperature (22 ± 2 °C) on a 12h light/dark cycle with free access to food and water. Animal care and research protocols were in accordance to the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA), were approved by the Biomedical Science Institute/UFRJ – ethical Committee for Animal Research, and received the number DFBCICB-015. The tetrahydropyran derivative was administered by intraperitoneal injection (ip) at doses of 6 to 600 µmol/kg in a final volume of 0.2 ml. Morphine (10 µmol/kg) was used as reference drug, and was administered by the same route as the tetrahydropyran derivative. The control group was composed of vehicle. To evaluate the site of action of the tetrahydropyran derivative, the effect of naloxone was assessed after ip administration of 15 µmol/kg of the opioid receptor antagonist and 15 min before the administration of the tetrahydropyran derivative or morphine. The mice had been submitted to the models of acetic acid-induced abdominal writhing, formalin test, tail-flick test, hot plate test and induction of tolerance. **Results**: The effects of tetrahydropyran derivative were evaluated in models of peripheral and central pain and were compared to those obtained with morphine. Intraperitoneal tetrahydropyran derivative (60 to 600 µmol/kg, i.p.) significantly reduced the nociceptive effects induced by acetic acid or formalin in mice. The tetrahydropyran derivative also demonstrated an anti-hyperalgesic effect in the tail flick model. The opioid receptor antagonist naloxone (at 15 µmol/kg, i.p.) reverted the tetrahydropyran derivative's antinociceptive activity in all of the models evaluated. Morphine and the tetrahydropyran derivative induced tolerance in mice. However, the tolerant effect induced by the tetrahydropyran derivative had its onset retarded when compared to that induced by morphine. **Discussion**: The present study demonstrated the antinociceptive activity of tetrahydropyran derivative in the test models of chemical nociception induced by acetic acid and formalin, as well as in the test model of nociception induced by thermal stimuli, and further suggested that antinociceptive activity of tetrahydropyran derivative might be related to the involvement of the opioid pathway. Although the tetrahydropyran derivative induced tolerance, it was achieved later when compared to morphine. Studies regarding the precise site and the mechanism of action are necessary. **Financial support:** FAPERJ

05.013

(±)-trans-4-hydroxy-6-propyl-1-oxocyclohexan-2-one: a novel substance with antinociceptive properties. Marinho BG[1], Miranda, LSM[2], Costa JS[2], Delle Monache F[3], Leitão SG[4], Vasconcellos, MLAA[5], Pereira, VLP[2], Fernandes PD[6] [1]UFES − Medicina Veterinária, [2]NPPN-UFRJ, [3]UIN − Farmacologia, [4]UFRJ − Farmácia, [5]UFPB − Química, [6]ICB-UFRJ − Farmacologia

Introduction: Although there are many effective pharmacological and non-pharmacologic pain treatments available, opioids are essential for the medical management of moderate to severe acute pain and pain due to cancer. However the adverse effects related to the use of opioids are constipation, confusion, respiratory depression, sedation and nausea. Therefore the development of new analgesic drugs is basic for an improvement in the quality of life of carrying patients of chronic pain. The objective of this study was to investigate antinociceptive effects of the (±)-trans-4-hydroxy-6-propyl-1-oxocyclohexan-2-one. The isomeric lactone emerged as the mayoritary structure for the isolated substance from the dichloromethane extract of *Vitex cymosa* (Verbenaceae). **Methods:** Male Swiss mice (20-22 g) were maintained in a room with controlled temperature (22 ± 2 °C) on a 12h light/dark cycle with free access to food and water. Animal care and research protocols were in accordance to the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA), were approved by the Biomedical Science Institute/UFRJ – ethical Committee for Animal Research, and received the number DFBCICB-015. The (±)-trans-4-hydroxy-6-propyl-1-oxocyclohexan-2-one was administered by intratechal injection (it) at doses of 10 to 600 nmol in a final volume of 5 µl. Morphine (10 nmol, EC50) was used as reference drug, and was administered by the same route as the substance. The control group was treated with vehicle alone. To detect potential targets of analgesic action of the substance, the antagonists naloxone, glibenclamide, atropine and L-NAME were administered (ip) in the concentrations of 10µmol/kg, 5 µmol/kg, 5 µmol/kg and 10 µmol/kg, respectively, 15 min before the administration of the substance. The mice were submitted to the formalin and hot plate tests . Results The effects of (±)-trans-4-hydroxy-6-propyl-1-oxocyclohexan-2-one were evaluated in models of inflammatory and non-inflammatory pain and were compared to those obtained with morphine. Intratechal (±)-trans-4-hydroxy-6-propyl-1-oxocyclohexan-2-one (10 to 600 nmol, it) significantly reduced the nociceptive effects induced by formalin in mice. The substance also demonstrated an antinociceptive effect in the hot plate model. The antagonists naloxone and glibenclamide not reverted the antinociceptive activity of the substance. The antagonist atropine partially reverted the antinociceptive activity, while the administration of L-NAME showed synergistic activity in the models of formalin and Hot plate. Discussion The present study demonstrated the antinociceptive activity of (±)-trans-4-hydroxy-6-propyl-1-oxocyclohexan-2-one in the test model of chemical nociception induced by formalin, as well as in the test model of nociception induced by thermal stimuli (Hot plate test), and further suggested that antinociceptive activity of substance might be related to the involvement of the nitric oxide pathway, beyond the involvement of the cholinergic system. **Financial support:** FAPERJ

05.014

CB_1 and CB_2 cannabinoid receptors are involved in the effect of crotalphine, an opioid-like analgesic peptide. Machado FC[1], Zambelli VO[1], Fernandes ACO[1], Heimann AS[2], Cury Y[1], Picolo G[1] [1]IBu − Dor e Sinalização, [2]Proteimax Biotecnologia Ltda. − P&D

Introduction: Although morphine and other opioid-like drugs are considered the main option for the treatment of moderate to severe pain, the use of opioids is limited because of the observed undesirable effects. Therefore, efforts have been made on the search of new analgesic compounds. Recently, our group demonstrated that crotalphine, a 14 amino acid-peptide synthesized based on the structure of the natural analgesic factor isolated from the venom of the South American rattlesnake *Crotalus durissus terrificus*, features a long-lasting analgesic activity when evaluated in experimental models of acute and chronic pain (Konno, K. Peptides, 29:1293, 2008). This effect is mediated by activation peripheral kappa- and delta-opioid receptors. Despite presenting opioid activity, the amino acid sequence of crotalphine displays no homology to any known opioid peptide (Gutierrez, VP. Eur J Pharmacol, 594:84, 2008). Furthermore, preliminary results indicate that crotalphine does not directly activate opioid receptors. Behavioral and molecular studies have demonstrated a great interaction between opioid and cannabinoid systems (Ibrahim, M.M. Proc Natl Acad Sci USA 102:3093, 2005) and also that cannabinoids may induce the release of endogenous opioids in the same manner that opioids may induce the release of endocannabinoids (Welch, S.P. Int Rev Psychiatry 21:143, 2009). Then the aim of this work is to evaluate the involvement of cannabinoid receptors in the antinociceptive effect of crotalphine. **Methods:** Male Wistar rats and male Swiss mice (Butantan Institute) were used. All procedures were approved by the Institutional Animal Care Committee of the Butantan Institute (CEUAIB, protocol number 622/2009). Hyperalgesia was induced in Swiss mice by carrageenin (Cg, 100 µg/paw) and in Wistar rats by prostaglandin E2 (PGE2, 100 ng/paw). The antinociceptive effect of crotalphine (0.04, 0.2, 1 and 5 µg/kg, p.o.), ACEA (CB1 agonist, 5, 10, 20 and 50 µg/paw, i.pl.) or AM1241 (CB2 agonist, 5, 10, 20 and 50 µg/paw, i.pl.) was determined using the paw pressure test in rats (Randall, L.O. & Selitto, J.*J. Arch Int Pharmacodyn Ther* 111:209, 1957) or an electronic pressure-meter test for mice (Cunha, T.M. *Braz J Med Biol Res* 37:401, 2004). The involvement of cannabinoid receptors in the antinociceptive effect of crotalphine was investigated using selective antagonists of CB1 (AM251, 5, 10 and 80 µg/paw) and CB2 (AM630, 5 and 50 µg/paw) receptors, injected by intraplantar route. The activation of CB2 cannabinoid receptors was confirmed by imunoblotting assays (Gupta, A. J Biol Chem 282:5116, 2007) using conformation-state sensitive antibodies (Proteimax Biotechnology, Brazil). **Results and Discussion:** The results demonstrated that crotalphine, ACEA and AM1241 induce antinociception in both models of pain evaluation. Both CB1 and CB2 receptor antagonists inhibited the antinociceptive effect of crotalphine and of their respective agonists. In addition, crotalphine increased the activation of CB2 receptors in the skin tissue from rat paw. These results indicate that peripheral CB1 and CB2 receptors are also involved in antinociception induced by crotalphine. **Acknowledgment**: Supported by grants from FAPESP (2009/14203-5) and INCTTOX program.

05.015

Antinociceptive and anti-inflammatory activities of novel *N*-acylhydrazone derivatives designed as piroxicam analogues. Bispo Junior W[1], Miranda AS[2], Queiroz AC[1], Cavalcante-Silva LHA[1], Matta CBB[1], Lima LM[2], Barreiro EJ[2], Alexandre-Moreira MS[1]
[1]UFAL − Farmacologia e Imunidade, [2]FF-UFRJ − LASSBio

Introduction: The aim of this study was to carry out a pharmacological evaluation of the new N-acylhydrazone derivates, structurally designed with two pharmacophoric groups, with 4-hydroxy-2H-1, 2 benzotiazina 1.1 dioxide, present in piroxicam structure a nonsteroidal anti-inflammatory drug belonging to the class of oxicams. The anti-inflammatory properties of these compounds will be investigated. **Methods:** Experiments were conducted using adult Swiss mice (20-30g), males or females, 6–8 weeks of age. All animals came from the BIOCEN – UFAL breeding unit and approved by the Ethics Committee –UFAL (number: 026681/2009-23) for animal handling. Functional models of nociception and inflammation were performed in vivo: abdominal writhing induced by acetic acid (Collier; Brit. Jour. Pharm., 32; 285, 1968), nociception induced by formalin (Hunskaar; Pain, 30; 103, 1987) and peritonitis induced by carrageenan (Ferrandiz; Infla. Res., 32, 2838, 1991). All the compounds and drug standards were administrated orally 40 minutes before the tests were initiated, at a dose of 100 µmol/kg. The results were analyzed using the t test in a tutorial Prism® (*p <0.05, *p <0.01) and were expressed as standard error of the mean. **Results:** The results showed that in abdominal writhing induced by acetic acid, the compounds LASSBio-1604, LASSBio-1605, LASSBio-1606, LASSBio-1607, LASSBio-ASM50 and LASSBio-ASM66 induced inhibition by 83.1% (±3.2, p<0.01), 75.1% (±5.1, p<0.05), 72.1% (±2.1, p<0.01), 62.4% (±2.9, p<0.01), 36.9% (±2.0, p<0.05), 56.9% (±3.4, p<0.01), and 89.9% (±2.3, p<0.01), respectively. In the formalin test, only LASSBio-ASM66 significantly inhibited (51.6% ±3.9, p<0.01) nociceptive activity in the neurogenic phase. In the inflammatory phase, LASSBio-1604, LASSBio-ASM66 and the piroxicam significantly inhibited nociceptive activity at 31.1% (±14.9, p<0.05), 42.6% (±23.9, p<0.05) and 21.6% (±15.9, p<0.05), respectively. The anti-inflammatory activity was evaluated by peritonitis induced by carrageenan. This result showed that the compounds LASSBio-1604, LASSBio-1605, LASSBio-1606, LASSBio-1607, LASSBio-ASM66 and piroxicam inhibited leukocyte migration by 74.2% (±0.62, p<0.01), 61.9% (±0.83, p<0.01), 57.4% (±0.5, p<0.01), 42.3 % (±1.5, p<0.01), 22.4 % (±0.66, p<0.01) and 28.5% (±0.19, p<0.01), respectively. **Conclusion:** The results suggest that these compounds present peripheral antinociceptive and anti-inflammatory activity. Further studies are needed to establish the comparative potency of piroxicam and its analogues.
Financial Support: INCT-INOFAR (573.564/2008-6), PROSUL (#490.600/07-7), CNPq, FAPEAL, UFAL, PPSUS MS.

05.016

Anti-hypernociceptive effect of dichloromethane and methanolic extracts obtained from *Piper variabile* C. DC. (Piperaceae) in mice. Alves DR[1], Silva S[1], Cechinel Filho V[2], Cruz SM[3], Caceres A[3], Alvarez L[4], Quintão NLM[2] [1]UNIVALI − Ciências da Saúde, [2]NIQFAR-UNIVALI – Ciências Farmacêuticas, [3]USAC − CCQQ y Farmacia, [4]UAEM – Investigaciones Químicas

Introduction: *Piper variabile* C. DC. (Piperaceae) grows in Guatemala being widely used to treat several ailments. We selected this plant as part of an Iberoamerican program (Ribiofar/CYTED) to search for bioactive natural products from plants, with the aim of evaluating the possible anti-hypernociceptive effects in mice. **Methods:** The plant was collected in Chisec, A.V., Guatemala and then extracted sequentially with dichloromethane and methanol by percolation and concentrated by rotavapor. Male Swiss mice were used throughout this study (25-35g, N=6-8). The animals were pre-treated intraperitoneally (i.p.) with methanolic and dichloromethane extract from *P. variabile* leaves (0.1-30 mg/kg) or saline, and after 30 min, they were submitted to the induced of inflammatory pain, mice received an i.pl. injection of 50mL l-carrageenan (300 mg/paw) under the surface of the right hind paw. The mechanical nociception was evaluated using electronic pressure-meter (1, 3, 4, 6, 24 and 48h after carrageenan injection). Mice were individually observed in acrylic cages with a wire grid floor, where the flexion reflex behavior, followed by a clear flinch response after mechanical stimulation was considered as hypernociceptive index. All the procedures used in the present study were approved by the Animal Ethics Committee of UNIVALI (Protocol numbers 001/2008 UNIVALI). **Results:** The pre-treatment with dichloromethane extract of the *P. variabile* (0,3-3 mg/kg, i.p.) was able to significantly reduce the carrageenan-induced mechanical hypernociception, with inhibition of 55 ± 6% with the dose of 3 mg/kg. The pre-treatment of mice with *P. variabile* methanolic extract (1-10 mg/kg, i.p.) was capable of inhibiting the paw sensitization induced by carrageenan, with inhibition of 28 ±10% with the dose of 3 mg/kg. **Discussion:** These results show for the anti-hypernociceptive effect of the methanolic and dichloromethane extract obtained from *P. variabile*, as demonstrated by the reduction of hypernociceptive behavior in mice submitted to carrageenan injection. However, additional studies are necessary to better delineate the anti-hypernociceptive effects of this plant, as well as to identify its active principles and the respective mechanism of action. **Financial Support:** CNPq; FAPESC-SC; ProPPEC/UNIVALI; Programa Iberoamericano de Ciência y Tecnologia para el Desarrollo (CYTED) – Red 0284 RIBIOFAR; Dirección General de Investigación (DIGI), USAC.

05.017

Inosine reduces pain-related behavior in mice: involvement of adenosine A_1 and A_{2A} receptor subtypes and protein kinase C pathways. Nascimento FP[1], Macedo Junior SJ[2], Lopez SMF[2], Martins DF[3], Cerutti M[1], Marcon R[1], Santos ARS[2] [1]UFSC − Farmacologia, [2]UFSC − Ciências Fisiológicas, [3]UFSC − Fisiologia

Inosine, an endogenous purine, is the first metabolite of adenosine in a reaction catalyzed by adenosine deaminase. This study aimed to investigate the antinociceptive effects of inosine against several models of pain in mice and in rats. In mice, inosine given by systemic or central routes inhibited the acetic acid-induced nociception. Furthermore, inosine also decreased the late phase of formalin-induced licking and the nociception induced by glutamate. Inosine produced inhibition (for up to 4 h) of mechanical allodynia induced by Complete Freund´s Adjuvant (CFA) injected into the mouse's paw. Given chronically for 21 days, inosine reversed the mechanical allodynia caused by CFA. Moreover, inosine also reduced the thermal (cold stimuli) and mechanical allodynia caused by partial sciatic nerve ligation (PSNL) for 4 h; when inosine was chronically administered, it decreased the mechanical allodynia induced by PSNL for 22 days. Antinociception caused by inosine in the acetic acid test was attenuated by treatment of mice with DPCPX (a selective adenosine A1 receptor antagonist), 8-PT (a nonselective adenosine A1 receptor antagonist) and ZM241385 (a selective adenosine A2A receptor antagonist). In rats, inosine inhibited the mechanical and heat hyperalgesia induced by bradykinin and phorbol myristate acetate (PMA), without affecting similar responses caused by prostaglandin E2 or forskolin. These results indicate that inosine induces antinociceptive, antiallodynic and antihyperalgesic effects in rodents. The precise mechanisms through which inosine produces antinociception are currently under investigation, but an involvement of adenosine A1 and A2A receptors and blockade of the protein kinase C (PKC) pathway seem to largely account for inosine's antinociceptive effect.

05.018

Mechanisms through which endogenous ATP via P2X3 and P2X2/3 receptors activation contributes to inflammatory nociception induced by formalin on rat´s hind paw. Krimon S[1], Parada CA[2], Oliveira MC[3] [1]UNICAMP − Fisiologia e Biofísica, [2]UNICAMP − Farmacologia, [3]UNICAMP − Ciências Fisiológicas

Introduction: It has been demonstrated that P2X3 receptors are involved in inflammatory nociception induced by formalin on rat´s hind paw (McGaraughty S, *Br J Pharmacol* 146, 180, 2005). However, the mechanisms through which endogenous ATP via P2X3 and P2X2/3 receptors activation contributes to this response are unknown. Considering that formalin-induced inflammatory nociception is mediated by TRPA-1 (Andrade EL, Neuroscience, 152, 511, 2008) and 5-HT3/1A (Parada CA, Neuroscience, 102, 2001) receptors activation, the aim of this study was to verify whether, during the formalin-induced inflammatory nociception, the activation of TRPA-1 and 5-HT3/1A receptors is dependent on previous release of endogenous ATP and P2X3 and P2X2/3 receptors activation. **Methods:** formalin or abmeATP was administered into the subcutaneous tissue of rat´s hind paw and the behavioral nociceptive responses characterized by the number of flinches of the affected hind paw were quantified for 60 minutes (Parada CA, Neuroscience, 102, 2001). The selective P2X3 and P2X2/3 receptors antagonist A-317491 was used to confirm the involvement of these receptors in formalin-induced inflammatory nociception. In order to verify whether the activation of TRPA-1 and 5-HT3/1A receptors is dependent on previous release of endogenous ATP and P2X3 and P2X2/3 receptors activation, the selective TRPA-1, 5-HT3 or 5-HT1A receptors antagonists HC 030031, tropisetron and WAY 100,135, respectively, were co-administered with the non-selective P2X3 receptor agonist abmeATP. All experimental procedures were previously approved by the Ethics Committee in animal research at the State University of Campinas (protocol number: 1995-389-1). **Results:** Formalin (1%/paw, n=5, 447±60,7) or abmeATP (750µg/paw, n=5, 199,8±42,0) induced behavioral nociceptive responses significantly greater ($p<0.05$, Tukey test) than that induced by NaCl 0.9% (n=5, 19±8,5). Administration of A-317491 (60 µg/paw) into the ipsilateral ($p<0.05$, Tukey test) but not into the contralateral hind paw ($p>0.05$, Tukey test, n=4) significantly reduced the behavioral nociceptive responses induced by formalin (1%/paw, n=9, 70,1±22,4) or abmeATP (750µg/paw, n=5, 97,7±29,9). Administration of HC 030031 (300µg/paw, n=5, 91,6±13,1), tropisetron (150µg/paw, n=5, 46,6±12,4) or WAY 100,135 (450µg/paw, n=5, 19,2±1,8) on ispilateral ($p<0.05$, Tukey test) but not into contralateral hind paw ($p>0.05$, Tukey test, n=4) significantly reduced the behavioral nociceptive responses induced by abmeATP (750µg/paw, n=5). **Discussion:** The results of this study confirm the involvement of endogenous ATP via activation of P2X3 and P2X2/3 receptors in formalin-induced inflammatory nociception. Also, they suggest that, during the formalin-induced inflammatory nociception, the activation of TRPA-1 and 5-HT3/1A receptors is dependent on previous release of endogenous ATP and P2X3 and P2X2/3 receptors activation. **Financial Support:** FAPESP (2009/53440-2).

05.019

Nociceptive responses and thermal hyperalgesia evoked by substance P and CGRP in the rat trigeminal system. Teodoro FC, Tronco Junior MF, Cruz L, Dotto G, Zampronio AR, Chichorro JG UFPR − Farmacologia

Introduction: Activation and sensitization of trigeminal afferent fibers are thought to play a central role in most forms of pain arising from the face and head. A variety of transmitters and their receptors have been described in different subsets of TG neurons, including SP and CGRP (Ma et al. Eur J Neurosci , 13:2099.2001). However, the ability of these neuropeptides (i.e. SP and CGRP) to induce nociceptive, as well as hyperalgesic responses in the trigeminal system remains to be elucidated. **Methods:** Male Wistar rats (200-250 g) were used in all protocols, which were previously approved by UFPR's Committee on the Ethical Use of Animals of (authorization number 424). Rats received a subcutaneous (s.c.) injection of either Substance P (SP, 1-100 mg/50 ml), Calcitonin Gene Related Peptide (CGRP, 1 -10 mM) or Vehicle (saline, 50 ml) and were placed in observation cages for evaluation of the facial grooming behavior, which was registered in 5-min intervals up to 15 min. Thirty minutes after the injection of the substances, rats were submitted to ipsilateral application of either heat (~50°C heat source placed 1 cm from vibrissal pad) or cold (1-s tetrafluoroethane spray) stimuli to the snout. Heat and cold hyperalgesia were estimated as decreases in the latency to display head withdrawal or vigorous snout flicking, or increases in duration of bilateral facial grooming behavior, respectively. An additional group of rats received a s.c. injection of the selective NK1 receptor antagonist (SR140333, 10 mg/50 ml) or vehicle (50 ml) 15 min before a s.c. injection of carrageenan (50 mg/50 ml) or vehicle (saline, 50 ml) into the ipsilateral upper lip and the carrageenan-induced heat hyperalgesia was evaluated up to 240 min. **Results and Discussion:** SP (1 -100 mg/50 ml) or CGRP (1-10 mM) injected into the upper lip did not evoke significant facial grooming compared to vehicle-treated rats. Prior treatment of rats systemically with captopril (5 mg/kg, i.p.) 30 min before SP (1 -100 mg/50 ml) also did not resulted in significant facial grooming compared to the respective vehicle-treated rats.. SP (1-10 mg/50 ml) or CGRP (1-10 mM) also failed to evoke hyperalgesia to a cold stimulus applied to the vibrissal pad. On the other hand, SP at 10 mg/50 ml induced heat hyperalgesia starting at 180 min after its injection and persisting up to 240 min. CGRP (1 and 10 mM) also induced thermal hyperalgesia to heat stimulus starting at 60 min up to 180 min. Moreover, heat hyperalgesia induced by carrageenan injected into the upper lip at 50 mg/50 ml was not affected by prior local treatment with a selective NK1 receptor antagonist (SR140333, 10 mg/50 ml, 15 min beforehand). Our results demonstrated that SP and CGRP induce heat hyperalgesia, but not nociception or cold hyperalgesia, in the orofacial region. Further experiments are underway to explore the role of these neuropeptides on experimental pathological conditions, such as trigeminal neuropathic pain, that are associated with heat hyperalgesia. **Financial Support:** Sanofi-Aventis, CNPq.

