05. Pain and Nociception Pharmacology

05.001 Activation of satellite glial cells and P2X7 receptors of dorsal root ganglion contribute to signaling of inflammatory muscle pain. Aquino BM¹, Fusaro C², Oliveira-Fusaro MCG¹ ¹LABEDI-FCA-UNICAMP – Health, ²USF

Introduction: The dorsal root ganglion (DRG) is an active participant in the signaling of painful process. Specifically, activation of satellite glial cells and purinergic P2X7 receptors of DRG seems to be important for signaling of inflammatory and neuropathic pain to central nervous system. However, the contribution of satellite glial cells and P2X7 receptors of DRG in the signaling of muscle pain is unknown. Considering the clinical relevance of muscle pain, the aim of this study was to analyze the involvement of satellite glial cells and P2X7 receptors of DRG in the signaling of inflammatory muscle pain induced by carrageenan in the gastrocnemius muscle of rats. Methods: Inflammatory muscle pain was induced by injection of carrageenan into the gastrocnemius muscle of rats. Mechanical muscle hyperalgesia was quantified with a Randall Sellito pressure analgesimeter, applied to the gastrocnemius muscle before and three hours after carrageenan. Male Wistar rats were used and methods were approved by the Ethics Committee in Animal Research of the UNICAMP. To investigate the involvement of satellite glial cells, fluorocitrate, an inhibitor of the function of satellite glial cells, was injected into DRG (L5) five minutes before injection of carrageenan into gastrocnemius. Similarly, to investigate the involvement of P2X7 receptors of DRG, A438079, a selective P2X7 receptors antagonist, was injected into DRG (L5) five minutes before the injection of carrageenan into gastrocnemius. Results: Carrageenan (100µg/muscle) produced mechanical muscle hyperalgesia 3h after its injection (p<0.05, Two Way ANOVA). Pre-treatment with fluorocitrate (2 and 20nM/5µL, i.g.l.) or A438079 (100 and 1000µM/5µL, i.g.l.) prevented, in a dose dependent manner, the mechanical muscle hyperalgesia induced by carrageenan (p>0.05, Tukey test, n=5). Injection of fluorocitrate or A438079 (i.g.l.) alone did not affect mechanical nociceptive threshold (p>0.05). Conclusions: These data demonstrated that activation of satellite glial cells and P2X7 receptors of DRG contribute to signaling of inflammatory muscle pain. Therefore, it is plausible to suggest satellite glial cells or P2X7 receptors of DRG as pharmacological targets to control inflammatory muscle pain. Financial Support: FAPESP (2011/11064-4), CNPQ (473790/2013-0). Animal Research Ethical Committee (CEUA = 3959-1).
Introduction: Fibromyalgia is a chronic disease, mainly characterized by widespread pain. Pain is usually initiated by a painless stimulus, thereby defining the allodynia present in fibromyalgia. The syndrome is accompanied by symptoms such as fatigue, sleep and cognitive disorders, as well as depression (Ceko M., Pain Res Treat. 2012:585419. 2012). Several studies have demonstrated antinociceptive activities for either agonists or antagonists of nociceptin/orphanin FQ receptor (NOP), depending on the route of administration and the experimental pain model (Schröder W., Br J Pharmacol. 171:3777. 2014). The NOP ligands also display modulatory effects on anxiety and depression parameters in rodents. The present study aimed to evaluate the relevance of NOP in experimental fibromyalgia. Methods: The fibromyalgia model was accomplished in adult female CF-1 mice by the administration of reserpine (0.25 mg/kg), given by subcutaneous route (s.c.), once a day, during 3 consecutive days. Control groups received vehicle, employing the same schedule of administration. The animals were subjected to the behavioral tests (mechanical allodynia, hot plate, forced swimming test and elevated plus-maze) on the 4th day. Mice were acutely treated with the selective NOP agonist nociceptin (N/OFQ), or antagonist UFP101 (5.4-9.5 µg/kg), given by intraperitoneal (i.p.) route, 30 min before the experimental sessions. Moreover, the N/OFQ levels were analyzed in serum samples of reserpine-treated mice or in saliva of fibromyalgia patients by ELISA assay. Results: Our data demonstrated that treatment with N/OFQ at 9 µg/kg, but not at 5.4 µg/kg, produced a slight increase (by 26±4.5%) of reserpine-induced mechanical allodynia. Of note, the systemic administration of UFP101 at 5.7 and 9.5 µg/kg was able to inhibit the mechanical allodynia, when compared with reserpine group (36±5% and 34±8.5%, respectively). On the other hand, the i.p. injection of N/OFQ (5.4 and 9 µg/kg) or UFP-101 (5.7 and 9.5 µg/kg) did not produce any significant effect on the latency time to thermal stimulus in the hot plate, when compared to control animals. The NOP ligands also failed to modify depression- or anxiety-related parameters in the forced swimming test and the elevated plus-maze paradigms, respectively. Finally, the levels of N/OFQ were not significantly different when comparing the saliva of control and fibromyalgia patients, whereas there was a slight reduction of N/OFQ serum levels in reserpine-treated mice. Conclusion: For the first time, our results show the ability of the selective NOP antagonist UFP101 to prevent the mechanical allodynia in the mouse model of fibromyalgia induced by reserpine. However, this antagonist failed to alter the thermal nociception or even depressive- or anxiety-like symptoms in experimental fibromyalgia. Further studies are in progress in order to assess the site of action of UFP101, and whether the levels of N/OFQ are altered at the spinal level. Financial support and acknowledgments: FINEP, CAPES, CNPq, PUCRS Animal Research Ethical Committee: 15/00487 Human Research Ethical Committee: 844208
Study on the participation of the adrenergic system in the modulation of peripheral pain. Gonzaga ACR¹, Romero TRL¹, Castor MGM¹, Lemos VS², Silva GC², Duarte IDG¹ UFMG – Farmacologia, ²UFMG – Fisiologia