05.020

Evaluation of potential antinociceptive the benzofuranones. Gonçalves CJ[1], Lenoir AS[1], Padaratz P[1], Cechinel Filho V[2], Niero R[3], De Campos-Buzzi F[3] [1]UNIVALI − Ciências da Saúde, [2]NIQFAR-UNIVALI − Ciências Farmacêuticas, [3]NIQFAR-UNIVALI

Introduction: Chalcones represent an important group of natural and synthetic compounds with a wide range of biological actions, including anti-inflammatory and antinociceptive activities. The present study evaluates the antinociceptive effects of some synthetic benzofuranones obtained synthetically in different models of pain in mice. **Methods:** All the procedures used in the present study were approved by the Institutional Animal Ethics Committee of UNIVALI under number 329/2009. Male Swiss mice (25-35 g, n= 6-8) were treated with the benzofuranones (3 to 60 mg/kg; i.p.) 30 min before each experiment. Controls were injected with saline and tween 80. Abdominal writhing induced by acetic acid: Acetic acid was injected (0.7%) and, after 30 min, the abdominal writhing was counted by 20 min. Formalin test: Formalin was injected (2.5%) and time the animals spent licking the injected paw was annotated in the first 5 min (1st phase − neurogenic) and within 20-25 min (2nd phase − inflammatory). Capsaicin test: Capsaicin was injected (1.6 mg/paw) and time the animals spent licking the injected paw was measurement in the first 5 min. Glutamate test: Glutamate was injected (30mmol/paw) and time the animals spent licking the injected paw was measurement in the first 15 min. Hot-plate test: The animals were pre-selected 24 hours before the test, and the time of the animals reaction was monitored at 56 ºC for 30 second. The results were expressed as mean ± SEM, except the ID50 which was presented as geometric mean accompanied by its corresponding confidence limit of 95%. **Results and Discussion:** All the benzofuranones exhibited significant antinociceptive activity. However, 3 − [2 − (4-chlorophenyl)-2-oxoetil]-2-benzofuran-1 (3H)-one (1) was the most active with ID50 values of 33.73 (29.19 – 38.96) mmol/kg. The benzofuranones evaluated followed the physical-chemical parameter 2p − p2, according Topliss method. Based on the substituents proposed by Topliss, we have tested one derivative from (1), the 3 − [2 − (3-methoxyphenyl)-2-oxoetil]-2-benzofuran-1 (3H)-one (2). This compound showed greater antinociceptive activity than (1) against writhing test, the model of pain induced by acetic acid with ID50 value of 9.64(7.84 – 11.88) mmol/kg. In formalin test was assessed two phases of pain. In the first phase, compound (2) showed maximum inhibition of 38.3% and in the second phase the activity was more pronounced, with an ID50 value of 174.27 (166.27 − 182.61) mmol/kg, suggesting that its mechanism may be involved in the inflammatory process. In capsaicin test, compound (2) showed an ID50 of 63.69 (59.17 − 68.54) mmol/kg, suggesting any action on the neurogenic pain. When evaluated in glutamate test, compound (2) showed a D50 value of 66.49 (58.32 − 72.82) mmol/kg, suggesting action towards glutamatergic system. On the other hand, in hot plate test, compound (2) was not effective in increasing the latency time. In summary, the use of Topliss method permitted to obtain a molecule with interesting analgesic potential. **Financial Support:** CNPq, ProBIC, ProPPEC/UNIVALI.

05.021

Effect of hemopressin on Fos and Egr-1 expression on an experimental model of neuropathic pain. Maique ET[1], Alves AS[2], Ferro ES[3], Heimann AS[4], Britto LRG[2], Dale CS[1] [1]IEP-HSL − Neuromodulação e Dor Experimental, [2]ICB-USP − Fisiologia e Biofísica, [3]ICB-USP, [4]Proteimax Biotecnologia Ltda. − P&D

Introduction: Hemopressin (Hp), a nonapeptide, derived from the α1 chain (95-103) of hemoglobin, is an inverse agonist of type 1 cannabinoid receptors and mediates signaling induced by these receptors (Heimann et al. PNAS.104: 20 588 2007). Data obtained by our group demonstrate that Hp inhibits pain in different experimental models of acute hyperalgesia (Dale et al. Peptides. 26: 431, 2005). Herein we evaluate the antinociceptive activity of hemopressin on an experimental model of neuropathic pain. **Methods:** male Wistar rats (180-200 g) were submitted to the chronic constriction injury model (CCI; Bennett and Xie, Pain, 33:87, 1988) and after 14 days, in the presence of neuropathic pain, animals received different treatments with Hp and were evaluated at the paw pressure test (Randall and Selitto, *Arch. Intern. Pharmacodyn*, 111:209,1957), After in vivo evaluation, animals were sacrificed and the spinal cords submitted to immunohistochemistry evaluation of egr and c-fos. A group of rats submitted to surgical procedure and treated with saline or the sham group were submitted to same protocols and evaluated as control groups. Results e **Discussion:** Oral administration of 0.25mg/Kg of Hp inhibited mechanical hyperalgesia from the 1st to the 6th h. After the 6th h, animals received a second administration of Hp were again evaluated on the nociceptive test. The second treatment with Hp inhibited mechanical hyperalgesia only at the 1st h of testing with the effect decreasing on the subsequent evaluated times. Immunohistochemical analysis of the spinal cords demonstrated that CCI model induced an increase in both egr-1 and c-fos expression at the superficial layers of the dorsal horn of the spinal cord of animals. Hp inhibited both egr-1 and c-fos expression, suggesting that it interferes with mechanisms involved in nociception induced by CCI, possibly by acting directly on sensory neurons. These data may assist in clarifying the antinociceptive role of Hp, a potent candidate for therapeutic purposes. **Financial Support:** Instituto Sírio-Libanês de Ensino e Pesquisa e Proteimax Biotecnologia Ltda. Licença CEUA sob nº 2008/22

05.022

Antinociceptive and anti-inflammatory effects of apocynin, an NADPH -oxidase inhibitor. Castor LRG[1], Ximenes VF[2], Hiruma-Lima CA[3] [1]UNESP-Botucatu − Farmacologia, [2]FC-UNESP-Bauru, [3]UNESP-Botucatu − Fisiologia

Introduction: Apocynin is a phytochemical originally extracted from the Himalayan herb Picrorhiza Kurroa. This plant has been used in popular medicine for treatment of asthma and hypertension. Moreover, apocynin is widely used as a non-toxic inhibitor of NADPH-oxidase, the enzymatic complex responsible by production of superoxide anion in phagocytes and endothelial cells. Objectives: Here we aimed to evaluate and compare the antinociceptive and anti-inflammatory effects of apocynin and the structurally related compounds vanillin and vanillic acid. Material and **Methods:** Formalin-induced nociception: Animals (mice weighting 25 to 35 g) received 20 µL of 2.0 % formalin (0.92 % formaldehyde in saline), injected intraplantar (i.pl) in the ventral surface of the right hind paw. Animals were observed from 0 to 5 min (neurogenic phase) and from 15 to 30 min (inflammatory phase), and the time spent licking the inject paw was recorded considered as indicative of nociception. One hour before the administration of formalin, the animals received the compounds (100 mg/Kg, p.o) and the control groups received vehicle (saline) or piroxicam (30 mg/Kg). **Results:** Apocynin, vanillin and vanillic acid did not inhibit the pain in the neurogenic phase. However, apocynin and vanillin inhibited significant the inflammatory pain (33% and 30%, respectively, $n = 9$, $p < 0.05$), but vanillic acid did not have effect. The classic anti-inflammatory piroxicam inhibited 27%. **Conclusion:** These results show that besides its action as a NADPH oxidase inhibitor, apocynin also produces antinociceptive effect, which is, probably related to COX inhibition. Moreover, the absence of effect when vanillic acid was used is this experimental model is consistent with its lack of effect as NADPH oxidase inhibitor. In conclusion, there are some special feature in the apocynin molecule that makes this phytochemical a very attractive and promising anti-inflammatory substance and its mechanism of action is not restrict to NADPH oxidase inhibition. **Financial Support:** Capes

05.023

Antinociceptive properties of a new series of indan-hydrazine compounds. Reis RC[3], Motta NAV[1], Canal PF[1], Ávila RMD[2], Miranda ALP[3], Veloso MP[3], Brito FCF[1] [1]UFF − Fisiologia e Farmacologia, [2]UNIFAL − Ciências Farmacêuticas, [3]FF-UFRJ − LASSBio

Introduction: The various substituents acyl- and aryl-hydrazone are important pharmacophore groups related to inflammation process inhibition. Searching for new bioactive compounds, a new series of indane-hydrazine derivatives (SH, SHA, SHB, SHC, SHE and SHM2) was synthesized. These compounds were synthesized from safrole (4-allyl-1,2-methyldioxybenzene), an abundant Brazilian natural product obtained from Ocotea pretiosa. This compound can be found in abundance in Sassafras oil, obtained from different species of cinnamon, found in southern of Brazil (Barreiro EJ. *Quím. Nova*, 22, 5, 1999). We have evaluated the antinociceptive properties of the indane-hydrazine derivatives employing the writhing model of nociception. **Methods:** 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 SH compound (100µmols/ 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 indane-hydrazine derivatives at a dose of 100 µmols/ kg. 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 Bonferroni´s test ($p < 0.05$). **Results and Discussion:** At the screening dose employed (100µmols/ kg), the indane-hydrazine derivatives presented a significant inhibition of writhing induced by acetic acid when compared to control group (SH, 32.9%; SHA, 27.3%; SHB, 51.0%; SHE, 48.6%; SHM2, 38.4%). From all compounds studied only compound SHC did not present any activity. The oral treatment of animals with indane-hydrazine compounds induced antinociception when assessed by the acetic acid-induced constrictions, a useful method to screen both peripherally and/or centrally acting analgesic activities. We are evaluating other doses of the compounds for a better assessment of their antinociceptive profile. In addition, other methods, such as the hot-plate test will be applied to evaluate the supraspinal response. These results contribute to elucidate the potent analgesic profile of indane-hydrazine compounds. **Financial Support:** FAPERJ, PROPPI/UFF, CAPES, FAPEMIG.

05.024

Sensitivity of cisplatin-induced sustained mechanical hyperalgesia of face and hind paw to inhibition by classical analgesics. Guginski G, Rae GA UFSC − Farmacologia

Introduction: Cisplatin, an antineoplasic drug widely used to treat solid tumors, especially those affecting ovaries, bladder, testes and lungs, causes neuropathic pain in about 50% of the patients that receive continued treatment. Like other forms of neuropathic pain, this long lasting condition induced by cisplatin is frequently refractory or poorly responsive to treatment with currently available analgesics (O´Connor AB and Dworkin RH, Am J Med, 122 (10), p. 22, 2009). The current study aimed to investigate the antinociceptive effects of classical analgesic drugs and of an anti-epileptic drug given at different stages of a murine model of cisplatin-induced neuropathic pain. **Methods:** Male Swiss mice received weekly injections of cisplatin (3 mg/kg, i.p.) for 4 weeks. Responsiveness to mechanical stimulation of the forehead and hind paw was assessed beforehand (basal) and then repeatedly at 1, 2 or 4 weeks of cisplatin treatment, using von Frey hairs. At these time points we tested the susceptibility of the mechanical sensory changes to the antinociceptive effects of indomethacin (non-selective COX inhibitor, 1 mg/kg, i.p.), celecoxib (selective COX-2 inhibitor, 30 mg/kg, i.p.), dexamethasone (glucocorticoid, 2 mg/kg, s.c.), morphine (opioid receptor agonist, 2 mg/kg, s.c.) or gabapentin (an anti-epileptic structural analogue of GABA, 10 mg/kg, s.c.). All the procedures were approved by UFSC's Ethics Committee on Animal Use (protocol number 23080.018994/2008-39). **Results:** Cisplatin treatment induced pronounced and sustained mechanical hyperalgesia of both forehead and hind paw, from Week 1 onwards. The antinociceptive effects of indomethacin, celecoxib, dexamethasone, morphine and gabapentin displayed on Weeks 1, 2 and 4 are shown in the Table (results expressed as maximal inhibition afforded – in % − relative to the mechanical hyperalgesia detected in cisplatin-mice treated with the vehicle; n = 7 per group). region (Test used) treatment antinociceptive effect (% inhibition) Week 1 Week 2 Week 4 FOREHEAD (% frequency of withdrawal responses to 0.04 g von Frey filament) Indomethacin 56 ± 15 65 ± 17 no effect Celecoxib 85 ± 11 95 ± 5 no effect Dexamethasone 62 ± 19 68 ± 11 no effect Morphine 100 88 ± 8 no effect Gabapentin 72 ± 21 75 ± 10 79 ± 6 HIND PAW (50% withdrawal threshold force – Dixon's up and down method) Indomethacin 71 ± 16 no effect no effect Celecoxib 71 ± 22 74 ± 20 no effect Dexamethasone no effect no effect no effect Morphine 100 43 ± 3 no effect Gabapentin 53 ± 15 38 ± 6 60 ± 22 **Discussion:** The results obtained confirm that cisplatin induces a state of prolonged hind paw mechanical hyperalgesia in mice, and reveals that the same occurs with respect to the forehead. Moreover, there are marked differences between susceptibility of mechanical hyperalgesia in both regions to inhibition by analgesics. Finally, the progressive loss in analgesic efficacy of the COX inhibitors and morphine suggests that the mechanical hyperalgesia induced by cisplatin on Week 1 (early phase) displays a strong inflammatory profile is substituted by a typically neuropathic profile by Week 4, when gabapentin is effective. **Financial Support:** CNPq, CAPES, PRONEX, UFSC.

05.025

Fractalkine expressed in dorsal root ganglion mediates inflammatory pain. Souza GR[1], Cunha TM, Lotufo CMC, Talbot J, Bozzo TA, Cunha FQ, Ferreira SH − FMRP-USP − Farmacologia

Glial activation in the central nervous system has been implicated in the development of neuropathic and inflammatory pain. More recently, it was found that activation of satellite cells present in dorsal root ganglion (DRG) seems also to play a role in nociception. The chemokine, Fractalkine (CX3CL1) is expressed by primary sensory afferent, whereas its receptor (CX3CR1) is predominantly found in the glial cells, indicating that fracktalkine could participate in the process of glial activation. This chemokine is tethered to the extracellular surface of neurons and when released constitute a diffusible signal that contributes to neuron-glial interaction. The aim of this study was to test whether activation of satellite cells by fractalkine is involved in the cascade of events responsible for the genesis of the inflammatory pain. Mechanical nociceptive threshold was evaluated with an electronic version of von-Frey test (electronic pressure-meter paw test) in Wistar rats weighing 150-200 g. Firstly, it was observed that the decrease in mechanical nociceptive threshold (hypernociception) observed during peripheral inflammation of rat paw by intraplantar injection of carrageenan was inhibited by the anti-CX3CR1 administered into the dorsal root ganglion (DRG-L5). Furthermore, intraplantar injection of carrageenan induced significant increase in GFAP mRNA expression (DRG). GFAP mRNA expression induced by intraplantar injection carrageenan was blocked by the treatment with a specific antibody against CX3CL1 (10µg/i.gl). In agreement, direct administration of fractalkine into the DRG (L5) of rats produced mechanical hypernociception of hind paws in a dose- and time- dependent manner. Regarding the mechanism by which glanglionar fractalkine mediates inflammatory hypernociception, it was observed that fractalkine hypernociceptive effect was blocked by the treatment with a specific antibody against CX3CR1 (10µg/i.gl) and CX3CL1 (10µg/i.gl), thalidomide (45 mg/kg), IL1-ra (100ng/i.gl.), indomethacin (3 mg/kg) as well as dexamethasone (2 mg/kg). In vitro, incubation of isolated satellite cells in culture with fractalkine induced the release of TNF-a. Overall, these results suggest that during peripheral inflammation fractalkine is released in DRG and contributes to the genesis of inflammatory hypernociception by a mechanism dependent on stimulation of satellite cells to produced cytokines (TNF-α and IL-1β). Finally, these cytokines might activate COX enzyme triggering the release of prostanoids which are responsible for the nociceptor sensitization and possibly maintaining inflammatory pain.

05.026

Evaluation of the analgesic effect of bupivacaine-hydroxypropyl-β-cyclodextrin inclusion complex in association to sufentanil, after intrathecal administration in rats. Queiroz VA[1], de Araújo DR[2], Cereda CMS[1], de Paula E[1] [1]UNICAMP − Bioquímica, [2]UFABC − Ciências Naturais e Humanas

Introduction: Local anesthetics (LA) are substances that cause loss of the ability to feel pain in a particular part or parts of the body without loss of consciousness. In spite of their extensive use, LA present limitations due to their relatively short duration of action (up to 2-4 hours) and toxicity to the central nervous and cardiovascular systems. The aminoamide local anesthetic Bupivacaine (BVC) is the drug of choice in surgical procedures worldwide. Aiming to prolong the duration of action and to reduce the systemic toxicity of LA, we have previously reported the complexation of BVC with hydroxypropyl-β-CD (HP-β-CD); Bupivacaíne forms stable, 1:1 (mole:mole) inclusion complexes with HP-β-CD that, in comparison to plain BVC of equivalent doses induced a significant (2.0 fold) improvement in the time of anesthesia (Araújo et al., *Rev Bras Anestesiol*, 55:316, 2005). In the present work, the analgesic efficacy of the BVC:HP-β-CD inclusion complex in association to the opioid sufentanil was evaluated after intrathecal administration in rats. We investigate LA:opioid association to combine the blockade effectiveness of BVC with the prolonged antinociceptive effect of sufentanil. **Methods:** BVC:HP-β-CD (1:1 molar ratio) inclusion complex was prepared by mixing BVC and HP-β-CD in aqueous solution. After equilibrium, the preparation was freeze-dried and stored at -20ºC. Male Wistar albino rats (250 – 300g, n=7 / group) were treated by intrathecal injection in the region of L5-L6 vertebra with HP-β-CD, BVC (0.5%), BVC:HP-β-CD (0.5%), BVC (0.5%) or BVC:HP-β-CD (0.5%) plus sufentanil (1µg/kg). Paw Withdrawal Threshold to Pressure (PWPT) tests were used to evaluate the sensory blockade evoked by the formulations with a cut-off value of 350g, established in order to avoid stress-induced analgesia (Protocol #1957-1 CEEA, UNICAMP). **Results and Discussion:** Injection of BVC:HP-β-CD in association to sufentanil prolonged the analgesic effect ($p<0.001$) in relation to the other groups of animals (BVC; BVC:HP-β-CD and BVC plus sufentanil). These results indicate a potential clinical application for the association of BVC:HP-β-CD and sufentanil which can be used to reduce the administration frequency as well as the necessary BVC doses to induce the same effect. Supported by CNPq and FAPESP (Proc. 06/0121-9).

05.027

Resistance exercise induces antinociception in rats with participation of nitric oxide/$_{C}$GMP/K_{ATP} pathway. Galdino GS, Silva GC, Almeida RT, Duarte ID, Perez AC
UFMG − Farmacologia

Introduction: Resistance exercise is characterized by increased strength, tone, mass, and/or muscular endurance and also for more beneficial effects, such as blood pressure and osteoporosis reduction, diabetes mellitus control, and analgesia1, 2, 3. The aim of this study was to evaluate whether resistance exercise induces antinociception and the participation of the nitric oxide/CGMP/KATP pathway in this effect. **Methods:** Wistar rats were submitted to acute resistance exercise (RE) in a weight-lifting model adapted for rat4. The nociceptive threshold was measured by the nociceptive test of paw-withdrawal5. To investigate the involvement of the NO/CGMP/KATP pathway the following nitric oxide synthase (NOS) unspecific and specific inhibitors were used: N-nitro-L-arginine (NOArg), Nω-Propyl-L-arginine (L-NPA), N5-(1-Iminoethyl)-L-ornithine dihydrocloride (L-NIO), Aminoguanidine; guanylyl cyclase inhibitor, 1H-[1,2,4]oxidiazolo[4,3-a]quinoxalin-1-one (ODQ); and KATP channel blocker, Glybenclamide; all administered subcutaneously at a dose of 2 mg/kg 10 min before resistance exercise started. Plasma and cerebrospinal fluid (CSF) nitrite levels were determined by spectrophotometry. **Results:** A significant increase ($P<0.05$) of nociceptive threshold was demonstrated after RE. The NOS unspecific (NOArg) and specific (L-NPA, L-NIO and Aminoguanidine) inhibitors, guanylyl cyclase inhibitor (ODQ) and KATP channel blocker (Glybenclamide) reversed this effect ($P<0.05$). Nitrite levels in plasma and cerebrospinal fluid (CFS) were also increased after acute resistance exercise. Discussion and **Conclusion:** The findings presented demonstrated that acute resistance exercise protocol produced antinociception, with a possible involvement of the NO/CGMP/KATP pathway in this effect. Animal experimentation ethics committees protocol (CETEA/UFMG): 185/2007 **Financial Support:** FAPEMIG and CNPQ

05.028

Peripheral sensitization increases opioid receptor activation and expression in both dorsal root ganglia and nerve paw of rats. Zambelli VO[1], Gutierrez VP[1], Fernandes ACO[1], Parada CA[2], Cury Y[1] [1]IBu − Dor e Sinalização, [2]UNICAMP − Farmacologia

Introduction: Besides their central mechanisms of action, opioids also exert analgesia through peripheral mechanisms. This peripheral action allows for analgesia after application of systemically inactive doses of opioids directly into injured peripheral tissue, minimizing adverse central effects. Several data have shown that the peripheral efficacy of opioid drugs is enhanced in the presence of tissue injury, but the mechanisms involved in this phenomenon are not well known. Previous data of our group showed that, in rats, prostaglandin E2 (PGE2, intraplantar/i.pl.) and chronic constriction injury (CCI) of sciatic nerve increase the peripheral analgesic efficacy of opioid agonists and of crotalphine (CRP), a peptide obtained from C. d. terrificus snake venom. CRP induces peripheral analgesia mediated by activation of k- opioid receptor in PGE2-induced hyperalgesia or k- and d- opioid receptor in CCI model. This study aims to characterize some of the mechanisms involved in the increase of the analgesic efficacy of opioids caused by inflammation/tissue injury. For this purpose the effect of PGE2-induced hyperalgesia and CCI on opioid receptor expression and activation in dorsal root ganglia (DRG) and nerve paw (NP) of male Wistar rats was evaluated. **Methods:** Expression and activation of opioid receptors were evaluated by rt-PCR, immunoblotting and ELISA assays, 3h after i.pl. injection of PGE2 (100 ng/paw) or 14 days after CCI. In vitro studies were carried out in DRG cell culture incubated with PGE2 and/or opioid agonists (1 mM). The protocols were approved by the Butantan Institute Ethical Committee (386/07). **Results:** PGE2 increases genic and proteic expression of m − and k-opioid receptors in NP (43% and 71%, respectively) and decreases (30%) the expression of d-opioid receptors, when compared to naïve rats. m-opioid receptor expression is also increased in the ipsilateral and contralateral DRG (79 and 27%, respectively), while k-opioid receptor expression is increased only in the ipsilateral DRG (168%). CCI up-regulates m-opioid receptors in NP (27%) and DRG (ipsi and contralateral, 49 and 20%, respectively) and d-opioid receptors in DRG (ipsilateral, 35%). In contrast, k-opioid receptors are down-regulated by CCI in NP (51%) and DRG (21%). Despite the increase in receptor expression, PGE2 did not cause receptor conformational changes. Activation of m- and k-opioid receptors was observed after treatment with CRP or opioid agonist. The activation caused by CRP or opioid agonist was enhanced in NP slices under PGE2 (16 and 20%, respectively) or CCI sensitization (15 and 30%, respectively) or DRG cells incubated with PGE2 (43 and 45%, respectively). **Discussion:** The results indicate that peripheral opioid receptor expression and activation are distinctly regulated by the presence of acute or chronic injury. The different pattern of k and d opioid receptors expression caused by acute and chronic injury may contribute to the activation of distinct opioid receptors by CRP, in the presence of PGE2 and CCI. These data also point out that drugs that activate peripheral opioid receptors, including substances derived from animal toxins, might have therapeutic potential as peripherally analgesics. Support: FAPESP, INCTTOX Program

05.029

The sesquiterpene lactone, budlein A, inhibits antigen induced-arthritis inflammation in mice. Zarpelon AC[1], Pinto LG[2], Souto FO[2], Turato W[3], Arakawa NS[4], Da Costa FB[4], Cunha TM[2], Ferreira SH[2], Cunha FQ[2], Silva JS[5], Verri Jr WA[1] [1]UEL − Ciências Patológicas, [2]FMRP-USP − Farmacologia, [3]FCFRP-USP − Análises Clínicas, Toxicológicas e Bromatológicas, [4]FCFRP−USP, [5]FMRP-USP − Imunologia

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by articular lesions, recruitment of inflammatory cells and cytokine production. In this sense, cytokine targeting therapies such as anti-TNF and anti-IL-1 inhibit arthritis inflammation. Glucocorticoids, which inhibit cytokine production by inhibition of NFkB activation and/or activity is also effective. Nevertheless, although inhibition of NFkB activity is an effective anti-inflammatory mechanism of glucocorticoids, their adverse hormonal effects limit their use. Therefore, a drug that inhibits NFκB activation without the adverse side effects of glucocorticoids would be a suitable strategy. Recent studies demonstrated that the sesquiterpenic lactone, budlein A, inhibits innate inflammation induced by carrageenin. In this study we evaluated the therapeutic effect of budein A in antigen induced-arthritis (AIA). **Methods:** C57BL/6 mice were immunized with 500µg of methylated bovine serum albumine (mBSA) in 0.2ml of an emulsion containing saline and complete Freund's adjuvant by subcutaneous (s.c.) route on the day 0 and 7. AIA was induced by intraarticular (i.a.) injection of mBSA (30µg/cavity) on day 21 and 24. Immunized mice were pre-treated with dexamethasone (2mg/Kg) as control, budlein A (1 or 10 mg/kg, p.o.) or vehicle (20% Tween 80 in saline) 30 min before intrarticular injection of mBSA and then, every 24 h. Articular hypernociception was determined at various time-points by electronic pressure meter test and the edema was evaluated using a caliper. The articular cavities were washed three times with 3.3µL PBS containing EDTA to evaluated leucocytes migration at 48hr after second challenge. The total number of leucocytes was determined in Neubauer chamber using Turk's solution. Differential cell counts were determined by Rosenfeld stained slices. The proteoglycans dosage was determined by DMMB method. This study was approved by the Ethics Committee on Animal Studies of the Universidade Estadual de Londrina (protocol no. 05761). **Results:** The oral pretreatment with budlein A (1 or 10mg/Kg) dose-dependently inhibited mBSA-induced mechanical hypernociception (17 and 55% respectively), oedema (27 and 42%, respectively), total leucocytes (57 and 80%, respectively), neutrophils (64 and 91%, respectively) and proteoglycans loss (42, and 60%, respectively) 48hr after the second challenge (day 26). **Discussion:** These results demonstrate that budlein A treatment inhibits prolonged inflammatory arthritis and the resultant proteoglycan loss in knee cartilage. Therefore, it is suggested that budlein A treatment might be a suitable approach in rheumatoid arthritis therapy. **Financial Support:** FAPESP, CNPq and CAPES and Fundação Araucária.

05.030

LASSBio-294 has partial agonist and antagonistic actions on TRPV1. Munaro DV[1], Barreiro EJ[2], Fraga CAM[2], Castro NG[3], Guimarães MZP[4] [1]UFRJ − Biofísica, [2]FF-UFRJ − LASSBio, UFRJ, [3]UFRJ − Farmacologia Molecular, [4]UFRJ − Farmacologia Básica e Clínica

Introduction: TRPV1 is an ion channel present in nociceptors which is activated by capsaicin, the pungent compound in chili peppers, acid pH and high temperatures, among other painful stimuli. This non-selective cationic ion channel has become an interesting target for the development of novel analgesics. In previous work we proposed that LASSBio-881, an N-acylhydrazone derivative effective against acute, inflammatory and neuropathic pain, was a TRPV1 antagonist (TRIBUTINO JLM, Br. J. Pharmacol. 2010 159: pp. 1716-1723). LASSBio-881 was synthesized via the molecular hybridization of LASSBio-294 and nimesulide. Nothing is known about the structure-activity relationships between N-acylhydrazones and their ability to modulate TRPV1 activity. To begin mapping moiety(ies) underlying the activity of N-acylhydrazones at the TRPV1 receptor, we sought to evaluate the effects of the parental compound, LASSBio-294, on heterologously-expressed TRPV1. **Methods:** *Xenopus laevis* oocytes were obtained from adult female frogs by performing a small abdominal incision through anesthesia. After collagenase treatment and sorting, oocytes were injected with approximately 50nL of cRNA encoding rat TRPV1. Following 5 to 9 days of expression, these cells were used in electrophysiological experiments in which cells were clamped at -60mV and continually perfused with ND-96 pH 7.4 or pH 5.5, containing or not the test substances. We tested the effects of LASSBio-294 alone and against activation of TRPV1 by 1 mM capsaicin. The experimental procedure was approved by CEUA UFRJ (DFBCICB 009). Results expressed in terms of mean ± SEM and the statistical significance was determined with One-Way ANOVA followed by Tukey's test. **Results** Alone, LASSBio-294 was capable of causing currents in TRPV1-expressing oocytes. For instance, at 100 microM, it elicited a current that was 18.44% ± 5.62 ($P<0.001$, n=11) of maximal capsaicin stimulation. When the same concentration of LASSBio-294 was applied at pH 5.5, the current became 56.3% ± 5.1 of maximal, statistically different from the compound alone ($P<0.001$, n=5). LASSBio-294 activation of TRPV1 is concentration-dependent with a steep slope, however, the highest concentration tested (250 microM) only achieved 41.2% ± 5.25 (n=6) of maximal capsaicin current. Activation of TRPV1 by LASSBio-294 was blocked by capsazepine. When 50 microM LASSBio-294 was used concomitantly with 1 microM capsaicin, it caused a significant decrease in amplitude relative to the vanilloid response alone (27.65% ± 3.80%, n = 3, $P < 0.05$). **Discussion:** LASSBio-294 was capable of activating TRPV1 with less efficiency than capsaicin and of inhibiting the vanilloid response, suggesting it acts as a partial agonist and antagonist. These effects contrast with the ones for LASSBio-881, which acts only as an antagonist. We are currently investigating intermediate compounds, which will allow the identification of functional moieties in this class of molecules. Supported by: CNPQ, UFRJ, FAPERJ.

05.031

Study of anti-inflammatory and antinociceptive properties of new derivatives rationally designed as PPAR agonists. Santos BLR, Lima CKF, D´Andrea ED, Lima LM, Barreiro EJ, Miranda ALP FF-UFRJ − LASSBio

Introduction: The research for new therapeutic targets to control the inflammatory response and to maintain the homeostasis lead to the development of new innovative drugs. After the advent and failure of biological therapies and Coxibs, a strong trend emerges in the search for targets that modulate physiological anti-inflammatory response, such as adenosine, IL-10 receptor and peroxisome proliferator-activated (PPAR) (Medzhitov, Nature, 454, 428, 2008). Therefore, the aim of this study is to evaluate the anti-inflammatory and antinociceptive profile of new compounds designed as prototypes of PPAR modulators. **Methods:** The antinociceptive activity was evaluated using the acetic acid (0.1 N; 0.1 ml/10g animal weight) induced writhing test and formalin (2.5%; 20 µl/paw) induced nociception test. Painful stimuli were administered 1h after administration of test compounds (100 µmol/kg, p.o.). The writhing were recorded 10 min after the stimulus for 20 min. The formalin test is divided in two phases and the time spent licking was recorded: 0-5 min (first or neurogenic phase) and 15-30 min (second or inflammatory phase) after formalin injection (Tjolsen, Pain, 51, 5, 1992). 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 –80ºC until determination of TNF-α (t = 24 h) by enzyme immunoassay (Gallily, J Pharmacol Exp There, 283, 918, 1997). **Results and Discussion:** Among seven derivatives evaluated in the writhing test, we emphasize the inhibitory effect displayed by LASSBio-1474 and LASSBio-1470, which inhibited the writhing by 50% and 30% respectively. The PPAR-α agonist fenofibrate at a dose of 100 µmol/kg inhibited by 30%. Taking into account the ability of PPAR agonists to modulate inflammatory cytokines, we evaluated the effect of compounds on TNF-α. LASSBio-1473 and LASSBio-1474 inhibited by 83% the production of TNF-α at 100 µM concentration, being more effective than thalidomide (300 µM; 67% of inhibition), a drug described as inhibitor of TNF-α. In order to better characterize the antinociceptive and anti-inflammatory profile of the compounds, we performed the formalin-induced nociception test. LASSBio-1523 (100 µmol/kg) showed a significant antinociceptive activity inhibiting the first phase by 30%. However, it was not observed any effect on the inflammatory phase. LASSBio-1471 and LASSBio-1474 inhibited only the inflammatory phase in 42% and 53% respectively (n = 7-10 animals, * p <0.05 one-way ANOVA). This study identified derivatives with anti-inflammatory and antinociceptive activities that can be useful in treating inflammatory conditions and neuropathies associated with chronic diseases such as diabetes. CNPq, CAPES, INCT-INOFAR. Table 1 – Anti-inflammatory and antinociceptive profile of LASSBio compounds. % of inhibition Compounds TNF-α production Writhing test Formalin test 1st phase 2nd phase Thalidomide 67* NT NT NT Fenofibrate NT 32* 28* 0 Indomethacin NT 54* 0 54* LASSBio-331 0 52* 0 72* LASSBio-1470 0 31* NT LASSBio-1471 0 0 0 42* LASSBio-1473 83* 0 0 0 LASSBio-1474 83* 52* 0 53* LASSBio-1503 0 0 0 0 LASSBio-1523 NT 0 28* 0 All test compounds, indomethacin and fenofibrate were evaluated in vivo at 100 µmol/kg (p.o.) (n = 7-10 animals/group) and in vitro at 100 µM (n = 3-4 experiments). Thalidomide was tested at 300 µM. *p<0.05 (one-way ANOVA).

05.032

Ketamine/fentanyl administration in infant rats promotes analgesia associated with increased hydrolysis of nucleotides. Medeiros LF[1], Souza A[2], Rozisky JR[1], Santos VS[1], Netto CA[2], Battastini AMO[2], Torres ILS[1] [1]UFRGS − Farmacologia, [2]UFRGS − Bioquímica

Objective: several therapeutic agents are used in pediatrics, among them we highlight anesthetics and analgesics. The challenge of anesthesia in neonates is the immaturity of the organs and systems; it can promote changes in biochemical and behavioral responses until adulthood. Fentanyl is an opioid analgesic with ability to provide a rapid analgesia and a relevant cardiovascular stability, and ketamine is a dissociative anesthetic agent with amnesic properties and hemodynamic stability for its sympathetic side effects. The objective of this study was to evaluate nociceptive response and enzymatic activity of ectonucleotidases of rats submitted to the administration of general anesthetic with or without a surgery procedure at P14. **Methods:** this study was approved by the Ethical Committee of HCPA (n°08149). Forty-day-old male Wistar rats (P14) were divided into 3 groups: control (C), ketamine S+/fentanyl (KF), and ketamine S+/fentanyl+surgery (KF+SUR). We used 0.09 mg/kg fentanyl and 20 mg/kg ketamine S+. The surgery model used was described by Levine, modified by Rice et al. (Ann Neurol 9:131, 1981) without ischemia. The nociceptive responses were evaluated by tail-flick test [n= P14(6-7), P30(11-14)], and the ectonucleotidases by the method of Battastini et al., (Neurochem Res 16:1303, 1991) [n= P14(2-3), P30(2-3)]. The Data analyzed by one-way ANOVA followed by Student-Newman-Keuls (SNK). The results were expressed as a percentage of control for Tail Flick Latency and mean±SEM for enzymatic activity (nmol Pi/min/mg protein), it was considered significantly different with $P<0.05$. **Results:** tail flick latency: in P14, it was not observed difference among the groups (ANOVA $P>0.05$); in P30, the KF and KF+SUR groups showed an increase in relation to the control group (KF:26.16% and KF+SUR:21.49%; ANOVA/SNK $P<0.05$). In the enzyme assay: in P14, it was not observed significant difference among the groups (ANOVA $P>0.05$); in P30, the KF group showed an increase in ATP hydrolysis when compared to other groups (C:100.86±0.90;KF:146.18±8.92;KF+SUR:104.95±1.82; ANOVA/SNK $P<0.05$); and both groups that received ketamine/fentanyl (KF and KF+SUR) showed an increase in AMP hydrolysis when compared to the control group (C:6.0±0.09; KF:14.97±1.71; KF+SUR:12.72±0.79; ANOVA/SNK $P<0.05$). **Conclusion:** These results demonstrate that anesthetics administration in early phase of life promotes analgesia and alterations in the hydrolysis of nucleotides at medium duration. Considering that anesthetic agents interact with several neuronal systems, including GABAergic, glycinergic, cholinergic, glutamatergic and purinergic systems, we suggest when using them in the maturation period of central nervous system they can promote changes in those neurotransmission systems. The NTPDase 2 probably is involved in the increase of ATP hydrolysis in P30, since this enzyme prefers nucleotide triphosphates as substrate. The analgesia observed in the Tail Flick Latency at P30 may be related to the increased levels of adenosine, an antinociceptive neuromodulator, which are the result of the increase of AMP hydrolysis in P30. **Financial Support:** CAPES; FIPE/HCPA, PROPESQ/UFRGS; FAPERGS; CNPq.

05.033

Evaluation of some mechanisms involved in antinociceptive effect of (-)epicatechin obtained from *Combretum leprosum* Mart. & Eicher (Combretaceae) in models of acute pain. Lopes LS[1], Pereira SS[1], Marques RB[1], Ayres MCC[2], Chaves MH[2], Almeida FRC[3]
[1]NPPM-CCS-UFPI, [2]UFPI − Chemistry, [3]UFPI − Biochemistry and Pharmacology

Introduction: Previous results from our laboratory showed that (-) epicatechin (EPI), a flavonoid of catechins group, obtained from *Combretum leprosum* Mart. & Eicher, showed antinociceptive effect in different models of chemical nociception in mice. This study aims to evaluate the possible mechanisms involved in the antinociceptive effect of EPI in models of acute pain. **Methods:** Swiss male mice (20-30 g, n = 6-10) were treated with EPI (EPI50: 50 mg/kg po) 60 min before the intraplantar (ipl) injection of glutamate (10µmol/paw). It was quantified the time in seconds of licking or biting the injected paw for 15 min. The MK 801 (0.03 mg/kg ip) was used as positive control. Animals received vehicle (0.1 mL/10 g) as a negative control (NC). In the study of mechanisms involved in the antinociceptive effect were used naloxone (opioid antagonist, 2 mg/kg sc), glibenclamide (ATP- sensitive channels inhibitor, 2 mg/kg sc) ondansetron (5-HT3 antagonist, 0.3 mg/kg sc), ketanserin (5-HT2A antagonist, 0.5 mg/kg sc), pindolol (5-HT1 and beta adrenergic antagonist, 0.1 mg/kg sc), yohimbine (α2 adrenergic antagonist, 0.15 mg/kg sc), caffeine (A1 antagonist, 3 mg/kg sc), atropine (muscarinic antagonist, 0.1 mg/kg sc) and L-NO arginine (NOS inhibitor, 75 mg/kg ip), and after 60 minutes the animals were stimulated with glutamate and the groups were compared. The protocols were approved by the Ethics Committee in Animal Research with No 011/08. The significant level was considered $p < 0.05$. **Results:** The antinoceptive effect of EPI (EPI50 = 38.86 ± 8.0**, **$p < 0.01$) was reversed by naloxone (98.63 ± 13.67), glibenclamide (76.43 ± 13.81), ketanserin (61.40 ± 6.35), pindolol (92.68 ± 15.39), yohimbine (92.10 ± 13.10) and atropine (98.45 ± 13.07). However, there was no reversal of the EPI50 effect in the presence of caffeine or L-NO arginine. **Discussion:** The results show that the antinociceptive effect of EPI probably involves the opioid, serotonergic and cholinergic systems, but not purinergic or nitrergic participation. **Financial Support:** PROCAD-CAPES/FINEP/RENORBIO/UFPI.

05.034

Preliminary studies of possible mechanisms involved in the antinociception presented by α-terpineol, a major constituent of essential oil from *Protium heptaphyllum* March. resin.

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Introduction: Previous studies at the Medicinal Plants Research Center (UFPI) with the essential oil from Protium heptaphyllum March. resin and its main constituent α-terpineol demonstrated antinociceptive effect in mice. The α-terpineol has also presented anti-inflammatory, antifungal and vasorelaxant activities. This study aimed to evaluate some possible mechanisms involved in the antinociceptive effect of α-terpineol. **Methods:** Male Swiss mice (25-35 g)(n = 6-7) were intraperitoneally treated with Ketanserin (K, 5-HT2A antagonist, 1 mg/kg), Pindolol (P, 5-HT1 and beta adrenergic antagonist, 1 mg/kg) or Naloxone (N, opioid antagonist, 2 mg/kg) before oral administration of water (W, 0.1 mL/10 g) or α-terpineol (T, 12.5 mg/kg). After 1h, all the animals received acetic acid (0.75%, ip) and were observed during 20 minutes when the number of writhings was counted. Morphine (2.5 mg/kg, sc) was used as positive control. **Results:** Antinociceptive effect of α-terpineol was not reversed by Ketanserin (W = 63.5 ± 2.5, K = 55.7 ± 5.4, T = 30.7 ± 4.7, C + T = 42 ± 4.2) or Naloxone (W = 58.2 ± 3.2, N = 50.5 ± 3.7, T = 33.3 ± 4.7, N + T = 23.3 ± 4 2). On the other hand, Pindolol reversed the effect of α-terpineol (W = 62 ± 2.6, P = 57 ± 3.8, T = 23 ± 1.3, P + T = 39.7 ± 3.3) ($p < 0.001$). **Discussion:** These preliminary results suggest a possible involvement of adrenergic (β receptor) or serotonergic (5-HT1, but not 5-HT2A receptor) systems in the antinociceptive effect of α-terpineol, but not of opioidergic one. However, further studies are necessary to investigate other possible mechanisms involved in the monoterpene action. **Financial Support:** CNPq/PROCAD-CAPES/RENORBIO/UFPI.

05.035

Antinociceptive effect of (-) epicatechin obtained from *Combretum leprosum* Mart. & Eicher (Combretaceae) in models of acute pain. Lopes LS[1], Fernandes, HB[1], Pereira SS[1], Marques, RB[1], Ayres MCC[2], Chaves MH[2], Almeida FRC[1] [1]NPPM-CCS-UFPI, [2]CCN-UFPI − Química

Introduction: (-)Epicatechin (EPI), which belongs to the group of flavonoids and catechins obtained from *Combretum leprosum* Mart. & Eicher, is known for its antioxidant property, nutritional value and preventive action in cardiovascular diseases. Recent studies show an effect of blocking sodium channels in vitro, however, there are no studies evaluating the antinociceptive action of this substance in animal models of acute nociception. The present study examined the antinociceptive effects of the EPI obtained from *Combretum leprosum* Mart. & Eicher. **Methods:** Swiss male mice (20-30 g, n = 6-10) were used in the method of writhing (CA) induced by acetic acid (0.75% ip), and quantified the number of contortions during 20 min, 60 min after the treatment with EPI (12.5 − 50 mg/kg po). In the formalin model, animals were treated with EPI (25-50 mg/kg po) 60 min before formalin administration (2%, 20 µL/paw − ipl) and the time (s) spent licking the injected paw that received nociceptive stimulus was quantified during 5 min (1st phase) and 15-30 min (2nd phase). In the capsaicin test mice (n = 6-10) were given EPI (12.5 − 50 mg/kg po) 60 min before administration of capsaicin (20 µL, 2 µg/paw). Nociception was evaluated immediately after injection and quantified by paw licking time during a 5 minutes period. Morphine (2.5 or 5 mg/kg sc) was used as positive control in these protocols. A volume of 20 µL of glutamate (10 µmol/paw i.pl.) was injected in the hind paw 60 min after the treatment with EPI (25-50 mg/kg po) and animals were observed individually for 15 min following glutamate injection. The amount of time they spent licking the injected paw was recorded and considered as indicative of nociception. The MK 801 (0.03 mg/kg ip) was used as positive control. All the animals received vehicle (0.1 mL/10 g) as negative control (NC). The protocols were approved by the Ethics Committee in Animal Research with No 011/08. The significant level was considered $p < 0.05$. **Results:** Administration of EPI reduced the nociceptive response in the writhing model at different doses (EPI25 = 20.0 ± 4.6 ***, EPI 50 = 21.0 ± 5.1 **, CN = 52.8 ± 3.9, ** $p < 0.01$). In the formalin test, the EPI was effective only in the second phase of testing at 25 mg/kg (EPI25 = 48.4 ± 11.2 *, CN = 94.7 ± 14.4, * $p < 0.05$). In the capsaicin model EPI was effective in two doses (EPI25 = 13.1 ± 2.2 ***, EPI50 = 10.3 ± 3.1 ***, CN = 35.6 ± 2.4, *** $p < 0.001$) and in the glutamate induced nociception, the effect of EPI was only observed at a dose of 50 mg/kg (EPI50 = 38.86 ± 8.0 **, CN = 91.6 ± 8.0, ** $p < 0.01$). **Discussion:** The EPI was effective in different models of chemically induced acute pain in mice.

05.036

Antinociceptive activity of new isatin derivatives. Figueiredo GSM[1], Zardo RS[1], Silva BV[2], Matheus ME[1], Pinto AC[3], Fernandes PD[1] [1]UFRJ − Farmacologia Básica e Clínica, [2]IQ-UFRJ − Química Orgânica, [3]UFRJ − Química

Introduction: Isatin (1H-indol-2,3-diona) is an indole substance, endogenous, present in mammalian tissues and fluids. Structurally, it's very versatile for the synthesis of new compounds. Moreover, its synthesis is easy and with high yield. Isatin and its derivatives have several biological activities including anti-inflammatory and antinociceptive actions. In this study our objectives were to evaluate the antinociceptive activity and the possible mechanism of action of new isatin derivatives. **Methods:** The antinociceptive activity was evaluated in models of abdominal writhing induced by acetic acid (2%) and formalin (2.5%). Swiss mice (males, 18-22 g, n = 5-8) were orally pre-treated with the isatin derivatives (ISA003, ISA127, or ISA147) at doses that varied between 0.1 and 10 mg/kg. To evaluate the mechanism of action, animals were pre-treated com nitric oxide pathway inhibitor (L-NAME, 3 mg/kg, ip) or opioid antagonist (naloxone, 1 mg/kg, ip) 30 min prior to isatin derivatives administration. The results are presented as the mean ± S.D. and statistical analysis was ANOVA followed by Bonferroni (*p<0,05). The experimental protocols were approved by the Ethics Committee in Animal Experimentation from Health Sciences Center (# ICBDFBC-015). **Results:** In the model of abdominal writhing, an inhibitory effect was observed for all substances at the doses of 0.1 mg/kg (48.3 ± 3.0 in control group vs 30.5 ± 1.7*; 15.8 ± 2.8; 28.8 ± 2.2 in animals treated with ISA003, ISA127, or ISA147, respectively), 1 mg/kg (48.3 ± 3 in control group vs 33.7 ± 4.3*; 24.5 ± 1.8; 32 ± 4.2 in animals treated with ISA003, ISA127, or ISA147, respectively, or 10 mg/kg (48.3 ± 3.0 at control group vs 19.3 ± 2.6*; 33.0 ± 1.3*; 35.8 ± 4.1* at groups treated with ISA003, ISA127, or ISA147, respectively). An antinociceptive effect was also observed in the second phase of the licking response induced by intraplantar injection of formalin. In this model, ISA003 (267.1 ± 48.1 sec in control group vs 68.1 ± 9.3* sec; 181.6 ± 13.7* sec; and 155.5 ± 5.3 sec in animals treated with 0.1 mg/kg, 1 mg/kg, and 10 mg/kg) and ISA127 (267.1 ± 48.1 sec in control group vs 129.4 ± 16.7* sec; 135.2 ± 9,8* sec; and 101.7 ± 8.2* sec in animals treated with 0.1 mg/kg, 1 mg/kg, and 10 mg/kg). Although naloxone has not reversed the anti-hipernociceptive effect of any isatin derivative in the abdominal writhing model, the pre-treatment of mice with L-NAME was able to reverse the antinociceptive activity of 1 mg/kg ISA127 (48.3 ± 3 in control group, 24.5 ± 1.8 in ISA127-treated mice vs 33.7 ± 2.9 in L-NAME pre-treated mice). Conclusions: Our results suggest that the new molecules derivates from isatin (ISA003, ISA127, and ISA147): 1) have significant antinociceptive activity; 2) at least part of ISA127 effect is mediated by nitric oxide pathway; 3) the ability in reducing the licking response at the 2nd phase of the formalin model indicates a possible anti-inflammatory activity. Financial Support: CNPq, CAPES, FAPERJ.

05.037

Involvement of adenosinergic system in the antinociceptive effect of ethanolic extract of *Cipura paludosa* Aubl. in mice. Macedo Junior SJ[1], Lucena GMRS[2], Nascimento FP[3], Cerutti M[3], Santos ARS[1] [1]UFSC − Ciências Fisiológicas, [2]UnB − Ciências da Saúde, [3]UFSC − Farmacologia

Introduction: The genus Cipura belong to the family Iridaceae, comprises nine species, distributed from southern Mexico in the north to Bolivia, southern Brazil and south of Paraguay. The tea of *Cipura paludosa* bulbs' is recommended in traditional medicine for the treatment of inflammatory and kidney diseases, dolorous process and diarrhoeal. Our group already demonstrated the antinociceptive and anti-inflammatory activity of ethanolic extract (EE) of *C. paludosa* in a several models of nociception, besides elucidating the participation of some mechanisms involved in this activity. The present study investigated the role of adenosinergic system in the antinociceptive effect of EE of *C. paludosa* in the glutamate-induced nociception. **Methods:** Experiments were conducted using Swiss mice (25-35g). First, animals were treated with EE of *C. paludosa* by intraperitoneal pathway (i.p.) in the doses of 0.3, 1, 3 and 10 mg/kg and by oral pathway (p.o.) in the doses of 10, 30, 100 and 300 mg/kg. After 25 minutes animals received 20µL of glutamate (10 µmol/site) by intraplantar pathway. After the obtaining of the curve dose-response of the EE of *C. paludosa*, we were able to investigate the involvement of adenosinergic system in this effect. For this, mice were pre-treated with caffeine (a non-selective antagonist of adenosine receptors, 10 mg/kg, i.p.) and after 20 minutes animals received an injection of EE (3 mg/kg, i.p.), adenosine (10 mg/kg. i.p.) or vehicle (10ml/kg, i.p.). Finally, after 25 minutes animals received an intraplantar injection of glutamate 10 µmol/site. The time spend licking and/or biting the injected paw was considered indicative of nociception, which was evaluated from 0 to 15 minutes. All the protocols were approved by Ethics Committee for Animals Use UnB under the following number: 33897/2009. **Results:** EE of *C. paludosa*, administrated i.p. and p.o, inhibited in a dose-dependent way the glutamate-induced nociception, with a ID50= 2,7 (1.6–4.6) and 76 (69.5–84.5) mg/kg and inhibition of 77±6 and 67±6%, respectively. The antinociceptive effect promoted by this EE was totally reverted by the pre-treatment with caffeine. **Discussion:** The present study demonstrated that systemic administration (i.p. or p.o.) of EE obtained from *C. paludosa* bulbs' inhibited in a dose-dependent way the glutamate-induced pain in mice. Besides this, those results indicate that the antinociceptive action of the EE of *C. paludosa* depends, in part, on the interaction with adenosinergic system. **Financial Support:** CNPq and CAPES.

05.038

Direct blockade of inflammatory hypernociception by peripheral activation of the A1 adenosine receptor: involvement of the NO/cGMP/PKG/KATP signaling pathway. Cunha TM[1], Lima FO[1], Souza GR[1], Verri Jr WA[2], Parada CA[3], Ferreira SH[1], Cunha FQ[1] [1]FMRP-USP − Farmacologia, [2]UEL − Ciências Patológicas, [3]UNICAMP − Farmacologia, [5]FMRP-USP

Introduction: Through activation of the A1 adenosine receptor (A1R) at both the central and peripheral level, adenosine produces antinociception in a wide range of tests. However, the mechanisms involved in the peripheral effect are still not fully understood. Therefore, the mechanisms by which peripheral activation of A1R reduces inflammatory hypernociception (a decrease in the nociceptive threshold) were addressed in the present study. **Methods:** and **Results:** Experiments were carried out with male Wistar rats (180 − 200 g, n = 5 – 6 per group). The mechanical nociceptive threshold was evaluated an electronic pressure meter in rats. Immunofluorescence of rat dorsal root ganglion revealed significant expression of A1R in primary sensory neurons associated with nociceptive pathways. Functionally, peripheral activation of A1R down-regulated inflammatory hypernociception because intraplantar (i.pl.) administration of an A1R antagonist (DPCPX) enhanced carrageenan-induced paw hypernociception. On the other hand, local (paw) administration of CPA (a selective A1R agonist) reversed mechanical hypernociception induced by carrageenan or by the directly acting hypernociceptive mediator prostaglandin E2 (PGE2). Down-regulation of A1R expression in primary nociceptive neurons by intrathecal treatment with antisense oligodeoxinucleotides significantly reduced the peripheral antinociceptive action of CPA. Direct blockade of PGE2 inflammatory hypernociception by activation of A1R depends on the nitric oxide/cGMP/PKG/KATP signaling pathway, because the peripheral antinociceptive effect of CPA was prevented by pretreatment with inhibitors of neuronal nitric oxide synthase (N-propyl-L-arginine), guanylyl cyclase (ODQ), and PKG (KT5823) as well as with a KATP blocker (glibenclamide). However, this effect of CPA was not reduced by naloxone, excluding the participation of endogenous opioids in the mechanism of A1R. Discussion These results suggest that peripheral activation of A1R plays a role in the regulation of inflammatory hypernociception by a mechanism that involves the NO/cGMP/PKG/ATP-sensitive K+ channel intracellular signaling pathway. **Financial Support:** This work was supported by grants from CNPq and FAPESP.

05.039

Ropivacaine gel for topical anesthesia: *in vitro* permeation skin and cytotoxic effects. Stoco SM[1], Grillo R[2], Mello NFS[2], Guilherme VA[1], Franz-Montan M[1], Tófoli GR[3], Fraceto LF[2], de Paula E[1], de Araújo DR[4] [1]UNICAMP − Bioquímica, [2]UNESP – Engenharia Ambiental, [3]UNIFAG − Farmacologia Clínica, [4]CCNH-UFABC − Farmacologia

Introduction: Topical anesthesia is widely used to reduce discomfort and pain associated to dermatological procedures such as venipuncture, curettage and biopsy. Commercially available local anesthetics (LA) present slow absorption or permeation across the skin providing ineffective anesthetic and/or analgesic effects. In order to overcome this problem it is necessary to minimize the stratum corneum barrier optimizing the skin permeation of LA agents [Harmatz A. Surg. Clin. North Am. v. 89, p.587, 2009]. Ropivacaine (RVC) is a long-acting amino-amide novel LA largely used on surgical procedures. In recent study, a liposomal-encapsulated RVC gel was efficient to reduce pain during needle insertion for anesthesia in Dentistry [Franz-Montan M. Anesth. Analg. v. 104, p. 1528, 2007]. However, topical anesthesia with RVC was not studied considering its skin permeation properties and cytotoxic effects. Since RVC is a long-acting LA not associated to allergic reactions or methemoglobinemia, such as benzocaine and prilocaine, traditional topically used LA, in this study we used a combination of penetration enhancers to evaluate the skin permeation and cytotoxic effects of RVC from different gel-based new formulations. **Methods:** Acrylic acid based-gels containing 2% RVC were prepared with different penetration enhancers: 15% (polyethylene glycol, PEG 400 or 600, 0.5% Span 20®, 5% menthol alone or in combination). Alternatively to those conventional penetration enhancers, a gel containing 2% RVC encapsulated in alginate-chitosan nanoparticles was also prepared. Permeation assays were performed during 6 h using vertical Franz-type diffusion cells with 0.6 cm2 permeation area. At predetermined time intervals, aliquots were withdrawn and analyzed by HPLC for drug content determination. Pig ear full thickness skin was obtained from a local slaughter-house and served as barrier for all experiments. Cytotoxicity was evaluated in cultured-3T3 fibroblasts by the tetrazolium reduction test (MTT test) for all formulations (0.42 to 8.04 mM). **Results and Discussion:** From the permeation profiles (infinite-dose condition) flux, lag time and permeability coefficient were calculated. The flux values obtained for the 2% RVC in PEG400/PEG600 (8.17 ± 1.35 µg.cm-2), nanoparticles (6.23 ± 0.49 µg.cm-2) and mentol (9.44 ± 1.03 µg.cm-2) gels were similar, but significantly different when compared to PEG400 (2.84 ± 0.35 µg.cm-2) or PEG600 gels (2.01 ± 0.3 µg.cm-2; $p<0.001$). The addition of PEG400/PEG600 combination or menthol significantly reduced the time lag (0.66 ± 0.21 h and 0.76 ± 0.09 h, respectively) in relation to the other formulations ($p<0.001$). Cytotoxicity assays showed that the PEG400 or PEG600-Span 20® association reduced the cell viability (25% cell viability, $p<0.01$). The use of solubilizers and permeation enhancers, such as PEG and menthol (alone or in combination) enhanced also the percentage of permeated RVC from different gels, possibly increasing the solubility of the drug into the formulation and its concentration gradient in solution. Results from our study indicate the possible use of RVC-gels for topical anesthesia and pain treatment associated to dermatological procedures. Acknowledgements: Fapesp (06/00121-9) and Oxiteno Ind. Prod. Quim.

05.040

The armed spider toxin TX3-3 restores the analgesic effect of morphine in neuropathic and opioid-tolerant mice. Dalmolin GD[1], Rigo FK[1], Silva CR[2], Gomez MV[1], Ferreira J[2] [1]UFMG − Farmacologia, [2]UFSM − Química

Introduction: It has generally been accepted that neuropathic pain is somewhat resistant to alleviation by morphine both in animal experimental models and in clinical studies. Besides, compelling evidence indicate that similar mechanisms within the spinal cord are implicated in development of tolerance to analgesic effect of morphine and hyperalgesic state induced by neuropathy. In fact, continuous morphine administration leads to a paradoxical hyperalgesia. Here, we tested the effect of the blockade of spinal voltage dependent calcium channels (VDCC), by intrathecal administration of the armed spider toxin Tx3-3 in morphine analgesic effect in opioid-tolerant and neuropathic hyperalgesic state. **Methods:** Neuropathic pain was achieved by tying one-third to one-half of the dorsal portion of the mice right sciatic nerve, using a procedure previously described by Malmberg and Basbaum (1998). Opioid tolerance was induced by three days repeated morphine administration, according to Marshall and Weinstock (1971). After neuropathy induction (7 days post-surgical procedure) or repeated morphine administration, male and female adult Swiss mice were pre-administered with the VDCC blocker Tx3-3 (30 pmol/site) or the calcium/calmodulin-dependent protein kinase II (CaMKII) inhibitor KN62 (10 nmol/site) by intrathecal route 15 min before intraperitoneal morphine injection (10 mg/kg). Antinociception was assessed by tail-flick test. Results are expressed in seconds (s) or as maximum possible effect (MPE%). All protocols employed have been approved by the Ethics Committee of UFSM (process number: 23081.005024/2010-88). **Results and Discussion:** Both experimental model of neuropathy and repeated morphine administration determined a hyperalgesic state, denoted as a reduction in tail flick latency in relation to baseline latency (the latency fell from 11.5±1.95 to 6.22±0.78s and from 10.8±1.6s to 4.5±0.5s in neuropathic and tolerant mice respectively). Administration of a usual analgesic dose of morphine (10 mg/kg) did not alter neuropathic or opioid-induced hyperalgesia (the MPE% values before and after systemic morphine was -0.4±1.9 and 2.6±3.6 or 1.6±2.0 and 0.5±3.5 for neuropathic or tolerant mice, respectively). Previous intrathecal administration of Tx3-3 (30 pmol/site) reestablished the morphine effect in animals treated chronically with opioid (37.3±8.3 and 30.0±11.0 of MPE% after systemic morphine for non tolerant mice and tolerant mice pre-treated with Tx3-3) and allowed morphine analgesic effect in neuropathic mice (2.6±3.6 and 53.8±11.5 of MPE% after systemic morphine for neuropathic mice pre-treated with PBS and Tx3-3, respectively). Similarly, previous inhibition of the enzyme CaMKII, by intrathecal administration of KN62 (10 nmnol/site), restored morphine effect in opioid tolerant (88.1±11.9 and 71.6±11.9 of MPE% after systemic morphine for non tolerant mice and tolerant mice pre-treated with KN62 respectively) and neuropathic animals (0.9±2.8 and 47.8±9.5 of MPE% after systemic morphine for neuropathic pre-treated with PBS and KN62, respectively). These data show that previous blockade of VDCC, and ultimately the lack of CaMKII activation by calcium, enable morphine analgesia in hyperalgesic states usually unresponsive to opioids. These preliminary results from bench could help in management of neuropathic pain in the bedside, where its painful state tends to exhibit a relatively poor response to traditional analgesics. References: Malmberg AB, J Neurosc 14:4882 (1994); Marshall I, Nature 234: 223 (1971). Fellowship: Instituto do Milenio MCT/CNPq, Capes, Pronex, Fapemig.

05.041

Celecoxib induces analgesia by release of B-Endorphin in rat paws. Paiva-Lima P[1], Queiroz Junior CM[1], Rezende RM[2], Machado-Silva LDF[2], Caliari MV[3], Bakhle YS[4], Francischi JN[1] [1]UFMG − Farmacologia, [2]UFMG − Fisiologia e Farmacologia, [3]UFMG − Patologia, [4]Imperial College − Leukocyte Biology

Introduction: We have previously suggested that the hypoalgesia induced by selective cyclooxygenase (COX)-2 inhibitors (Francischi et al., Br. J. Pharmacol. 137:837; 2002), was related to endogenous opioids release (França et al., Neuropharmacol. 51:37; 2006). Therefore, the aim of the present study was verify whether β-endorphin (β-END) could be one of the opioids released peripherally by celecoxib (CX). **Methods:** Based on study of Ibrahim et al. (PNAS. 102(8):3093; 2005) who have shown the release of β- END through the activation of type 2 cannabinoid receptors (CB2) on cultures of keratinocytes, an immunohystochemical analysis was conducted to determine the β-END content in the epithelial cells from skin of the inflammation site (rat paws) under various experimental conditions. The immuno-procedures were followed, in parallel, by mechanical nociceptive response measurements (Randall & Selitto, Arch. Intern. Pharmac.; 113:233; 1957) of the study rats under the same experimental conditions. All animal procedures were approved by the UFMG Ethics Committee for Animal Experimentation (180/07). For this, Holtzman rats (180-210 g) were initially intraplantarly (i.pl.) injected with opioid or cannabinoid antagonists or respective vehicles at -30 min, followed by CX, β-END or JWH015 (an agonist of CB2 receptors) at -5 min, and by carrageenan (CG; 250 µg) at time zero. Both procedures were performed at time 30, 60 and 120 min following CG injection. The total content of β-END on the paw excised tissues was also obtained by an immunoenzymatic assay at timepoint 60 min. **Results and Discussion:** CG administration in rat paws induced a drastic increase on the β-END content as compared with control skin samples at all measured times, which was related to hyperalgesia at definite timepoints. The CX or the compound JWH015 reduced the β-END imunnostaining in samples from rat paws injected with CG, this reduction was related to the occurrence of hypoalgesia. Moreover, the compound SR144528 (SR; an antagonist of CB2 receptors) prevented the reduction on β-END imunnostaining by CX only at time 30 and 60 min, which was timely-related with prevention of CX-induced hypoalgesia. Moreover, direct administration of β-END, JWH015 or CX induced similar hypoalgesia in rat paws inflamed by CG, which peaked at 15, 30 and 60 min, respectively. Conclusions: This study suggests that CX induces hypoalgesia, at least partially, by the release of β-END at the inflammatory site, in rat paws. It is also suggested that the release of β-END by CX derives from the activation of CB2 receptors located on keratinocytes, present in skin of the peripheral site of inflammation. **Financial Support:** Capes, CNPq and FAPEMIG.

05.042

Antinociceptive and anti-inflammatory effect of electroacupuncture in zymosan-induced arthritis in the rat temporomandibular joint. Gondim DV[1], Chaves, HV[1], Costa JL[2], Rocha SS[2], Brito GAC[3], Vale ML[2] [1]UFC − Medicina Clínica, [2]UFC − Fisiologia e Farmacologia, [3]UFC − Morfologia

Introduction: Electroacupuncture (EACP) activates neuroendocrine responses and is a method used in acute and chronic-degenerative diseases. This work verifies the antinociceptive and anti-inflammatory effect of electroacupuncture in the zymosan (Zy) - induced acute arthritis in the rat temporomandibular joint (TMJ). **Methods:** Wistar male rats (160- 220g) were utilized and separated in 4 groups of 6 animals. This work was submitted to Animal Ethical and Research Committee of the Federal University of Ceará (protocol number 027/10). Saline (40µl) or Zy (control group; 2mg; 40µl) were administrated at the left TMJ. EACP (rectangular pulses; f1=10Hz; f2=15Hz, 3mA, 30min) was done in the acupoints (LI4, LI11, ST36, ST44) or sham points (random points in pelvic region) 2h after Zy administration. Von Frey test was used to assess mechanical hipernociception (4th hour) and the animals were sacrificed 6h after Zy administration. After this time, the joint was removed for histopathological analysis, myeloperoxidase (MPO) activity, vascular permeability observation and to verify immunohistochemical evidence of inflammatory and nociceptive mediators. **Results:** The results show that EACP inhibited hypernociception induced by zymosan by 122% ($p<0,01$) when compared to control group and 64% when compared to sham group ($p<0,05$). In the evaluation of the inflammatory parameters EACP inhibited the neutrophil migration in MPO assay with 68 % MPO activity inhibition when compared to control group and 63% when compared to sham group ($p<0,05$). In the edema evaluated by vascular permeability, EACP inhibited the Evans' blue extravasation in 145 % when compared to sham group ($p<0, 05$). The histopathological analysis showed that EACP significantly inhibited the edema and periarticular infiltrate ($p< 0, 05$) when compared to control and sham groups. EACP showed immunohistochemical detection to TNF ($p< 0,05$) when compared to control group, to COX-2 ($p< 0,05$) when compared to control and sham groups and to NOSi ($p< 0,01$) when compared to control and sham groups. **Discussion:** The Traditional Chinese Acupuncture literature emphasizes the precise localization and correct selection of acupoint combinations to elicit an adequate therapeutic response. Our data suggest that EACP on the points LI4, LI11, ST36 and ST44 produced an antinociceptive and anti-inflammatory effect in zymosan-induced acute arthritis in rat TMJ when compared sham EACP **Financial Support:** FUNCAP and CAPES

05.043

Macrophage Migration Inhibitory Factor (MIF) is involved in a cascade of events leading to inflammatory hypernociception in mice. Costa VV[1], Amaral FA[2], Sachs D[3], Tavares LD[4], Scopa IP[2], Morcatty TQ[1], Teixeira MM[1], Souza DG[2] [1]UFMG − Bioquímica e Imunologia, [2]UFMG − Microbiologia, [3]FMRP-USP − Farmacologia, [4]UFMG − Fisiologia e Farmacologia

Introduction: Different types of inflammatory mediators are involved in nociceptor sensitization (hypernociception). After carrageenan injection into the paw in mice, a sequential cascade of mediators is triggered (participation of TNF-α, IL-1β and CXCL1) leading to the releasing of final products (prostaglandins and sympathetic amines) that act on peripheral nerve endings promoting hypernociception. MIF is an important pro-inflammatory cytokine that participate of many inflammatory conditions. Thus, its properties suggest a correlation with that cytokine and inflammatory hypernociception. Objectives: Our aim was to analyze the participation of cytokine MIF in the development of inflammatory hypernociception in mice. **Methods:** This project was previously approved by CETEA/UFMG on number access 165/2008. Hypernociception was quantified using an electronic version of the von Frey filament test in wild type (BALB/c) and MIF-deficient (MIF-/-) male mice before and after the i.pl injection of MIF (10, 30 or 300 ng/paw) or Carrageenan (CG, 100 µg/paw). In another set of experiments, treatments with Il-1 receptor antagonist (IL-1ra; 500 ng/paw), dexamethasone (5 mg/paw), CXCR2 allosteric antagonist (DF2162; 15 mg/kg) or vehicle (sterile saline, 200 ml) were given 30 minutes before MIF (100 ng/paw) and hypernociception quantified after 3 hours. **Results and Discussion:** MIF was able to induce hypernociception in a dose and time-dependent manner. Mechanical hypernociception was reduced in MIF-/- mice after CG injection compared to WT ones. Still, the treatment with IL-1ra, dexamethasone or DF2162 reduced the hypernociception induced by MIF injection. Altogether, these results suggest that MIF has an important participation of inflammatory hypernociception and it is inserting in the cascade of mediators initiated by carrageenan injection. **Financial Support:** CNPq , Fapemig and CAPES.

05.044

Role of TRPV1 and NK1 receptors on nociception and edema induced by monosodium urate crystals in rats (MSU). Trevisan G, Rossato M, Hoffmeister C, Ferreira J UFSM − Química

Introduction: Gout is characterized by the deposition of monosodium urate (MSU) crystals. Despite being one of the most painful forms of arthritis, gout and the mechanisms responsible for its acute attacks are poorly understood. Therefore, the present study aimed to investigate the role of TRPV1 and NK1 receptors on the nociceptive and edematogenic response to MSU crystals. **Methods:** Adult male Wistar rats (200-300 g) were used in all experiments. All experiments were approved by the local ethics committee of our university (process number: 23081.003640/ 2009-61). To observe the possible nociceptive and edematogenic effects produced by MSU, 100 µl of MSU suspension (0.015-2 mg/paw) or vehicle (PBS) was administered subcutaneously (s.c.) under the plantar surface of the right hind paw of unanesthetized animals. Animals were placed individually in chambers (transparent glass) and adapted for 20 min before injection. The number of flinches responses were observed during 10 minutes after the injection and it was recorded and considered as indicative of nociception. The edema formation was assessed as an increase in paw thickness measured by a digital caliper after the s.c. injection of MSU or vehicle. To investigate the possible involvement of TRPV1 and tachykinin NK1 receptors in the spontaneous pain and edema produced by MSU, SB 366791 (10 nmol/paw, a selective TRPV1 receptor antagonist) and the NK1 receptor antagonist RP 67580 (20 nmol/paw), were co-injected s.c. with MSU (0.25 mg/paw), and nociception and edema were observed as described above. As a positive controls we also evaluated the effect of SB 366791 and RP 67580 on nociception and edema caused by capsaicin (0.01 or 1 nmol/paw, respectively) and substance P (0.1 nmol/paw), respectively. To further explore the role of capsaicin-sensitive fibers in the nociceptive and edematogenic effect induced by MSU, animals were submitted to a perineural capsaicin desensitization protocol. First, animals were anesthetized with ketamine (90 mg/kg) and xylazine (3 mg/kg), and an incision was made over the hip joint to expose the sciatic nerve. Once isolated, 10 µL of 2% capsaicin or vehicle (10% ethanol, 10% Tween 80 and 80% PBS) was injected directly inside the sheath covering the nerve using a micro-syringe. After 7 days, animals were submitted to a subcutaneous injection of MSU (0.25 mg/paw), capsaicin (0.01 nmol/paw, used as positive control) or PBS (100 µl/paw). **Results and Discussion**: In the present study, we found that MSU caused dose-related nociception (DE50=0.04 (0.01-0.11) mg/paw) and edema (DE50=0.08 (0.04-0.16) mg/paw) when injected into the hind paw of rats. Treatment with the selective TRPV1 receptor antagonist SB366791 largely inhibited nociceptive and edematogenic responses to MSU, with calculated DI50 values of 0.34 (0.07-4.22) nmol/paw and 0.07 (0.001-5.8) mg/paw and maximal inhibition of 90±6% and 100%, respectively. The NK1 receptor antagonist RP 67580 (20 nmol/paw) partially reduced MSU-induced nociceptive (55±9%) and edematogenic (48±6%) responses. Moreover, the desensitization of capsaicin-sensitive afferent fibers also significantly reduced MSU-induced nociception and edema, in 88±4% and 50±13%, respectively. Collectively, the present findings demonstrate that MSU produces a nociceptive and edematogenic response and these effects might be mediated by TRPV1 and NK1 receptors activation. **Apoio financeiro**: CNPq, CAPES.

05.045

The antinociception observed during glycogen-induced inflammation in rat paws is mediated by neutrophil migration and is independent of opioid peptides. Nogueira TO[1], Spadacci-Morena DD[1], Santoro ML[1], Pagano RL[2], Giorgi R[1] [1]IBu − Fisiopatologia, [2]IEP-HSL

Introduction: Glycogen-induced peritonitis has been shown to induce antinociception in mice evaluated by the test of abdominal contortion (Pagano et al., Mediators Inflamm 36765:1, 2006). Herein, nociception control by neutrophils was investigated after intraplantar (i.pl.) injection of glycogen in rats, using the paw pressure test. The involvement of opiod peptides in such effect was also investigated. The procedures were approved by Butantan Institute Ethics Committee (protocol, 529/08). **Methods:** Male Wistar rats were injected or not with fucoidan, which inhibits neutrophil migration, (5 mg/kg, i.v., 500 µL/animal) 15 min before i.pl. injection of 5% glycogen solution (100 µL/animal). At different periods of time, the paw pressure test was evaluated. The histological analysis of plantar tissue was undertaken using the same protocol described above. To verify the participation of opioid peptides in glycogen effect, naloxone (1 mg/kg, s.c.) was administrated 15 min before evaluating nociception in animals previously injected with glycogen. In other experiment, naloxone was injected i.pl. 15 min before glycogen administration. In both protocols animals were submitted to the nociceptive test at 2, 3 and 4 h after glycogen injection. **Results:** Glycogen induced an increase in pain threshold at 2 (16%), 4 (26%), 6 (36%), 8 (26%) and 12 h (22%) in comparison with the control group injected with saline i.pl. ($p<0.001$), indicating the antinociceptive effect of glycogen. The pre-treatment with fucoidan inhibited antinociception between 2 and 6 h after glycogen injection, compared with rats injected only with glycogen, and induced hyperalgesia in 2 (38%), 4 (32%) and 6 h (28%) in comparison with control group ($p<0.001$). At 8 h after the glycogen injection, it was only observed the reversion of antinociception in 25% in rats pre-treated with fucoidan and submitted to the nociceptive test. At 12 h after glycogen administration, the pre-treatment with fucoidan failed to inhibit antinociception. The qualitative histological analysis of plantar tissue demonstrated an increase in migration of polymorphonuclear cells between 2 and 8 h after glycogen administration, which was inhibited in rats pre-treated with fucoidan. Neither subcutaneous nor intraplantar injection of naloxone interfered in the glycogen antinociceptive effect in any time assessed. **Discussion:** The intraplantar injection of glycogen induced antinociception in rats evaluated by the paw pressure test. Pre-treatment with fucoidan not only reverted glycogen-induced antinociception, but also induced hyperalgesia in initial time periods of the inflammatory process. Neutrophils were the predominant cells accumulated in footpads after glycogen administration, and fucoidan induced a reduction in migration of these cells to the inflamed tissue. Once the administration of naloxone did not inhibit glycogen-induced antinociception, it is plausible to suggest that opiod peptides did not participate in the observed effect. Thus, glycogen likely induces antinociception in rats by inducing accumulation of polymorphonuclear cells, particularly neutrophils, and such effect is not related to the release of opiod peptides. Supported by: FAPESP, Fundação Butantan

05.046

Ropivacaine gel for topical anesthesia: *in vitro* permeation skin and cytotoxic effects. Stoco SM[1], Grillo R[2], Mello NFS[2], Guilherme VA[1], Franz-Montan M[1], Tófoli GR[3], Fraceto LF[2], de Paula E[1], de Araújo DR[4] − [1]UNICAMP − Bioquímica, [2]UNESP – Engenharia Ambiental, [3]UNIFAG − Farmacologia, [4]CCNH-UFABC − Farmacologia

Introduction: Topical anesthesia is widely used to reduce discomfort and pain associated to dermatological procedures such as venipuncture, curettage and biopsy. Commercially available local anesthetics (LA) present slow absorption or permeation across the skin providing ineffective anesthetic and/or analgesic effects. In order to overcome this problem it is necessary to minimize the stratum corneum barrier optimizing the skin permeation of LA agents [Harmatz A. Surg. Clin. North Am. v. 89, p.587, 2009]. Ropivacaine (RVC) is a long-acting amino-amide novel LA largely used on surgical procedures. In recent study, a liposomal-encapsulated RVC gel was efficient to reduce pain during needle insertion for anesthesia in Dentistry [Franz-Montan M. Anesth. Analg. v. 104, p. 1528, 2007]. However, topical anesthesia with RVC was not studied considering its skin permeation properties and cytotoxic effects. Since RVC is a long-acting LA not associated to allergic reactions or methemoglobinemia, such as benzocaine and prilocaine, traditional topically used LA, in this study we used a combination of penetration enhancers to evaluate the skin permeation and cytotoxic effects of RVC from different gel-based new formulations. **Methods:** Acrylic acid based-gels containing 2% RVC were prepared with different penetration enhancers: 15% (polyethylene glycol, PEG 400 or 600, 0.5% Span 20®, 5% menthol alone or in combination). Alternatively to those conventional penetration enhancers, a gel containing 2% RVC encapsulated in alginate-chitosan nanoparticles was also prepared. Permeation assays were performed during 6 h using vertical Franz-type diffusion cells with 0.6 cm2 permeation area. At predetermined time intervals, aliquots were withdrawn and analyzed by HPLC for drug content determination. Pig ear full thickness skin was obtained from a local slaughter-house and served as barrier for all experiments. Cytotoxicity was evaluated in cultured-3T3 fibroblasts by the tetrazolium reduction test (MTT test) for all formulations (0.42 to 8.04 mM). **Results and Discussion:** From the permeation profiles (infinite-dose condition) flux, lag time and permeability coefficient were calculated. The flux values obtained for the 2% RVC in PEG400/PEG600 (8.17 ± 1.35 µg.cm-2), nanoparticles (6.23 ± 0.49 µg.cm-2) and mentol (9.44 ± 1.03 µg.cm-2) gels were similar, but significantly different when compared to PEG400 (2.84 ± 0.35 µg.cm-2) or PEG600 gels (2.01 ± 0.3 µg.cm-2; $p<0.001$). The addition of PEG400/PEG600 combination or menthol significantly reduced the time lag (0.66 ± 0.21 h and 0.76 ± 0.09 h, respectively) in relation to the other formulations ($p<0.001$). Cytotoxicity assays showed that the PEG400 or PEG600-Span 20® association reduced the cell viability (25% cell viability, $p<0.01$). The use of solubilizers and permeation enhancers, such as PEG and menthol (alone or in combination) enhanced also the percentage of permeated RVC from different gels, possibly increasing the solubility of the drug into the formulation and its concentration gradient in solution. Results from our study indicate the possible use of RVC-gels for topical anesthesia and pain treatment associated to dermatological procedures. Acknowledgements: Fapesp (06/00121-9) and Oxiteno Ind. Prod. Quim.

05.047

Cannabinoid and opioid receptors activation induces peripheral antinociception by noradrenaline release and α_{2C} adrenoceptor interaction. Romero TRL[1], Duarte IDG[2]
[1]UFMG − Fisiologia e Farmacologia, [2]UFMG − Farmacologia

Introduction: Cannabinoid and opioid agonists induce peripheral antinociception by several different mechanisms in the presence or absence of inflammation. The interaction of cannabinoidergic and opioidergic systems with adrenergic system has been described. Beyond its peripheral pronociceptive effect in primary afferent nociceptors, noradrenaline also contribute in part to peripheral antinociception by an interaction with the immune system. Thus, the aim of this study was verified if cannabinoid and opioid agonists could induce the peripheral antinociception by noradrenaline release and α2 adrenoceptor activation. **Methods:** The rat paw pressure test was used and hyperalgesia was induced by intraplantar injection of prostaglandin E2 (2 µg/paw). All drugs were administered locally into the right hind paw of Wistar male rats. **Results:** CB1 (anandamide, 50 ng/paw) and CB2 (PEA, 20 µg/paw) agonists, µ (morphine, 40 µg/paw), δ (SNC80, 20 µg/paw) and κ (bremazocine, 20 µg/paw) opioid agonists elicited a local antinociception. The non selective α2 adrenoceptor antagonist yohimbine (05, 10 and 20 µg/paw) and the selective α2C adrenoceptor antagonist rauwolscine (10, 15 and 20 µg/paw) inhibited the antinociceptive effect of all drugs used. The selective α2A-B-C adrenoceptor antagonists BRL 44 480, imiloxan and RX 821002, respectively, were ineffective at blocking the effect of a local drugs injection. In addition, using guanethidine (30 mg/Kg; intraperitoneal) to depletion of peripheral noradrenaline, it was verified a reduction (30-50%) of antinociceptive effect of all drugs tested. **Discussion:** The results provide evidences that cannabinoid and opioid agonists probably induce their peripheral antinociceptive effect by release of endogenous noradrenaline and selective activation of the α2C adrenoceptors. **Financial Support:** CNPq (473758/2007-5), FAPEMIG and fellowships from CNPq. Ethics Committee on Animal Experimentation (CETEA/UFMG) protocol No. 41/2007.

05.048

Antinociceptive effects of *Parkia platycephala* Benth in diabetic rats. Amorim VR[1], Brito SRMC[2], Sales Filho HLA[3], Piauilino, CA[4], Chaves MH[5], Bezerra RDS[5] [1]UFPI − Farmacologia, [2]UFPI − Bioquímica e Farmacologia, [3]UFPI − Farmacologia, [4]UFPI-NPPM-UFPI, [5]UFPI − Química

Introduction: *Parkia platycephala* Benth (Leguminosae-Mimosoideae), popularly known as "fava de bolota". Previous studies have been showed anti-inflammatory and antinociceptive effects of other species of Parkia gender (Kouadio, F., Phytother Res. (14): 635, 2000). In this study, we have proposed ourselves to investigate the antinociceptive activity of ethyl acetate fraction (F. AcOEt) obtained from leaves of *Parkia platycephala* Bent in diabetic rats. **Methods:** plant material: the dried and ground leaves of P. platycephala were submitted to 95% ethanol extraction in 5 consecutive steps at room temperature. The concentrated ethanol extract (300g) was suspended in methanol/water (15%) and successively extracted with solvents yielding the aqueous (F. H2O; 180g) and ethyl acetate (F. AcOEt; 23 g) phases, where this last fraction contains total phenols, flavonoids and triterpenes. Animals: Streptozotocin (STZ, 40mg/Kg, iv) was administrated in fasted male rats for 12h (250-290 g). Controls and diabetic rats (D) were randomly separated into groups after 21 days of STZ injection. Diabetic rats were treated as follows: DV (saline-DMSO, 0,5mL/Kg, v.o), DFA (F. AcOEt 150; 50; 12,5; 6,25 mg/Kg, v.o,), DM (morphine 5 mg/Kg, i.p.) and non-diabetic control "C" (saline-DMSO, 0,5mL/Kg, v.o). The painful threshold with mechanical stimuli were determinated in all groups by means of von Frey filaments in the times of 0, 60, 120 e 180 minutes after treatment. In other experiments, formalin test was used to investigate the anti-inflammatory activity 21 days after diabetes induction (Animal Ethics Committee, AEC-UFPI, 058/09). **Results and Discussion:** data are expressed as means ± S.E.M, n= 6-9; ANOVA-one way/Tukey ($p<0.05$, a vs C, b vs DV, c vs DM). Mechanical hyperalgesia: Glycemia 48 h and 21 days after STZ injection, respectively: (C: 104,5 ± 11,0; 120,5 ± 5,7); (DV: 345,6 ± 23,6a; 395 ± 61,3a); (DFA 150 mg/Kg: 380,0 ± 36,4a; 364,5 ± 50,9a); (DFA 50: mg/Kg 437,2 ± 94,5a; 349,2 ± 46,8a); (DFA 12,5 mg/Kg: 421,4 ± 24,0a; 569,2 ± 50,1a); (DFA 6,25mg/Kg: 443,4 ± 78,2a; 535,5 ± 30,9a); (DM 317,8 ± 18,24a; 294,8 ± 27,63a). Mechanical nociceptive threshold after 21 days of STZ injection in the times 0, 60, 120 and 180 minutes, respectively: C (12,1 ± 1,0; 11,1 ±0,9; 10,1 ± 0,6; 11,6 ± 1,0); DV (2,7 ± 0,5a; 3,0 ± 0,5 a; 3,3 ± 0,5 a; 3,8 ± 0,5 a); DFA 150 mg/Kg (2,7 ± 0,4 a; 7,6 ± 1,8 b; 7,0 ± 0,8 b ; 7,0 ± 0,8 ab); DFA 50 mg/Kg (2,4 ± 0,4 a; 8,1 ± 1,1 b; 7,0 ± 1,1 b; 6,0 ± 0,7 a); DFA 12,5 mg/Kg (3,1 ± 0,6 a; 8,6 ± 0,6 b; 6,9 ± 1,1 b; 6,6 ±1,0 a); DFA 6,25mg/Kg (2,9 ± 0,5 a; 8,1 ± 1,4 b; 7,0 ± 1,5 a; 4,0 ± 0,4 a); DM (3,7 ± 0,5 a; 11,3 ± 1,53b; 7,3 ± 0,75b; 6,5 ± 0,98 a). Formalin test: Glycemia 48 h and 21 days after STZ injection, respectively: (C: 87,6± 4,0;186,5 ± 8,5); (DV: 283,2 ± 33,3 a; 510,2 ± 91,0 a); (DFA 50: mg/Kg 375,1 ± 29,2 a; 699,4 ± 112,7 a); (DFA 12,5 mg/Kg: 314,7 ± 42,7 a; 459,9 ± 55,1 a); (DM 319,9 ± 52,9 a; 416,9 ± 58,7 a). Reaction time of animals in the first and second phases of formalin test, respectively: (C: 48,3 ± 11,4; 347,0 ± 30,1); (DV: 37,0 ± 5,8; 263,3 ± 25,2); (DFA 50: mg/Kg 33,5 ± 5,2; 138,0 ± 38,4 ab); (DFA 12,5 mg/Kg: 27,6 ± 2,5 a; 333,8 ± 27,1c); (DM 20,8 ± 3,7 a; 123,3 ± 35,0ab). **Conclusion:** data suggest antinociceptive effects of F. AcOEt from P. platycephala in diabetic rats. More investigation of anti-inflammatory action may be evaluated as a future perspective. Support by CAPES, FINEP, CNPq.

05.049

Effects of thalamic nucleus submedius inhibition on the stimulation-induced antinociception in rats Reis GM, Rossaneis AC, Fais RS, Prado WA FMRP-USP − Farmacologia

Introduction: Electrical stimulation (ES) of the cerebral cortex has been used for the management of intractable pain in many patients. However, the precise knowledge about the neural mechanisms involved in cortical pain modulation still remains a complex and challenging field for future research. We have earlier shown that the ES of the rat retrosplenial cortex (RSC) produces antinociception that depends on the activation of descending inhibitory pathways (Reis, G.M. J.Pain, 2010). Of considerable interest is the finding that in rats the RSC has reciprocal connections with the thalamic nucleus submedius (Sm), which is implicated in antinociception and is activated by persistent nociceptive inputs (Van Groen, T. J.Comp. Neurol. (4) p.593, 1990). This study (approved by the Commission of Ethics in Animal Research, Number159/2007) then utilized the tail-flick test (TFT) to examine whether the Sm is involved in the modulation of the antinociceptive effect of ES of the RSC. **Methods:** Male Wistar rats (160g; n = 8) were chronically (7 days) implanted with an unipolar electrode in the RSC, and a guide canulla in the Sm. The tail-flick test was conducted in independent groups of animals receiving vehicle (0.9% sterile saline; 0.25 µl), cobalt chloride ($CoCl_2$) (1mM; 0.25 µl, dissolved in 0.9% sterile saline, a nonselective synaptic blocker) or naloxonazine (5µg; 0.25 µl, dissolved in 0.9% sterile saline, m1 antagonist). **Results:** The ES of the RSC (20 µA, 20 seg) produced a strong and long lasting inhibition of the tail flick reflex. Pre-treatment of Sm with $CoCl_2$ or naloxonazine reduced the intensity but did not change the duration of the responses to the ES of the RSC. The time-course curves obtained were significantly different regarding treatment ($F_{2,28}= 15,25$; $p< 0,0001$), time ($F_{14,392}= 98,1$; $p< 0,0001$) and had significant treatment x time interaction ($F_{42,392}= 21,70$; $p< 0,0001$). **Discussion:** The present results suggest that the stimulation-produced antinociception from the RSC depends on the activation of µ1-opioid-mediated connection in the Sm. Supported by: FAPESP.

05.050

Amitriptyline increases the duration of the antinociceptive effect produced by 2 Hz electroacupuncture in rats. Fais RS, Reis GM[2], Dias QM[1], Silveira JWS[1], Prado WA[3] FMRP-USP − Farmacologia

Introduction: The eletroacupuncture (EA) is widely used to relieve various types of pain. Antidepressants, particularly the tricyclic antidepressants (TCA) are widely used for chronic pain control. Analgesia induced by EA and TCA may be complementary. By this reason, the present study (142/2009) examines whether the antinociceptive effect induced by EA in a rat model of incision pain is changed by the previous intraperitoneal administration of amitriptyline (AMT). **Methods:** The pain induced by a surgical incision of the plantar aspect of the right hind paw was evaluated using an electronic Von Frey apparatus. Male Wistar rats (160g ; n = 6 per group) were used. Lightly anesthetized rats, using inhalatory isoflurane (0,5%), pretreated with saline (1 ml/Kg) or AMT (a non-seletive serotonine-norepinephrine reuptake inhibitors, 0.8 mg/kg, 1ml/kg) were submitted to a 20-min of real or sham 2 Hz EA applied bilaterally to the Zusanli (ST36) and Sanyinjiao (SP6) accupoints 60-min after intraperitoneal administration of AMT. The experimental groups were AMT followed by EA 2 Hz, AMT followed by EA sham, saline followed by EA 2 Hz and saline followed by EA sham. **Results:** The surgical incision reduced significantly the mechanical threshold in the ipsilateral paw (IL) but produced no significant change in the contralateral paw. In saline-treated animals the EA produced a significant reduction of the incision hyperalgesia in the IL for up to 40 minutes as compared to the control group (rats treated with saline followed by sham EA). The intensity of the antihyperalgesic effect of EA in AMT-treated rats was not changed but lasted for up to 70 minutes as compared to control. The curves obtained were significantly different regarding treatment ($F_{3,20} = 65,37$; $p < 0,0001$), time ($F_{13,260} = 76,51$; $p < 0,0001$) and had significant treatment x time interaction ($F_{39,260} = 18,28$; $p < 0,0001$). Conclusions: The results led us to suggest that intraperitoneal AMT increased significantly the duration of the antinociceptive effect produced by low frequency EA in rats. Supported by FAPESP

05.051

Role of IL-33 / ST2 in carrageenin-induced innate inflammatory hypernociception in mice. Zarpelon AC[1], Cunha, TM[2], Xu D[3], Alves-Filho JC[3], Liew FY[3], Ferreira SH[2], Cunha FQ[2], Verri Jr WA[1] [1]UEL − Patologia, [2]FMRP-USP − Pharmacology, [3]University of Glasgow − Immunology, Infection and Inflammation

Introduction: Interleukin-33 (IL-33) is a novel cytokine of IL-1 family that signals through ST2 receptor. IL-33 mediates adaptive inflammation-induced hypernociception and neutrophil recruitment in a mice model of rheumatoid arthritis by inducing further production of cytokines. Herein, we addressed whether IL-33 is also relevant in innate (carrageenin) inflammation-induced hypernociception. Concerning the investigated mechanisms, we focused on the role of neutrophils, cytokines (TNFalpha, IL-1beta, CXCL1 and IL-10) and prostaglandins, which are known to be part of the mechanisms triggered by carrageenin. **Methods:** Mechanical hypernociception was evaluated using an electronic version of von Frey filaments test (0-5h after stimulus), cytokine (TNFalpha, IL-1beta, CXCL1 and IL-10) production by ELISA (2h), mRNA (IL-33 and ST2) expression by quantitative PCR (2h), and neutrophil recruitment by myeloperoxidase activity assay (5h). Sex matched Balb/c and ST2 deficient (-/-) mice of 20-25g received: IL-33 (30-300 ng/paw); carrageenin (0.1 mg/paw); fucoidin (binds L-selectin, 20 mg/kg/iv/30min pretreatment); anti-TNF (infliximab, 10 mg/kg/ip/48h and 1h pretreatment), IL-1ra (30 mg/kg/iv/15 min pretreatment); anti-CXCL1 antibody (700 ng/paw/15 min pretreatment) and/or indomethacin (cyclooxigenase inhibitor, 5 mg/kg/ip/40 min pretreatment). Procedures were approved by the Ethics Committee of Universidade Estadual de Londrina (Of. 47/10 − 02/10). Differences were considered significant for $P<0.05$ (One-way ANOVA followed by Tukey's t test). **Results and Discussion** ST2-/- mice presented reduced hypernociception (up to 66%) compared to control wild type (Balb/c) mice concomitantly with reduced neutrophil recruitment (33%) to paw skin after carrageenin stimulus. IL-33 injection in naïve mice induced dose-dependent hypernociception, which was dependent on neutrophil recruitment (inhibited by fucoidin, up to 49%) and absent in ST2-/- mice. IL-33 (100 ng)-induced hypernociception was diminished by anti-TNF (up to 54%), IL-1ra (up to 69%) or anti-CXCL1 antibody (up to 32%) pretreatments, demonstrating that IL-33-induced hypernociception depends on TNFalpha, IL-1beta and CXCL1 in naïve mice. In agreement, carrageenin-induced production of TNFalpha, IL-1beta and CXCL1 in the paw skin was reduced in ST2-/- mice (inhibition of 56, 69 and 67%, respectively) while IL-10 levels were not altered. IL-33-induced hypernociception was also inhibited by indomethacin (78%) pretreatment suggesting the role of prostaglandins. Further supporting the role of IL-33 in carrageenin model, carrageenin induced a 2 fold increase of IL-33 and ST2 mRNA expression in paw skin samples. Concluding, we demonstrated that IL-33 acting on ST2 is an important mediator of innate inflammatory hypernociception induced by carrageenin via the production of other hypernociceptive mediators including cytokines and prostaglandins. **Financial Support:** Fundação Araucária, FAPESP, and CNPq (Brazil), IASP Early Career Research Grants Program funded by Scan/Design by INGER & JENS BRUUN Foundation, Arthritis Research Campaign and Medical Research Council (UK), Chief Scientist Office (Scotland).

05.052

Antinociceptive property of selenothiazolidines administered by oral route in mice. Frasson NR[1], Donato F[1], Schneider PH[2], Savegnago L[1] [1]UNIPAMPA − Farmacologia e Toxicologia, [2]UFRGS – Química

Introduction: The interest in organoselenium biochemistry and pharmacology has increased in the last two decades due to a variety of organoselenium compounds that possess biological activity. Accordingly, diphenyl diselenide, an organoselenium compound, elicits anti-inflammatory and antinociceptive activities (Nogueira, C.W. et al Chem. Rev. 104:6255–6286, 2004). Therefore, due to the need for more effective analgesic or antiinflammatory compounds with low side-effects, many studies have been appearing in the literature involving the synthesis of new compounds which can provide alternatives to current therapeutic agents. Based on the considerations above, the goal of the present study was to verify the antinociceptive activity caused by selenothiazolidines given orally (p.o) in model thermical of pain in mice. **Methods:** The behavioral experiments were conducted using male Swiss mice (25 – 35g). Ethics Committee for Animal Experimentation is being evaluated. The antinociceptive property was evaluated in the thermal stimulus using hot plate test. In these experiments, the hot plate (model-DS 37; Ugo Basile) was maintained at 55±1 °C and animals were placed into a glass cylinder on the heated surface, and the time between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. Each animal was tested before administration of drugs to obtain the baseline. Control or pretreated with selenothiazolidines (1–100 mg.Kg-1, p.o) animals were injected 30 min earlier. The latency, Δt (s), was calculated for each animal according to the formula: Δt (s)=postdrug latency − predrug latency. In addition, the locomotor and exploratory activities were evaluated in the open field task. To this end, mice were evaluated 30 min after oral exposure with canola oil or selenothiazolidines (1- 100 mg.Kg-1, p.o.). Each animal was placed individually at the center of the apparatus and observed to record the spontaneous ambulation (number of segments crossed with the four paws) and exploratory activity (expressed by the number of time rearing on the hind limbs). **Results and Discussion:** In hot-plate test, a useful test to evaluate the role of supraspinally mediated responses to noxious heat, selenothiazolidines (10 – 50 mg.Kg-1) caused a significantly increase response latency suggesting an analgesic activity. Thus demonstrating that even at low doses, selenothiazolidines showed an antinociceptive effect in models of thermal nociception in mice, without altering the effect of locomotor and exploratory activities of these animals in open field test. In conclusion, this is a promising compound for more detailed pharmacological studies involving organochalcogens compounds. **Financial Support:** Fapergs (BIC), CNPq (PIBIC), UFRGS e UNIPAMPA. Acknowledgements: FAPERGS and CNPq for financial support and fellowships.

05.053

Evaluation of the antinociceptive profile of a series of *N*-acylhydrazones derivatives modified from the prototype LASSBio-294. Veloso RR, Nogueira MCO, Maia RC, Lima ML, Barreiro EJ, Miranda ALP FF-UFRJ- LASSBio

Introduction: The N-acylhydrazone has proved to be an important pharmacophoric group for anti-inflammatory and analgesic activities. The compound LASSBio-294 was identified as an important cardiotonic prototype, also presenting anti-inflammatory and analgesic profile (Barreiro, E. J, Quim. Nova, 25, 1172, 2002). Later studies have developed new N-acylhydrazones (NAH) derivatives conformationally restricted which have showed analgesic activity, emphasizing that structural changes may be effective in the development of actives analogs (Kummerle, A.E et al, Bioorg. Med Chem Letters, 19, 4963, 2009).To evaluate the profile of antinociceptive derivatives N-acylhydrazones modified from LASSBio-294, and analyze the influence of structural changes in the observed activity. **Methods:** The antinociceptive activity was evaluated in mice using the writhing test induced by acetic acid (0,1N; 10ml/g de animal; i.p.). The number of writhing was evaluated between 10-30 min after the painful stimuli. The test and reference compounds (celecoxib) were administered p.o. at a dose of 100 mmol/kg one hour before the nociceptive stimulus in a suspension of gum Arabic 5% (vehicle). The results were checked through the comparison between groups treated with derivatives and the control vehicle group (* $p < 0.05$, $n = 7$-10 animals). **Results and Discussion:** All derivatives were able to significantly inhibit the number of writhing from 25% to 58%. Derivatives LASSBio-1476, LASSBio-1498, LASSBio-1499 and LASSBio-1501 were the most actives with percentage of inhibition greater than 50% (*** $p < 0.001$), showing a similar profile that was presented by Celecoxib (49, 3% of inhibition). Interestingly, the most actives derivatives are conformationally restricted, consisting of N-methylated analogs, confirming the findings of Kummerle et al, 2009. LASSBio-294 inhibited the writhing by 46% and has an analgesic potency (ED50) of 8.2 mmol / kg (Miranda, A.L.P et al. Cong. Ann. Soc Franc. Pharmacol, 62, 2002), showing that structural modifications introduced in these new NAH derivatives did not lead to loss of the analgesic potential, therefore suggesting the importance of determining this parameter for the comparison of power between the derivatives and the prototype, and allow us a better understanding of structure-activity relationship for the antinociceptive effect. The results have showed that the compounds tested showed a significant antinociceptive activity. So, the perspective of this study comprehend to determine the ED50 of the most active compounds, to evaluate the occurrence of central analgesic effect and to determine the anti-inflammatory profile of these derivatives. FAPERJ, INCT-INOFAR, PIBIC/UFRJ/CNPq.

05.054

Effects of methysergide injected into the anterior pretectal nucleus on the stimulation-induced antinociception from the retrosplenial cortex. Rossaneis AC, Reis GM, Prado WA
FMRP USP − Farmacologia

Introduction: Several studies have demonstrated the participation of the anterior pretectal nucleus (APtN) in nociception. Efferents from the retrosplenial cortex (RC) to the rostral APtN were also demonstrated (Foster et. al., Neurosc. 29: 685- 694, 1989).We investigated whether pain inhibitory pathway activated from the RC by persistent noxious inputs depends on APtN serotonergic receptors. **Methods:** The experiments were performed and approved by the Ethics Committee on Animal Experiments of FMRP-USP (No. 069/2009). Male Wistar rats (140g-160g) were stereotaxically implanted with chronic monopolar electrode in the RC and guide cannula in APtN. One week later each animal was anesthetized with 1.5% halothane in oxygen and a 1-cm longitudinal incision was made through the skin and fascia of the plantar aspect of right hind paw, and the skin was sutured with two 5-0 nylon stitches. The mechanical threshold was measured with an electronic von Frey 2h after the incision, and 5, 10 and 15 min after injection of saline or serotonergic antagonist (methysergide) into the APtN and immediately after RC stimulation. **Results:** The plantar incision significantly reduced the mechanical threshold whereas the RC stimulation (20μA, during 15 sec) significantly increased the mechanical threshold for about 15 min. Serotonergic antagonist injected into the APtN significantly reduced the withdrawal thresholds to mechanical stimulation of the ipsilateral paw. Furthermore, the microinjection of methysergide into the APtN decreased the intensity and duration of the effect of RC stimulation. **Discussion:** The results suggest the involvement of serotonergic mechanisms in modulation of the antinociceptive effect of RC stimulation. **Financial Support:** Capes, FAPESP.

05.055

Crotalphine induces a long-lasting and opioid-mediated antinociceptive effect in an experimental model of bone cancer pain. Gutierrez VP[2], Brigatte P[1], Zambelli VO[2], Carvalho JS[2], Picolo G[2], Radin A[3], Marques FLN[3], Cury Y[2] [1]CEIS-UNESP, [2]IBu − Pain and Signaling, [3]FM-USP − Oncology

Introduction: Crotalphine, a peptide first identified and isolated from the South American rattlesnake *Crotalus durissus terrificus* venom, induces analgesia mediated by activation of d- and k-opioid receptors (Konno, K., peptides,29,1293,2008). The aim of this work is to characterize the analgesic effect of crotalphine in a new rat model of bone cancer pain induced by inoculation of Walker 256 carcinoma cells (4x106) into the rat femoral cavity. **Methods:** The presence of bone metabolic alterations was determined by scintigraphy, using 99mTc-MDP, which is significantly concentrated in areas of osteogenesis. Femoral images were obtained before and 7, 14 and 21 days after tumor cell inoculation. Bone cancer pain was characterized by the presence of hyperalgesia and allodynia, determined using the rat paw pressure test or von Frey filaments, respectively. **Results and Discussion:** Incorporation 99mTc-MDP was significantly increased 7, 14, 21 days after tumor cell injection, suggesting the development of bone cancer. Hyperalgesia and allodynia were detected one day after cell inoculation, persisting for at least 21 days. Interestingly, allodynia was detected not only in the ipsilateral hind paw inoculated with the tumor cells, but also in the contralateral one, demonstrating the existence of mirror-image pain. Crotalphine (8 mg/kg p.o) administered on day 21, blocked hyperalgesia and allodynia. The analgesic effect was detected for up to 2 days after peptide administration. This effect is mediated by activation of d- and k-opioid receptors. These results indicate that intrafemoral injection of Walker 256 cells causes bone cancer and pain. Crotalphine induces a potent, long-lasting and opioid mediated antinociception in this model of cancer pain. **Financial Support:** FAPESP and INCTTOX program Ethics Protocol: Comissão de Ética em Experimentação Animal – CEEA / Protocol number 32 nas fls 55 do livro 02 – Instituto de Ciências Biomédicas da USP and Comissão de Ética no uso de Animais do IBu – CEUAB / Protocol number 437/07).

05.056

Aspirin-triggered resolvin d1, AT-RVD1, and its precursor, possess anti-hyperalgesic properties in a model of arthritis in rats. Lima-Garcia JF[1], Motta EM[1], Campos MM[2], Calixto JB[1] [1]UFSC − Farmacologia, [2]PUCRS − Cirurgia-Odontologia

Introduction: Resolvins of the E and D series are derived from the polyunsaturated fatty acids (PUFAs) EPA (eicosapentaenoic acid) and DHA (docosahecosaenoic acid), respectively. Both PUFAs have recently been described to be produced during the resolution phase of acute inflammation, where they act as potent bioactive molecules with anti-inflammatory and proresolving properties (Serhan, Annu. Rev. Immunol., 25: 101, 2007). Resolvins from D series are also able to block IL-1b transcription induced by TNF-a in microglia and in inflammatory models (Serhan et al., J. Exp. Med., 196: 1025, 2002). Nonetheless, few studies have addressed, so far, the potential therapeutic involvement of the Resolvins in pain processing related to inflammatory pain (Xu et al., Nat. Med., 16: 592, 2010). Herein, we sought to investigate the therapeutic potential of AT-RvD1 as well as its precursor, namely 17(R)-hydroxy-docosahexaenoic acid [17(R)HDoHE], to modulate inflammatory pain in a model of arthritis in rats. **Methods:** Male adult Wistar rats (180-250 g, n=6) received an intraplantar (i.pl.) injection of CFA (1 mg.ml-1, 100 µl) suspended in saline (100 µl) into the right hind paw. The control group received 200 µl of sterile saline into the right hind paw. Animals were treated intraperitonealy (i.p.) with AT-RvD1 (100 or 300 ng/i.p.) or with the precursor 17(R)HDoHE (300 ng/i.p) 3 days after CFA injection. Also, a continuous protocol of treatment with AT-RvD1 (100 ng/i.p., given for 4 days, twice a day) or with 17(R)HDoHE (300 ng/i.p., given for 5 days, once a day) was adopted in order to assess their potential in modulating inflammatory pain as well as the levels of TNF-a and IL-1b in the hind paw tissue. Mechanical hyperalgesia was evaluated as the paw withdrawal response frequency (%, von Frey Hair, 8.0 g. The levels of the pro-inflammatory cytokines were determined using ELISA's kit. (Ethics Committee protocol number: 043/CEUA/PRPe/2008). **Results and Discussion:** The AT-RvD1 (100 ng/i.p.) post-treatment 3 days after CFA injection significantly decreased mechanical hyperalgesia induced by CFA for up to 6 hours (26.0±3.6%, $p<0.01$). However, the dose of 300 ng/i.p. elicited a similar effect in inhibiting mechanical hyperalgesia (32.5±8.7%, $p<0.01$). Thus, we next assessed the dose of 100 ng of AT-RvD1 for the continuous treatment. AT-RvD1 (100 ng/i.p.; given for 4 days, twice a day) induced a more pronounced antinociceptive effect after the second treatment (day 4 after CFA injection) (62.0±15.0% of inhibition). Animals treated with 17(R)HDoHE (300 ng/i.p.; given for 5 days, once a day) presented a significant overall inhibition of the mechanical hyperalgesia when compared to the control group (48.0±14.0% of inhibition). We show that efficacy of continuous treatment with either 17(R)HDoHE or AT-RvD1is related, at least in part, to a decrease of the cytokines TNF-a (60.3±17.2% and 90.9±4.8%, respectively) and IL-1b (63.3±1% 23.3±11.7%, respectively) in the hind paw tissue. The findings presented herein bring new evidence on the relevant anti-hyperalgesic properties of 17(R)HDoHE and AT-RvD1. We might suggest that such lipid mediators would be useful for treating painful states associated to chronic inflammatory diseases like arthritis. Supported by: CAPES/CNPq/FAPESC.

05.057

Endothelin-1 induces both itch and pain in the mouse cheek. Oliveira L, Hara DB, Rae GA UFSC − Farmacologia

Introduction: − Itch and pain are frequent events in diseases affecting skin and other systems. These sensations are mediated by distinct neural circuits and multiple mediators (Ikoma et al., Nat. Rev. Neurosci., 7: 535, 2003), yet it is difficult to discriminate between itch and pain in behavioral animal models. A new model has been proposed to discriminate behaviorally itch from pain, based on the finding that intradermal (i.d.) injections of capsaicin or histamine into the cheek of mice elicit, mainly and respectively, wipes with the forepaws or scratching with the hind paws of the injected cheek (Shimada et al, Pain, 139: 681, 2008). Endothelin-1 (ET-1) causes licking and flinches of the hind paw when injected into the foot pad (Piovezan et al., Br. J. Pharmacol., 129: 961, 2000) and scratching when injected i.d. into the scruff of the neck (Trentin et al., Exp. Biol. Med.,231: 1146, 2006), which are suggestive of overt pain and pruritus, respectively. The current study assesses if the behavioral responses to i.d. ET-1 in the "cheek model" better resemble those induced by capsaicin or histamine, i.e. if the mediator causes overt nociception or pruritus. The receptors underlying the actions of ET-1 were also examined. **Methods:** Male CD1 mice (2 months) were anesthetized with isofluorane and both cheeks were shaved. Two days later, each mouse was placed in an observation chamber to habituate for 1 h. They then received i.d. injections of either capsaicin (CPS, 10 ug), histamine (HIS, 50 ug), ET-1 (3, 10, 30 pmol), or vehicle into the left cheek. The number of forepaw wiping and hind paw scratching bouts, directed to the injected cheek, was counted over 40 min. In some experiments, endothelin ETA (BQ-123) and/or ETB BQ-788 receptor antagonists (each at 10 nmol, i.d.) were injected 5 min prior to ipsilateral ET-1 (30 pmol) injection. In another experiment, the cheek was removed 15 min after i.d. ET-1 or vehicle, and the number of degranulated mast cells was quantified by histology in 7 um sections stained with Toluidine Blue. Results were analyzed by one-way ANOVA followed by Bonferroni's test ($P < 0.05$; $n = 5$-7). The study was approved by UFSC's Ethics Committee on Animal Use (PP00299). **Results and Discussion** − Vehicle injection caused few wipes (10 ± 2) and scratches (9 ± 3), whereas CPS induced more wipes (53 ± 9) than scratches (12 ± 5) and HIS caused the opposite effect (9 ± 4 wipes, 38 ± 9 scratches). ET-1 (3-30 pmol) promoted dose-dependent bouts of wiping and scratching (at 30 pmol: 33 ± 3 wipes, 49 ± 3 scratches). Scratching had a faster onset than wipes. Treatment with BQ-123 prior to ET-1 (30 pmol) did not change wipes (25 ± 5), but blocked scratching (to 9 ± 3 bouts), whereas BQ-788 increased both responses to ET-1 (to 59 ± 6 wipes, 82 ± 10 scratches). When compared to values of BQ-788 + ET-1 mice, those given BQ-788 + BQ-123 displayed markedly fewer ET-1-induced wipes (to 24 ± 3) and scratches (to 19 ± 4). Cheek skin sections obtained 15 min after ET-1 showed more degranulated mast cells than those of vehicle-treated controls. Thus, ET-1 induces ETA receptor-mediated overt pain and itch in the mouse cheek model, and both responses are subject to modulation by inhibitory ETB receptors. These responses coincide in time with local mast cell degranulation. **Financial Support:** CAPES, CNPq, FAPESC, PRONEX.

05.058

The involvement of the transient receptor potential A1 (TRPA1) on norepinephrine-induced nociception in neuropathic mice. Pinheiro FV[1], Silva CR[2], Oliveira SM[2], Villarinho JG[3], Andre E[3], Ferreira J[2] [1]UFSM − Fisiologia e Farmacologia, [2]UFSM − Química, [3]UFRN − Biofísica e Farmacologia

Introduction: The sympathetic nervous system mediates some forms of neuropathic pain, where nociceptors may be activated by both endogenous and exogenous norepinephrine (NE) inducing overt pain and allodynia. However, the mechanisms involved in NE-induced pain in neuropathies are poorly known. Thus, the aim of this study was to investigate the involvement of TRPA1 receptor on NE-induced nociception in neuropathic mice. **Methods:** Male Swiss mice (N=6-10, 25-30g) were used in this study (Ethics Committee, process number 23081.005024/2010-88). Neuropathy was induced by chronic constriction injury (CCI) of the sciatic nerve. Loosely constrictive ligatures were placed around the right sciatic nerve under anesthesia (90 mg/Kg of ketamine plus 3 mg/Kg of xylazine, intraperitoneal route i.p.). Sham surgery was done by exposing the sciatic nerve without performing constriction. The paw withdrawal threshold (PWT) to mechanical stimulus in the right hind paw was measured before lesion and 7 days after, through the application of von Frey filaments, which verified the development of allodynia, characterized by the decrease of PWT 7 days after lesion. After PWT measurement, mice were treated with the TRPA1 antagonist HC-030031 (100 mg/kg, i.p) or vehicle (10 ml/kg, i.p.). After 30 min we injected subcutaneously into the right hind paw the vehicle (20 µl/paw), NE (30 ng/paw), or the TRPA1 agonist allyl isothiocyanate (AITC, 1 and 10 nmol/paw) in sham and CCI mice. The time of overt nociceptive behaviors was measured for 5 min. Results and discussion The injection of NE in sham-operated mice did not produce nociceptive response when compared with vehicle (4.7±6.4 and 3.7±2.6 s of response, respectively). On the other hand, injection of NE in neuropathic mice produced a marked nociceptive response when compared with vehicle group (40.8±2.1 and 7.8±3.3 s, respectively). As expected, CCI induced allodynia in mice when compared with sham-surgery (PWT of 0.35±0.06 and 2.89±0.17 g, respectively). Of interest, we observed a significant negative correlation (Pearson r=-0.64, R2 = 0.41, $P<0.01$) between PWT and NE-induced nociceptive response. The treatment with the TRPA1 antagonist HC-030031 largely reduced NE-induced nociception when compared with vehicle treatment (2.8±0.6 and 36.0±8.9 s of response, respectively). In addition, the injection of TRPA1 agonist was more potent and efficacious to induce nociceptive response in CCI than in sham mice (94.0±11.1 s with 1 nmol/paw and 62.2±8.8 s with 10 nmol/paw, respectively). Finally, the treatment with HC-030031 was more efficacious to reduce AITC-induced nociception in neuropathic than in control mice (inhibitions of 90±2 and 57±7 %, respectively). Taken together, the present data demonstrated a critical role of TRPA1 receptor in a model of neuropathic pain mice, especially in nociceptive responses evoked by NE. Thus, TRPA1 receptor could be a target to the development of new treatments for sympathetically-maintained neuropathic pain. Financial Support CAPES, CNPq, CCNE/UFSM, CCS/UFSM.

05.059

Peripheral antinociceptive effect of inosine depends of the A1 adenosine receptor but not of receptors A2A and A3. Cerutti ML[1], Nascimento FP[2], Macedo Junior SJ[1], Santos ARS[1]
[1]UFSC − Ciências Fisiológicas, [2]UFSC − Farmacologia

Background: Inosine is an endogenous metabolite resulting from the action of adenosine deaminase on adenosine. Our group previously demonstrated that inosine has antinociceptive effects (Nascimento et al., JPET, 2010). Thus, this study examined the involvement of adenosine receptors A1, A2A and A3 in the antinociception caused by inosine in a model of peripheral pain chemistry. **Methods:** We used in this experiment, male Swiss mice, weighing around 30-35g, in which we administered different adenosine receptor antagonists or agonists, such as CHA (A1 agonist), 8-PT (A1 antagonist); DPMA (A2A agonist), ZM241385 (A2A antagonist); HEMADO (A3 agonist) and inosine. We evaluated the involvement of adenosine receptors by performing combinations of the drugs mentioned above. Nociception was induced by intraplantar injection of glutamate (10µmol/paw) and was measured by the time the animals were licking or biting the injected paw (Protocol CEUA PP00484). **Results:** inosine (1 − 100 micrograms/paw) caused antinociception when co-administered with glutamate (10 mmol / paw) with ID50 of 44.5 micrograms/paw and inhibition of 54 ± 4%. Then the co-administration of adenosine A1 agonist, CHA (0.1 − 10 micrograms/paw) also caused the antinociceptive effect, with ID50 of 11.2 micrograms/paw and inhibition of 79 ± 3% . In contrast, the intraplantar injection of A2A agonists (DMPA, 1-10 micrograms/paw) and A3 (HEMADO, 0.1 -10micrograms/paw) did not reduce the nociception induced by glutamate. In another series of experiments, the antinociceptive effect of inosine was significantly reversed by intraplantar injection with 8-PT (1 micrograms/paw) 5 min before, but was not reversed by pretreatment with ZM241385 (15 micrograms/paw). **Conclusion:** In view of these results, it is concluded that inosine has an antinociceptive effect induced by peripheral injection of glutamate. But only the A1 receptor is involved in this mechanism, while the other adenosine receptors (A2A and A3) will not participate in the antinociception caused by inosine in this model of peripheral pain. **Financial Support:** UFSC, CNPq.

05.060

Analgesic effects of ethanolic extract from *Sinningia aggregata* tubers and from the isolated compound 3-prenyl-4-oxo-3´-methylnaphtho [1,2b] oxepin-1,3´(4H)-diol. Simas AS[1], Verdan ML[2], Kassuya CAL[3], Stefanello MEA[2], Zampronio AR[1] [1]UFPR − Farmacologia, [2]UFPR − Química, [3]UFGD − Ciências da Saúde

Introduction: Plants from the genus Sinningia (Gesneriaceae) are popularly used for their anti-inflammatory and antipyretic properties but there are no studies that support this use. In this study we investigated the antinociceptive, anti-inflammatory and antipyretic effects of the ethanolic extract from the tubers of *Sinningia aggregata* (Ker Gawl) (EESAg) and from the isolated compound 3-prenyl-4-oxo-3´-methylnaphtho[1,2b]oxepin-1,3´ (4H)-diol (PMNO). **Methods:** Male Swiss mice (25-35g) were treated with EESAg (10-100 mg/kg) or vehicle (10ml/kg) by oral route and after 1h they received carrageenan (Cg, 300µg) or formalin (2.5%) into the hind paw or lipopolysaccharide (LPS, 100µg/kg) intraperitoneally. Indomethacin (5 mg/kg, v.o.) was used as positive control. Cg-induced paw oedema, cell migration and mechanical hyperalgesia were evaluated using a digital micrometer (0.5,1,2,4h after Cg), the myeloperoxidase test (4h after Cg) and von Frey microfilaments, respectively (4h after Cg). Formalin-induced nociceptive behavior was evaluated for 30 min. LPS-induced fever was evaluated using remote data loggers implanted in the peritoneal cavity of the animals 1 week before. Mice were also treated with PMNO (0.021-2.1 mg/kg by oral route or 0.07-7ng/paw) or the respective vehicles (10ml/kg or 20µl/paw) and formalin-induced nociception and Cg-induced mechanical hyperalgesia were measured. Moreover, animals were submitted to rotarod and hot plate tests. The procedures were approved by the Institutional Ethics Committee (protocol nbr. 387) **Results:** Oral administration of EESAg did not change the Cg-induced edema and LPS-induced fever and reduced neutrophil migration by 40% only at the higher dose. In contrast, EESAg promoted a dose-dependent reduction of the phase II of the formalin-induced nociception (maximal inhibition of 71±2%) similarly to the reduction induced by indomethacin (61±3%). The isolated compound PMNO also reduced phase II of formalin-induced nociception (maximal inhibition of 57±3%). Both EESAg and PMNO completely inhibited Cg-induced mechanical hyperalgesia but were ineffective in hot plate test and did not change the motor performance of the animals. Local injection of PMNO reversed dose-dependently the reduction of the threshold for mechanical stimulation induced by Cg with a maximal inhibition of 115±14% at the higher dose. The injection of this dose of PMNO in the contralateral paw did not change the mechanical hyperalgesia induced by Cg. **Discussion:** These results show that EESAg from the tubers of the *S. aggregata* posses an important analgesic activity which seems to be related, at least in part to the reduction of the mechanical hyperalgesia. A new compound found in this species of Sinningia, PMNO shared similar activity by blocking the overt nociception induced by formalin and the Cg-induced mechanical hyperalgesia acting probably in peripheral sites. **Financial Support:** CNPq, CAPES and Fundação Araucária.

05.061

Cytokines and chemokines participate in the genesis of post-incisional pain. Carreira EU, Cunha FQ, Ferreira SH, Cunha TM FMRP-USP − Farmacologia

Introduction: A great number of patients suffer intense or very intense pain few days postsurgery. Postoperative pain is a common form of acute pain, and efficacious postoperative analgesia improves patient satisfaction, may decreases morbidity, and perhaps reduces mortality following surgery. Recent studies have demonstrated an inflammatory component in this type of pain. However, the mechanisms involved are not fully elucidated. We have demonstrated that release of pro-inflammatory cytokine (TNF-alfa and IL-1beta) plays a crucial role in genesis of inflammatory hypernociception. Recently, our group also showed that CXC chemokines acting on CXCR1/2 is important to induce neutrophil migration and inflammatory hypernociception. In this context, the purpose of this study was to evaluate the role of cytokines and chemokines in postoperative pain. **Methods:** Animals (C57BL/6 wild-type and TNFRI-deficient mice) were anaesthetized and a 0,5 cm longitudinal incision was made with a surgical blade through the skin and fascia of the plantar region, starting 0.5 cm from the proximal edge of the heel. The plantaris muscle was elevated, but its origin and insertion were left intact, after that the skin was opposed. Nociceptive mechanical threshold was measured with electronic von Frey and the mechanical stimulus was applied in an area adjacent to the wound. The role of TNF-alfa was evaluated in animals TNFRI-deficient and the role of IL-1beta was evaluated through administration of IL-1ra i.v. (30 mg / kg; 15 minutes before the surgery and 4 hours after). For the analysis of CXCR1/2 importance, mice were treated with a non-competitive allosteric inhibitor of this receptors, DF2156 i.v. (50 mg / kg; 15 minutes before the surgery). The levels of cytokines and chemokines into the plantar tissue were evaluated using sandwich ELISA (enzyme-linked immunosorbent assay). **Results:** The incision in the paw of mice produced a decrease in mechanical nociceptive threshold, which was statistically significant until 72 h after surgery. This decrease was associated with an increase of cytokines (TNF-alfa and IL-1 beta) and chemokines (CXCL1/KC) release. The TNFRI-deficient mice and the wild-type treated with IL-1ra have shown a reduction in mechanical hypernociception. Furthermore, the treatment with DF2156 also decreases the mechanical hypernociception after surgery. **Conclusion:** These results indicate that chemokines and cytokines play a crucial role in the genesis of postoperative pain. Therefore, we can suggest that the inhibition of CXCR1/2 and cytokine release could be a target in the control of this type of acute pain. **Financial Support:** CNPq and FAPESP Protocol Brazilian College of Animal Experimentation (COBEA): nº 119/2009

05.062

Effect of Oligopeptidases B from *Trypanosoma cruzi and Trypanosoma brucei* in an experimental model of pain in mice. Abrahão RQ[1], Juliano MA[2], Juliano L[2], Giorgi R[3], Dale CS[4] [1]UNIFESP − Biofísica, [2]UNIFESP − Farmacologia, [3]IBu − Fisiopatologia, [4]IEP-HSL

Introduction: Oligopeptidases B from *Trypanosoma cruzi* (OPTc) and *Trypanosoma brucei* (OPTb) play an important role in the pathogenesis of trypanosomiasis Chagas disease and sleeping sickness, respectively (Gorrão et al. Peptides. 28: 2146, 2007). The OPTc is related to the process of invasion of T. cruzi in the host cell and the activity of OPTb is involved in the disorderly degradation of peptides and hormones in the blood of patients with sleeping sickness (Gorrão et al. Peptides. 28: 2146, 2007). It has been proposed that peptidases may be important mediators in the pathogenesis of trypanosomiasis once it is demonstrated that the deletion of these enzymes from the parasites induces attenuation of virulence of trypanosomes (Caler et al. Embo J. 17:4957, 1998). The aim of this work was to evaluate the effect of purified forms OPTc and OPTb in a murine model of pain evaluation. **Methods:** Clones of oligopeptidase B from T. cruzi and T. brucei were provided by Dr. Silvia S. Gorrão from the Department of Biophysics of Federal University of São Paulo – SP. Enzymes were expressed as previously described (Burleigh et al. J. Cell Biol. 136(3):609, 1997; Morty et al. J. Biol Chem. 274(37): 26149, 1999). Male Swiss mice (18 to 22g) were evaluated at the writhing test (KOSTER et al. Fed. Proc.18:412, 1959). Nociceptive response evaluated was the number of abdominal contortions displayed by animals during a period of twenty minutes after injection of the irritant. **Results and Discussion:** OPTc and OPTb after expressed and purified were quantified and stored at a concentration of 5mg/ml at -20ºC, and both presented activity. Mice received intraperitoneal injections of OPTb or OPTc at different concentrations (150µg, 50µg and 16.6µg) and after 1 h were evaluated at the writhing test. OPTb decreased the number of abdominal contortions in all evaluated concentrations. On the other hand, OPTc decreased the number of abdominal contortions only at the concentrations of 150µg and 50µg. These data suggest that OPTb and OPTc might inhibit chemical mediators involved in the process. **Apoio financeiro**: CNPQ e Instituto Sírio-Libanês de Ensino e Pesquisa. Protocolo de Aprovação da CEUA – nº 2010/03

05.063

Central antinociceptive effects of ethanolic extract from the bark of *Pithecellobium cochliocarpum* on mice. Souza IA[1], Jesus RPFS[2], Bastos IVGA[2], Rodrigues GCR[2] [1]UFPE − Antibióticos

Ethnopharmacological relevance − *Pithecellobium cochliocarpum* has been used in Brazilian folk medicine. The present study examined the possible antinociceptive effect of the ethanolic extract from Pithecellobium cochliocarpum barks in chemical behavioral in four models of nociception, namely, acetic acid-induced abdominal writhing, formalin, tail immersion test and hot plate test. The extract at doses of 27.75, 51.5 and 103 mg/kg given intrapheritonially (i.p) produced inhibition of acetic acid-induced visceral pain. In the formalin test, the extract (27.75-103 mg/kg, i.p) also caused significant inhibition of both, the early (neurogenic pain) and the late (inflammatory pain), phases of formalin-induced licking. The antinociceptive action of the extract was tested against naloxone in the hot-plate test in a bid to further elucidate probable mechanisms of antinociception. Results showed that the extract at doses of 27.75, 51.5 and 103 mg/kg body weight caused significant ($P<0.05$) antinociceptive activity in all the nociceptive models. Furthermore, naloxone (2 mg/kg, i.p), significantly ($P<0.05$) antagonized the antinociceptive activity in all doses of the extract suggesting that the endogenous opioid system is involved in its analgesic mechanism of action. Thus, the study showed that the ethanolic extract of *Pithecellobium cochliocarpum* has analgesic effect in different experimental models of pain assessment and that this effect is mediated, at least in part, by activating opioid receptors in the CNS.

05.064

Role of NMDA receptors in carrageenin-induced hypernociception in rat temporomandibular joint: magnesium chloride modulator effect. Cavalcante ALC[1], Siqueira RMP[2], Colares MT[1], Chaves HV[1], Vale ML[2] [1]UFC − Ciências Médicas, [2]UFC − Fisiologia e Farmacologia

Temporomandibular disorder (TMD) is defined as functional change of the masticatory muscles and / or temporomandibular joint (TMJ) and is a common cause of chronic or acute orofacial pain. It is considered the main cause of non-dental chronic orofacial pain and a mandibular movement limitation factor. The TMJ nociceptive sensitivity is conduced mainly by the trigeminal via, which has NMDA receptors in resemblance with the somesthesic innervation of other parts of the body, but its role in orofacial pain, related to TMD, is still unclear. Considering the role of NMDA receptors (NMDA-R) in acute and chronic pain, we proposed to study the importance of this receptor in orofacial pain induced by TMJ inflammation. We also investigated the effect of magnesium chloride administration in the hypernociception of inflamed TMJ, since Mg++ is a physiological modulator of NMDA-R. Materials and **Methods:** The experimental protocol was previously approved by the Animal´s Ethics Committee of the Faculty of Medicine of the Federal University of Ceará (protocol number: 3010). Male Wistar rats (weighing 180-200g) received carrageenan injection (Cg) intrarticular (5% or 50 ug / articulation, 10 ul) in the left TMJ. We evaluated the behavioral nociceptive parameter, through the mechanical hypernociception test using electronic von Frey. The nociceptive threshold was measured before and after (4,6,10, 24 − 168 hours) the Cg injection. The NMDA receptor antagonist, MK-801 (0.1 − 0.5 mg/kg) was administered intraperitoneally 30 min before the Cg injection. Magnesium chloride (MgCl2, 90 mg/kg, divided in two oral administrations) was administered 3 days before the Cg and in another group for 5 days before the Cg. Both groups continued receiving MgCl2 up to 7 days after injection of Cg. The nociceptive test was performed as described above. **Results and Discussion:** the pre-treatment with MK-801 (0.1 − 0.5 mg / kg) inhibited hypernociception, increasing the nociceptive threshold by 83% at the hypernociception peak (6th hour) when compared to the control group ($p < 0.01$). The best response was reached with 0.5 mg/kg dose. The hypernociceptive effect of MK-801 lasted until 120 hours after a single administration (16%, $p < 0.05$). We observed that 3 day MgCl2 treatment increased the nociceptive threshold at 24% in hypernociception peak (6th hour), compared to the group that received only Cg ($p < 0.001$). There was a higher increase in nociceptive threshold at 56% in hypernociception peak (6th hour) in animals receiving MgCl2 for 5 days before injection of carrageenan, compared to the non treated group that received only carrageenan ($p < 0.001$). The antinociceptive effect was prolonged until the end of the experiment. Together these data suggest that NMDA-R probably are important for acute phase of hypernociception in TMJ inflammation, as well as for sustained hypernociception that occurs until the 7th day after a single Cg injection. The data also suggests that MgCl2 supplementation has antinociceptive effect and offers an alternative assay to prevent TJM inflammatory pain. Although, these are preliminary results and more experiments are needed to confirm our hypothesis. **Support**: CAPES and CNPq.

05.065

Involvement of B1 and B2 kinin receptors in painful neuropathy induced by paclitaxel in mice. Tamiozzo LLR[1], Dalmolin GD[2], Rigo FK[2], Ferreira J[1] [1]UFSM − Química, [2]UFMG − Farmacologia

Introduction: The chemotherapeutic agent paclitaxel is commonly used to treat solid tumors. Although effective, this drug often induces the development of painful neuropathy. Even though it is well established the involvement of kinin receptors in neuropathic pain induced by trauma or diabetes, their participation in neuropathic pain induced by chemotherapy has not been studied. Thus, the present study we evaluated the participation of B1 and B2 receptor in the placlitaxel model of chemotherapy-induced neuropathic pain. **Methods:** For the induction of neuropathy, male and female Swiss mice (25-35g, n=6-8) received administrations of paclitaxel (1 mg/kg) for 4 alternate days (1, 3, 5 e 7 days) intraperitoneally. The development of neuropathy was confirmed by the measurement of mechanical allodynia (painful hypersensitivity produced by previously innocuous stimuli) in the right hind paw of the animals through the application of von Frey filaments several days after the end of paclitaxel treatment. It was verified the involvement of B1 and B2 receptors in paclitaxel-induced allodynia by the intraperitoneal administration of the selective antagonists des-Arg9-[Leu8]-bradykinin (DALBK 150 nmol/Kg) and HOE 140 (100 nmol/Kg), respectively, in animals previously injected with paclitaxel. All experiments have been approved by the Ethics Committee of UFSM (process number: 23081.005024/2010-88). **Results and Discussion:** In the animals treated with paclitaxel, there was the appearance of allodynia on day 7 (the mechanical threshold reduction of 72± 20%), which intensified on the 14th day (94± 24%) and remained unchanged 21 days after the last paclitaxel administration (93±18%). Treatment with B2 receptor antagonist HOE 140 (100 nmol/ Kg) inhibited the mechanical allodynia observed 14 days after completion of paclitaxel injections. The anti-allodynic effect of HOE 140 was verified 30 minutes after its administration (44±12% of inhibition) and lasted about 1 hour (45±3%). Similarly, the treatment with the B1 receptor antagonist DALBK (150 nmol/Kg) also inhibited the mechanical allodynia induced by paclitaxel on day 14, an effect was observed 30 minutes after its administration (39±16%inhibition) effect lasted about 1 hour (46±13%). Taken together, our results demonstrated the involvement of both B1 and B2 receptors in the painful hypersensitivity induced by paclitaxel chemotherapy and confirmed the critical role of kinin receptors in neuropathic pain. Apoio Financeiro: CNPq, CAPES, CCNE/UFSM, CCS/UFSM.

05.066

Mechanism of peripheral antinociceptive effect of 15D-PGJ_2 on rheumatoid arthritis into the TMJ of rats. Quinteiro MS[1], Napimoga MH[2], Clemente-Napimoga JT[1] [1]UNIUBE − Biologia Molecular, [2]UNIUBE − Biologia Celular e Molecular

Introduction: Rheumatoid arthritis (RA) is an autoimmune chronic inflammatory disease that results in the destruction of cartilage and bone. Temporomandibular joints (TMJ) afflicted with RA may produce pain, joint stiffness, difficulties in opening the mouth, and open bite. This study evaluated the effect of peripheral administration of the 15d-PGJ2 into the rat TMJ on nociceptive behavioral induced by rheumatoid arthritis as well its mechanisms. **Methods:** Wistar rats were immunized with 500 µg of mBSA in 0.2 ml of an emulsion containing 0.1 ml phosphate buffered saline (PBS) and 0.1 ml Freund's complete adjuvant administered by subcutaneous injection. Booster injections of mBSA dissolved in Freund's incomplete adjuvant were given 7 and 14 days after the first immunization. Nonimmunized rats received similar injections but without the antigen (mBSA). Twenty-one days after the initial injection, arthritis was induced in the immunized animals by intraarticular injection of mBSA (1, 3, or 10 µg/TMJ). Nonimmunized and immunized rats were challenged with mBSA or with PBS. Animals were killed 6, 12, 24 or 48 hours after intraarticular injections of mBSA followed (15 min) by the injection of a low dose of formalin (0.5%) into the TMJ. A different set of immunized animals were challenged (mBSA followed by injection with 0.5% of formalin) and received different doses of 15d-PGJ2 (30, 100 or 300 ng/TMJ). The hyperalgesia was assessed by measuring the behavioral nociceptive responses, such as rubbing the orofacial region and flinching the head. After behavioral experiments, animals were terminally anesthetized and periarticular tissues were removed and homogenized. The supernatants were used to evaluate the levels of TNF-alpha, IL-1beta and KC by ELISA as well the expression of PKC and PKA by Western blot analysis. All animal experimental procedures and protocols were approved by the Committee on Animal Research of the University of Uberaba (059/2009). **Results:** The intraarticular injection of mBSA, but not PBS, in immunized rat induced dose- and time-dependent behavioral nociceptive responses in which the peak of behavioral nociceptive responses were obtained by using 10ug/TMJ of mBSA after 24h. Pretreatment (15min) with 15d-PGJ2 (30, 100 and 300 ng/TMJ) inhibited the RA-induced TMJ hyperalgesia in a dose-dependent manner (47%, 51% and 65% respectively, Tukey $p<0.05$). None of the three doses used reduced the expression of TNF-alpha and IL-1beta, but reduced the amount of KC (72%, 74% and 57% respectively, $p<0.05$). The western blot analysis demonstrated that using 100 ng/TMJ of 15d-PGJ2 in RA-induced TMJ was able to reduce the expression of PKA (67%, $p<0.05$) as well the expression of PKC (42%, $p<0.05$). Discussion/**Conclusion:** In the present study, we demonstrated that 15d-PGJ2 was able to reduce the RA-induced TMJ nociceptive behavior. This antinociceptive effect is related, in part, due to decrease of KC levels and PKA/PKC expression in the TMJ. **Financial Support:** PAPE/FAPEMIG 2010/005 and FAPEMIG PPM 097/09.

05.067

Involvement of kinin receptors in pronociceptive action of dynorphin A (1-17) in a mouse model of orofacial neuropathy. Schroeder SD[1], Luiz AP[2], Chichorro JG[3], Rae GA[1] [1]UFSC − Farmacologia, [2]UFSC − Ciências Fisiológicas, [3]UFPR − Farmacologia

Introduction: Endogenous opioids are well known for their analgesic properties. It has been recently demonstrated that Dynorphin A (1-17) [Dyn A], at high levels in the CNS, can induce nociception through activation of kinin B1 and B2 receptors and glutamate NMDA receptors (Lai et al.; Neurosci. Lett., 437: 175, 2008; Silverman SM; Pain Physician, 12: 679, 2009). Results from our laboratory have shown that constriction of the infraorbital nerve (CION) induces orofacial neuropathy in mice and that kinin receptor antagonists transiently diminish the hyperalgesia induced by CION (Luiz et al.; Neuropeptides, 44: 87, 2010). The current study assesses if changes in central levels of Dyn A could underlie the contribution of kinin receptor-mediated mechanisms to heat hyperalgesia induced by CION. **Methods:** Male Swiss mice (~35 g, 6-10 per group) underwent CION or sham surgery (Vos et al.; J. Neurosci., 14: 2708, 1994) or received subarachnoid administration (s.i.; between the occipital bone and C1) of Dyn A or vehicle (Fischer et al.; J. Neurosci. Meth., 148: 108, 2005). Animals were submitted repeatedly to (ipsilateral) application of a heat stimulus to the snout (heat source ~50°C placed about 1 cm from the vibrissal pad) and heat hyperalgesia was estimated as a decrease in the latency to display head withdrawal or vigorous snout flicking. Treatments included: Dyn A (1-17) (15 nmol, s.i.), antiserum against Dynorphin A (1-13) (200 mg, s.i.; anti-Dyn A), naloxone hydrochloride (opioid receptor antagonist, 10 mg/kg, i.p.), MK-801 (NMDA receptor antagonist, 0.025-0.01 mmol/kg, i.p.), (Des-Arg9,Leu8)-Bradykinin (DALBK, B1 receptor antagonist, 0.1-3 mmol/kg, i.p. or 25 nmol, s.i.), HOE-140 (B2 receptor antagonist, 0.1-1 mmol/kg, i.p or 100 pmol, s.i.) or vehicle. Protocols were approved by institutional ethics committee (23080.009342/2008-11). **Results:** Dyn A caused heat hyperalgesia, starting at 6 h of Day 1 and lasting up to Day 6 after treatment. Anti-Dyn A, injected s.i. 10 min prior to Dyn A, markedly reduced heat hyperalgesia from Day 1 to Day 5. Heat hyperalgesia on Day 3 after Dyn A was transiently reduced at 1 h after i.p. DALBK (1 mmol/kg): vehicle 11.1 + 0.6, Dyn A 4.9 + 0.6, Dyn A + DALBK 9.5 + 0.7 s), but was not influenced by i.p. HOE-140, naloxone or MK-801. Heat hyperalgesia at 6 h after Dyn A was significantly reduced in mice pretreated 10 min prior to Dyn A with DALBK or HOE-140: vehicle 9.8 + 0.6, Dyn A 5.5 + 0.4; DALBK (25 nmol, s.i.) 10.1 + 1.6; HOE-140 (100 pmol, s.i.) 9.7 + 1.0 (s). On the other hand, CION caused heat hyperalgesia from Day 2 to Day 17. Anti-Dyn A, given s.i. on Day 2 after CION, significantly decreased the heat hyperalgesia between 20 min and 2 h of its administration. **Discussion:** These results show that centrally-injected Dyn A induces a sustained orofacial heat hyperalgesia which is mediated in part by activation of central B1 and B2 kinin receptors. More importantly, as CION-induced heat hyperalgesia is inhibited by Anti-Dyn A, endogenous Dyn A appears to contribute to the neuropathic orofacial thermal hyperalgesia inflicted by CION by acting through B1 and B2 receptors. **Financial Support:** CNPq, CAPES, PRONEX, UFSC.

05.068

Analgesic effect of a novel allosteric antagonist of C5a receptors. Carneiro VL[1], Bertini R[2], Cunha FQ[1], Ferreira SH[1], Teixeira MM[5] [1]FMRP-USP − Farmacologia, [2]Dompé Research and Development

Introduction: Complement factor 5a (C5a), also named anaphylatoxin, is an important mediator of the inflammatory process, being involved in the recruitment and activation of leukocytes. Recently, we showed that C5a also play a crucial role in the cascade of events that trigger inflammatory hypernociception (increase in the sensitivity of nociception). In this context, the aim of this study was to evaluate the possible analgesic effect of a novel allosteric antagonist of C5a receptor (non-petidic, orally-acting) in different models of acute and long-lasting nociception. **Methods:** a) C57Bl6 male mice were used; b) mechanical nociceptive threshold was evaluated using the electronic version of the von Frey before and after the injection of different nociceptive stimuli into the mice paw; c) thermal nociception was evaluated using hot plate test d) the recruitment of neutrophils was determined indirectly by the quantification of myeloperoxidase activity; e) motor coordination was determined in rotarod f) This study was approved by Animal Ethics Committee of FMRP/USP (nº 173/2008). **Results:** Orally administration of C5a antagonist (50 min before) inhibited in a dose-dependent (0.3-3 mg/kg) manner carrageenin-induced hypernociception but did not alter neutrophil migration toward mice paw. C5a antagonist was also effective upon CFA- and TNF-induced hypernociception. The selective action of C5a antagonist on C5aR was confirmed by its effect upon C5a-induced hypernociception but not against PGE2, which effect does not depends on C5a.Moreover, C5a receptor antagonist neither produced central analgesic effect nor cause motor disturbance. **Conclusion:** These results confirm the involvement of C5a in the genesis of inflammatory pain, and they also indicate that this novel allosteric antagonist of C5a receptors could be a prototype of analgesic drugs. **Acknowledgement**: FAPESP, CNPq and FAEPA.

05.069

Hyponociceptive effect of H1 and H2 receptors in a model of articular inflammation induced by formalin. Souza-Silva E, Mascarin LZ, Eto C, Oliveira D, Tonussi CR UFSC − Farmacologia

Introduction: Histamine is found in synovial fluid of arthritic patients. Previous results showed that H1 antihistamines induce hypernociception probably acting in articular H1 receptor (P-05-058, SBFTE, 2009). Our aim was to obtain additional support on the role of H1, as well as H2 receptors in the articular incapacitation, edema, and plasma leakage after formalin injection into rat knee-joints. **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 induced two phases (P1: 0-5 min, and P2: 10- 60 min) of nocifensive behavior. Edema was evaluated by the articular diameter increase (AD; cm), and the plasma leakage was measured by the amount of Evans Blue (25 mg/kg, i.v., 30 min before the test) in synovial fluid (PL; µg/mL). The H1 antagonist cetirizine (0.01, 0.1, 1 and 10 mg/kg) and the H2 antagonist ranitidine (2.5 and 10 mg/kg) were administered systemically. Ranitidine (2 and 17 pmol/i.a), the H1 agonist 2-Pirydylethylamine (0.0492; 0.492, 4.92 and 492 nmol/i.a.), and the H2 agonist dimaprit (6, 34, 214 and 428 nmol/i.a.) were co-injected with formalin 1.5%. **Results:** Systemically given cetirizine (1 and 10 mg/kg) and ranitidine (10 mg/kg, as well as intraarticularly given ranitidine (2 and 17 pmol) increased P2 of formalin nociceptive response ($P<0.05$). In addition, 2-Pirydylethylamine (0.0492; 0.492, 4.92 nmol/i.a) decreased P2, while dimaprit (6, 214 nmol) decreased P1 and P2 (6, 34, 214, 428 nmol) of the nociceptive response ($P<0.05$). Cetirizine (0.01 mg/kg) prevented the hyponociceptive effect of 2-Pirydylethylamine (4.92 nmol/i.a). However, the highest dose of the H1 agonist (492 nmol/i.a. $P<0.01$) increased the PET in P1 and P2. None of the treatments changed the edema or plasma leakage, except for the lower dose of dimaprit (6 nmol/i.a) that increased the plasma leakage. **Discussion:** The present data confirm and extend the idea that articular H1 receptor activation, and also H2 receptors, can inhibit the formalin-induced nociception. In addition, this effect seemed to be unrelated to any vascular effect. This work was approved by the local ethical committee for animal use (CEUA 23080.034306/2009-69).Acknowledgments: Capes, Fapesc/Pronex/CNPq

05.070

Role of nitric oxide in nociception, edema and plasma leakage induced by intra-articular formalin. Eto C, Souza-Silva E, Mascarin LZ, Tonussi CR UFSC − Farmacologia

Introduction: The participation of nitric oxide (NO) has been demonstrated in several nociceptive cutaneous tests, and as a mediator of analgesic drugs. Furthermore, NO can change the permeability of blood vessels facilitating plasma leakage in different tissues. Our aim was to evaluate the role of nitric oxide in intrarticular nociception, edema and plasma leakage caused by formalin injection into rat knee-joints. **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 of articular incapacitation (P1: 0-5 min, and P2: 10-60 min). Edema was evaluated by the articular diameter increase (AD; cm), and the plasma leakage was measured by the amount of Evans Blue (25 mg/kg, i.v., 30 min before the test) in synovial fluid (PL; µg/mL), 1 hour after formalin injection. N-Nitro-L-Arginine (0,4; 4; 400; 800 nmol /i.a), an iNOS inhibitor, L-Arginine, a NOS substrate (0,57; 5,7; 57 nmol/i.a), and sildenafil, an inhibitor of type 5 phosphodiesterase (0,15; 1,5; 15 nmol/i.a.) were administrated into knee joint 20 minutes before formalin injection. **Results:** Lower N-Nitro-L-Arginine doses (0,4; 4 nmol) decreased nociception ($P<0,05$), but the higher (400 nmol) caused hypernocicepion in P2 (10-35 min) of formalin test. The highest dose of L-Arginine (57 nmol) caused hyponociception in P2 (10-45 min) ($P<0.001$). Sildenafil in the lowest dose (0.15 nmol) decreased nociception in P2 (10-20 min). None of the treatments changed the AD or PL. **Conclusion:** The present data suggest that nitric oxide can modulate the articular nociception induced by formalin without change AD or PL. Furthermore, the NO production seems to have dual role in nociception induced by intra-articular formalin. This work was approved by the local ethical committee for animal use (CEUA 23080.057206/2008-20). **Acknowledgments**: Capes, Fapesc/Pronex/Cnpq

05.071

Cafestol evokes peripheral antinociception via activation of α_{2C} adrenoceptors. Guzzo LS, Perez AC, Duarte IDG UFMG − Farmacologia

Introduction: Cafestol is a diterpene found only in the coffee's unsaponifiable lipid fraction. Studies in our laboratory showed the peripheral antinociceptive effect of cafestol and the involvement of endogenous opioid peptides in this effect. Considering that it has been proposed the existence of a μ/α2 receptor complex mediating antinociception in periphery, the aim of this study was to investigate the involvement of adrenoceptors in the peripheral antinociceptive action of cafestol. **Methods:** The rat paw pressure test was used and the hyperalgesia was induced by intraplantar injection of prostaglandin E2 (PGE2, 2 μg). The nociceptive threshold was measured in the right paw and determined as the average of three consecutive trials recorded before and 3 h after PGE2 injection. Cafestol was injected 2 h and 55 min after PGE2 administration. Yohimbine (inespecific α2-adrenoceptor antagonist) was administered 40 min before cafestol. BRL 44 480 (α2A antagonist), imiloxan (α2B antagonist), rauwolscine (α2C antagonist), and RX 821002 (α2D antagonist) were administered 30 min before cafestol. All drugs were administered subcutaneously into the plantar surface of male Wistar rats' hind paw. **Results and Discussion:** Intraplantar injection of cafestol (40 and 80 μg/paw) induced peripheral antinociception; however, a dose of 20 μg/paw did not significantly reduce the hyperalgesic effect of PGE2. The antinociceptive effect of cafestol was due to a local action, since even in the highest dose (80 μg/paw) did not produce any effect in the contralateral paw. Yohimbine (10 and 20 μg/paw) and rauwolscine (15 and 20 μg/paw) prevented the peripheral antinociceptive effect of cafestol (80 μg/paw). BRL 44 480, imiloxan and RX 821002 in the dose of 20 μg/paw were ineffective in blocking cafestol antinociception. The present results provide evidence that the α2C-adrenoceptor was involved in the peripheral antinociceptive effect of cafestol. It is probable that cafestol promotes opioid peptides release and the endogenous opioids act on the μ/α2 receptor complex inducing an antinociceptive effect. **Financial Support:** EMBRAPA, Fapemig and CNPq (473758/2007-5) Fellowships: CNPq Ethics Committee on Animal Experimentation (CETEA/UFMG) protocol nº 41/2007

05.072

Antinociceptive and anti-inflammatory activity of ethanol extract of *Polygala sabulosa* in mice. Borges FR, Pierosan L, Silva MD, Córdova MM, Santos ARS CFS-UFSC

Introduction: Polygala sabulosa, A. W. Bennett (Polygalaceae), popularly known as "timutu-pinheirinho" is a plant that has distribution in southern of Brazil (Wurdack & Smith, 1971). The plants of the genus Polygala are traditionally used in popular medicine to treat a large number of pathologies, including its use as a topical anaesthetic (Wasicky, 1945) and have reports of use of plants of this species with potential neuroprotective effects (Kwon et al. 2004). Data from our research group, reported that the hydroalcoholic extract of P. sabulosa, has antinociceptive and anti-inflammatory effect in animal models (mice) of acute pain (Ribas et al., 2007), evidenced mainly by glutamatergic antagonism. Additional studies from our group demonstrated the action of compounds isolated from the aqueous fraction of this same plant mediating the antinociceptive activity in an animal model of inflammatory visceral pain induced by intraperitoneal injection of acetic acid in mice (Meotti et al., 2006). Therefore, the aim of our study is to evaluate the anti-inflammatory activity of ethanol extract (EE) of P. sabulosa in several animal models of pain and inflammation. Experimental Protocol approved by CEUA − UFSC under code PP00503. **Methods:** and **Results:** A model of inflammatory nociception induced by intraplantar formalin injection (2.5%) in animals pretreated with ethanolic extract (EE) of P. sabulosa orally 1 h before, at doses of 30, 100 and 300 mg / kg was used. The formalin model is able to induce an acute inflammation in two phases, primarily neurogenic phase (5 min) and second phase called inflammation phase (15-30min). EE of P. sabulosa was not able to induce antinociception in any of the doses in the neurogenic phase and inhibited in a dose-dependent manner the nociception of inflammatory phase (inhibition 67% ± 11; DI50=38,95 mg/kg). Posteriorly, we used a model of visceral inflammation by intraperitoneal injection (i.p) of LPS (0.02 mg / kg), able to induces peritonitis. The EE was administered one hour before the LPS injection and four hours before the LPS administration, the animal were euthanized and the peritoneal exudate collected in order to proceed to count of the number of mononuclear and polymorphonuclear leukocytes. The positive control group received dexamethasone i.p. (5 mg/kg) 30 min before injection of LPS. The administration v.o of the ethanol extract of P. sabulosa, was able to inhibit in a dose dependent manner the migration of leukocytes (inhibition 58% ±3) **Discussion:** The EE of P. sabulosa, has antinociceptive and anti-inflammatory effect evidenced by the models described above, promoting reduction of leukocyte migration in the model of peritonitis, requiring further study about the mechanisms by which this effect takes place. Support: UFSC, CAPES, CNPQ

05.073

Visceral antinociceptive effect of HC-030031, an antagonist of TRPA1 ion channel, is independent of inflammatory cells and nitric oxide. Pereira LMS[1], Sá LG[1], Wong DVT[1], Callado RB[1], Teixeira CCG[1], Bem AXC[1], Larsen GR[2], Lima-Júnior RCP[1], Ribeiro RA[1]
[1]UFC − Fisiologia e Farmacologia, [2]Hydra Biosciences Biopharmaceutical Co − Biopharmacology

Introduction: Visceral pain (VP) is one of the most frequent reasons why patients seek medical care. The mechanism involved in the viscero-somatic model of nociception, writhing test, vary according to the algogenic substance used. For instance, the nociceptive response to the prostaglandin analogous, iloprost, involves the direct sensitization of the nociceptor. On the other hand, we have previously shown that acetic acid and zymosan mechanisms of nociception are dependent on inflammatory resident cells. (Ribeiro et al Eur J Pharmacol.; 387(1):111-8, 2000). Some studies have shown a somatic nociceptive response due to the TRPA1 activation which is effectively modulated by the experimental tool, HC-030031, a TRPA1 antagonist. However, as far as we know, there is no study showing the implication of TRPA1 receptors in VP. We then aimed to investigate the role of TRPA1 in the animal model of ifosfamide (IFO)-induced VP in bladder and the possible mechanisms involved. **Methods:** Swiss male mice (n=6) were given Carboxymethyl cellulose (CMC 0.25%, 1 mL/kg, p.o.), the compound HC-030031 (75mg/Kg, p.o.) or L-NAME (10mg/Kg, s.c.) alone or in combination with L-arginine (600mg/Kg, i.p.) 1h before the injection of IFO (400 mg/kg, i.p.). Visceral nociception was assessed by the von Frey test previously (T0) and 12h (T1) later IFO injection. The results were obtained in grams (T0-T1). In another experimental setting, the animals were treated with CMC 0.5% (1 mL/kg, p.o) or HC-030031 (18.75; 37.5 or 75mg/Kg, p.o.) previously an i.p. injection with acetic acid 0.6% (AA, 10 mL/kg), zymosan (Zym, 1 mg/cavity) or misoprostol (MPT, a stable prostaglandin analogous, 1µg/cavity) and immediately had the writhing responses counted for 30 min. Statistical analysis were performed with ANOVA/Student Newman Keul as appropriate. $p<0.05$ was accepted. (CEPA: Protocol 06/09). **Results:** IFO induced significant ($p<0.05$) visceral nociception (6.25±1.08) in comparison with saline treated group (1.97±0.89). Moreover, HC-030031 and L-NAME prevented ($p<0.05$) the nociceptive response (2.30±1.07; 1.58±0.86) respectively when compared with IFO-treated group. Although, the pretreatment with L-arginine was able to reverse the antinoceceptive effect of L-NAME (6.84±1.23), it failed to do the same ($p>0.05$) with HC-0300031 (0.72±0.69). In addition, AA, Zym and MPT induced significant writhing responses (43.71±4,43, 11.00±2.11, 9.00±2.30, respectively) which was significantly abrogated in HC-030031 treated mice in all the doses tested (29.07%, 53.35% and 41.59%, in the AA test, 55.85%, 61.03% and 71.20%, in the Zym test, 63.88%, 83.33% and 88.88%, in the MPT induced nociception, respectively to 18.75, 37.5 and 75 mg/kg doses. **Discussion:** Since prostaglandin activates the nociceptor directly, it was shown that HC-030031 inhibits visceral nociception possibly through the stabilization of the neuronal ends. The antinociceptive effect of HC-030031 seems to be independent of the inhibition of inflammatory resident cells and nitric oxide pathway. This study provides perspective for the effective management of visceral pain through the modulation of TRPA1 channels. **Financial Support:** CAPES/CNPq.

05.074

Synthesis and evaluation of antihyperalgesic activity of benzofuranone derived of xanthoxylin: hypothesis of the possible mechanism of action. Souza JP[1], Quintão NLM[1], Sonza DR[2], De Campos-Buzzi F[2], Niero R[2] [1]UNIVALI − Ciências Farmacêuticas, [2]NIQFAR-CCS-UNIVALI

Introduction: Benzofuranones are substances founded naturally in plants, but can be obtained by synthetic means. Represent an interesting group of compounds with a wide range of biological action including anti-inflammatory and antinociceptive activities. The present study evaluate the antinociceptive effect and a possible mechanism of action of a benzofuranone obtained synthetically in different models of pain in mice. **Methods:** The synthesis of a benzofuranone (2-(1-3-dihydro-benzofuran-2-1-yl)-1-(2-hydroxy-4,6-dimetoxy) ethanone) was performed using xanthoxylin and 2-carboxybenzaldehyde (0.95 mmol each) as starting reagents. Initially the xanthoxylin was solubilized in ethanol, then added a solution NaOH 10% (p/v) (2.5 mmol) and after a few minutes, added the benzaldehyde. The reaction was kept stirring at room temperature for 19 hours. The reaction was monitorated by TLC using a mixture of hexane / ethyl acetate (80:20) as eluent. Finally the reaction was neutralized with HCl (hydrochloric acid) until total precipitation and filtered in the Buchner funnel. For the pharmacological tests were used Swiss mice males and females (20 to 35g, n = 6-8). Hypernociception to induce inflammatory models were carried out by hypernociception induced by carrageenan (50 µl / paw) or PGE2 (20 µl/paw). A mechanical sensitivity was assessed using von Frey filament (0.6 g). All the procedures used in the present study were approved by the Animal Ethics Committee of UNIVALI (Protocol numbers 115/2010). **Results:** The pretreatment with benzofuranone (0,03, 0,1 and 0,3 mmol / kg, ip) was effective in reduce the mechanical sensitization induced by carrageenan (34,9% ± 11, 79,3% ± 9, 96,4% ± 11), as well as PGE2 (42,5% ± 8, 76,8% ± 7, 98,8% ± 7). **Discussion:** These results demonstrate that the use of 2-(1-3-dihydro-benzofuran-2-1-yl)-1-(2-hydroxy-4,6-dimetoxy)ethanone) was capable of interfering in both models, in the edema formation, while the mechanical sensitization induced by carrageenan, suggesting that substance can act in the inhibition of cascade the interleukins formation. **Financial Support:** CNPq, FAPESC − SC, ProPPEC/UNIVALI.

05.075

Evaluation of antinociceptive spinal activity of TX3-4, a toxin peptide purified from the spider *Phoneutria nigriventer* venom, in mice. Silva JF[1], Fontanini CEM[2], Lavor, MSL[3], de Souza AH[4], Pessoa FLC[2], Pinheiro ACN[4], Binda NS[4], Ferreira J[5], Gomez MV[4] [1]UFMG − Farmacologia Bioquímica e Molecular, [2]UFMG − Medicina, [3]UFMG − Clínica e Cirúrgica Veterinária, [4]UFMG − Farmacologia, [5]UFSM − Química

Introduction: The voltage-sensitive calcium channels (VSCCs) play a key role in several cellular and physiological functions like neurotransmitter release from both central and peripheral nerves. *Phoneutria nigriventer* toxins have shown a high potential to block these channels and one of these toxins, Tx3-4, blocks P/Q and N-type of VSCCs (Miranda et al, 2001). The present study investigates the Tx3-4 action on the inflammatory nociception. **Methods:** Experimental Animal Ethics Committee: 4201092005-6. Tests of behavior were performed on healthy Swiss mice (25-35 g) injecting intrathecally (i.t.) 5 µl of Tx3-4 (3-30 pmol/site) or omega-conotoxin MVIIC (3-30 pmol/site) , a control toxin, 0.5 h, 1h, 2h, and 3 hours before injection of formalin (2,5% formaldehyde in saline, 20 µl volume) into the plantar surface of the right hind paw. Tx3-4 and MVIIC (3 pmol/site) were administrated 0.25 to 4 h after plantar surface incision into the plantar surface of the right hind paw. The injected hind paw pain behavior's time and the postoperative mechanical allodynia were measured in the formalin test and incisional pain model, respectively. **Results:** The phonotoxin intratecally injected (30 pmol/site) presented an antinociceptive effect of 60± 8% , 1 hour before formalin administration ($P < 0.05$ compared with "placebo" treatment) and an antinociceptive effect of 80±12% , 1 h after plantar incision ($P < 0.001$ compared with vehicle). The MVIIC did not present antinociceptive activity on first test but inhibited 65±10% of allodynia 1 h after paw surgery ($P < 0.01$ compared with vehicle). Discussion and **Conclusion:** Tx3-4 blocks P/Q-type of VSCCs (Dos Santos 2002) and this is consistent with its action rising inflammatory antinociception. The toxin has been more powerful and effective than MVIIC, mainly in clinic model. Thus, these data suggest that Tx3-4 acts as a potent analgesic on in vivo models of experimental nociception and more studies are necessary to better clarify the mechanisms involved in its antinociceptive spinal activity. KEY WORDS: Nociception, calcium channels, toxins and inflammation

References: De Miranda DM., Brain Res Bull. 54(5):533 (2001) Dos Santos RG, J. Biol. Chem. 277:13856-13862 (2002) SUPPORTED BY: FAPEMIG, CNPq, Instituto Milênio, Grupo Santa Casa

05.076

Is there an involvement of endocannabinoid system in the peripheral antinociception of NSAID? Resende LC, Duarte IDG UFMG − Farmacologia

Introduction: Diclofenac and dipyrone are nonsteroidal anti-inflammatory drugs (NSAID) with potent anti-antinociceptive activity. It was initially suggested that its antinociceptive effect was due to inhibition of prostaglandin synthesis at the inflamed tissue. Studies show that others mechanisms are involved in its antinociceptive effect like opioids system and NO/cGMP/KATP pathway. Recently it was demonstrated the participation of endocannabinoid system on supraspinal antinociceptive activity of indomethacin. Thus, the aim of this work is to investigate the participation of endocannabinoid system in the peripheral antinociception effect of NSAIDs dipyrone, diclofenac and indomethacin. **Methods:** The rat paw pressure test was used and hyperalgesia induced by intraplantar injection of prostaglandin E2. All drugs were administered locally into the right hind paw of Wistar male rats. We used the antinociceptive drugs, dipyrone, diclofenac and indomethacin, the antagonists of CB1 and CB2 receptors, AM251[N-(piperidin-1-yl)-5-(4-iodophonyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide] and AM630 [6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)methanone], respectively. **Results:** Dipyrone 40µg/paw, diclofenac 20µg/paw and indomethacin 40µg/paw, elicited a local peripheral antinociceptive effect. This effect was not antagonized by CB1-AM251(80µg) and CB2-AM630 (100µg) antagonists. **Discussion:** The results provide evidences that the endocannabinoid system is not important for peripheral antinociceptive activity of NSAIDs dipyrone, diclofenac and indomethacin. **Financial Support:** Fellowships from CNPq and FAPEMIG. Ethics Committee on Animal Experimentation (CETEA/UFMG) protocol No. 41/2007

05.077

Antinociceptive effect of Riparin II in mice. Carvalho AMR[1], Leite CP[1], Moura BA[3], Vasconcelos LF[1], Melo TV[1], Bastos MVR[1], Barbosa Filho JM[2], Sousa FCF[2] [1]UFC − Fisiologia e Farmacologia, [2]UFPB − Tecnologia Farmacêutica

Introduction: *Aniba riparia* (NEES) MEZ, from the Lauraceae family, is popularly named "louro" in Brazil. It belongs to a genus mainly found in Central Amazonia and Guiana comprising approximately 40 species of lowland shrubs and trees. From the green fruit of *Aniba riparia*, collected from the Amazonas state of Brazil, were isolated three substances with broad spectrum antimicrobial activity: methyl ethers of N-benzoyl tyramine (riparin I), N-(2-hydroxybenzoyl) tyramine (riparin II) and N-(2,6-dihydroxybenzoyl) tyramine (riparin III) which were later synthesized. The present work was undertaken to evaluate the antinociceptive effect of riparin II, using animal models of antinociceptive activity. **Methods:** The effects of riparin II (ripII) were studied in two behavior animal models in mice: acetic acid-induced abdominal whrithing test, described by Koster et al. (Fed. Proc. 18: 412, 1959) and formalin test, described by Dubuisson and Dennis (Pain 4: 161, 1977). Riparin II (ripII) was administered orally at single doses of 25 and 50 mg/kg while indometacin 10 mg/kg and morphine (7.5 mg/kg) i.p. were used as standard drugs. This work was approved by Committee on Ethics in Animal Research (CEPA), of the Federal university of Ceara (protocol number 15/09). **Results and Discussion:** The results are presented as mean ± S.E.M. Data were analyzed by ANOVA followed by Student–Newman-Keuls´s post hoc test. Results were considered significant at $P<0.05$. In the acetic acid-induced abdominal whrithing test, groups treated with riparin II (25 mg/kg and 50 mg/kg) and indometacin (10 mg/kg) significantly decreased the number of acetic acid-induced abdominal whrithing as compared to control group [control: 28.29± 1.782 (8); ripII-25: 17.33± 0.7149 (8); ripII-50: 21.14± 1.455 (7); IND-10: 12.08±1.323 (10)]. In the formalin test, groups treated with morphine 7.5 mg/kg significantly decreased the paw licking time at the early phase [control: 51.29± 4.669 (8); MORP-7.5 mg/kg: 29.57± 1.152 (8)] and late phase [control: 17.83± 1.887 (8); MORP-7.5 mg/kg: 1.286± 0.9932 (8)] as compared to control. However, animals treated with riparin II 25 and 50 mg/kg significantly decreased the paw licking time only at the late phase [ripII-25: 3.667± 0.8819 (8); ripII-50: 1.750± 0.5901 (8)] as compared to control. In conclusion, acute treatment with riparin II at doses of 25 and 50 mg/kg seems to possess antinociceptive activity as demonstrated in the acetic acid-induced abdominal whrithing test and formalin test in mice. **Financial Support:** CNPq and CAPES

05.078

Crotalphine reduces peripheral sensitization evoked by activation of TRPV1 receptor in mice. Motta EM[1], Machado FC[2], Gutierrez VP[2], Lira[2], Gandra[2], Picolo G[3], Cury Y[3] [1]UFSC − Dor e Sinalização, [2]IBu − Dor e Sinalização, [3]IBu − Laboratório de Fisiopatologia

Introduction: Our group has demonstrated that treatment with crotalphine (CRP), a peptide first identified and isolated from *Crotalus durissus terrificus* snake venom, produces a potent and long-lasting analgesic effect in different models of inflammatory and chronic (neuropathic/cancer) pain. The antinociceptive effect of crotalphine seems to depend almost exclusively on the indirect activation of the opioid system. Interestingly, the high effectiveness and long-lasting action of CRP is observed only in the presence of inflammation/tissue lesion. In order to further characterize the role of previous sensitization in the action of CRP, the aim of this work is to evaluate the effect of this peptide in peripheral sensitization mediated by activation of TRPV1 receptors. **Methods:** Mice (30-40 g) received a 20 µl intraplantar (i.pl.) injection of either capsaicin (CPS; 0.03 or 1 nmol/paw), prostaglandin E2 (PGE2; 0.01 nmol/paw) or the corresponding vehicles. Immediately after the injection, the overt nociception [licking time (s)] was recorded in the first 5 min. The same animals were evaluated for paw sensitivity to mechanical stimulation through von Frey filaments (VFH) at different time intervals (1-3 h). The withdrawal response frequency (in %) was measured following 10 applications (with a duration of 3 s each) of VFH (0.6 g). **Results:** Pretreatment (1 h before) with CRP (50-200 ug/kg) did not alter nociception caused by i.pl. injection of CPS (1 nmol/paw). However, treatment with CRP reduced, in a dose-dependent manner, the mechanical allodynia caused by this algogenic agent. The higher dose of CRP (200 ug/kg) markedly reduced the mechanical allodynia at all times assessed (~80±7). Injection of a sub-threshold dose of PGE2 (0.01 nmol/paw) prior (1 h) to CPS (0.03 nmol/paw, sub-threshold dose) markedly sensitized the hind paw to the overt nociception (CPS: 18±3 s; PGE2: 16±4 s; CPS+PGE2: 59±7 s). The sub-threshold doses of CPS and PGE does not change the basal threshold of mechanical stimulation. The potentiation, by pre-treatment with PGE2, of mechanical allodynia and nociception induced by CPS was reduced by the treatment with CRP (200 ug/kg) in 63±5% and 50±6%, respectively. **Discussion:** The data from this study confirm and extend previous findings from our group which demonstrated that the antinociceptive effect of CRP depends on a prime sensitization. This important feature of CRP is of great clinical relevance, since various pathophysiological conditions have an inflammatory component. **Financial Support:** FAPESP and INCTTOX program Ethics Protocol: Comissão de Ética no uso de Animais do IBu – CEUAB / Protocol number 437/07).

05.079

Evidence for the Involvement of kinin B_1 and B_2 receptors in the neuropathic pain-like behavior after treatment with paclitaxel in mice. Motta EM[1], Costa R[2], Manjavachi MN[3], Dutra RC[2], Pesquero JB[3], Calixto JB[2] [1]UFSC − Dor e Sinalização, [2]UFSC − Farmacologia, [3]UNIFESP − Biofísica

Introduction: Paclitaxel is a commonly used cancer chemotherapic but its use is frequently associated with painful peripheral neuropathies, but the underlying mechanisms are poorly understood. Several literature data have demonstrated the involvement of kinin B1 (B1R) and B2 (B2R) receptors in inflammatory and neuropathic pain. OBJECTIVE: To assess the role of B1R and B2R receptors in the chemotherapy-evoked painful peripheral in mice by means of genetic and pharmacological tools. **Methods:** Male CD1, C57BL/6 WT, B1R-/-, B2R-/- and B1B2R-/- mice received Paclitaxel (PTX, 2 mg/kg) or vehicle intraperitoneally (i.p.) once a day for 5 consecutive days (d). Mechanical and thermal hypersensitivity were measured 7d, 9d, 11d and 14d after PTX. Different groups of animals were systemically post-treated (7d and 14d) with DALBK (100-300 nmol/kg) or HOE (30-100 nmol/kg), B1R or B2R antagonists, respectively. Other animal groups were repeatedly treated (0d-6d after PTX) with DALBK or HOE. Intraplantar, intrathecal or intracerebroventricular administration of those antagonists was conducted on 7d and 14d after induction of neuropathic pain. **Results:** PTX-induced mechanical and thermal hypersensitivity were significantly reduced in B1R-/-, B2R-/- and B1B2R-/- knockout mice. Moreover, the systemic pre- and post-treatment with B1 or B2 antagonists, DALBK or HOE significantly reduced both painful phenomena when assessed 7d and 14d after PTX. Both antagonists, administered by intrathecal or intracerebroventricular routes (but not intraplantar) 7d or 14d after neuropathy induction, significantly inhibited mechanical and thermal hypersensitivity. **Conclusions**: Together, present results provide new evidence implicating the role of spinal and supraspinal role of both kinin B1R and B2R in PTX-induced neuropathic pain. Therefore, kinin receptors antagonists might represent valuable tools for the management of chemotherapic-induced neuropathic pain. **Financial Support:** CNPq, FAPESC and CAPES (all from Brazil). Ethics Protocol: The experiments were performed with the agreement of the Institutional Ethics Committee (protocol number PP00032)-Universidade Federal de Santa Catarina.