Introduction: Noradrenaline is classic involved in endogenous modulation of pain at the spinal site. On the other hand, noradrenaline is also involved in the peripheral inflammatory pain and analgesia by a different mechanism. Therefore, this study proposed to investigate the involvement of the noradrenergic system in the peripheral modulation of inflammatory pain.

Methods: It was used the rat paw pressure test and the Western Blotting assay, in which hyperalgesia were induced by intraplantar carrageenan administered into the right hind paw of male Swiss mice. Results: The intraplantar administration of non-selective α₂ adrenoceptors antagonists yohimbine, at different times and doses induced a significant increase in carrageenan hyperalgesia (100 μg). Rauwolscina, a selective α²C-adrenoceptor antagonist, induced a significant increase in the hyperalgesia induced by carrageenan (100 μg). Moreover, imiloxane, a selective α₂B-adrenoceptor antagonist, reversed carrageenan (100 μg) induced hyperalgesia. The selective α₂A- and α₂D-adrenoceptor antagonists, BRL 44408 and RX 821002 respectively, did not alter carrageenan induced hyperalgesia. Administration of reboxetine, a selective noradrenaline reuptake inhibitor, induced a significant decrease in the Δ of nociceptive threshold in animals pretreated with carrageenan (200 μg). The Western Blotting assay showed a significant increase in of α₂C adrenoceptor expression with time. Conclusions: These data suggest that noradrenaline released by the inflammatory process activates α₂C adrenoceptor contributing to the peripheral control of inflammatory pain, at peripheral sites, whereas α₂B-adrenoceptor could be involved in the hyperalgesic effect induced by peripheral noradrenaline.

Financial support: CNPq, CAPES and FAPEMIG. Protocol of CEUA: 50/2013
Role of endocannabinoid system in aripiprazole induced-peripheral antinociception. Ferreira RCM, Almeida-Santos AF, Duarte IDG, Aguiar DC, Moreira FA, Romero TRL ICB-UFGM – Farmacologia e Fisiologia

Introduction: Aripiprazole has a peripheral analgesic component; however, the mechanism involved in this effect is not fully established. Therefore, the aim was to obtain pharmacological evidences for the involvement of cannabinoid system in the peripheral antinociceptive effect induced by aripiprazole. Methods: To induce hyperalgesia, rat paws were treated with intraplantar prostaglandin E$_2$ (PGE$_2$, 2 μg) injection. Nociceptive thresholds were measured, using the rat paw pressure test. All drugs were administered locally into the right hind paw of Swiss male mice with n = 4 animals per group. Results: Antinociceptive effect induced by aripiprazole (100 μg/paw) was blocked by CB$_1$ and CB$_2$ cannabinoid receptor antagonists AM251 (40, 80 and 160 μg/paw) and AM630 (100, 200 and 400 μg/paw), respectively. Administration of fatty acid amide hydrolase inhibitor, MAFP (0.5 μg/paw) and monoacylglycerol lipase inhibitor, JZL184 (3.8 μg/paw), was able to potentiate the peripheral antinociception effect induced by aripiprazole (25 μg/paw). Moreover, the same effect was observed with the anandamide uptake inhibitor, VDM11 (2.5 μg/paw). Conclusion: The results suggest that aripiprazole induces peripheral antinociceptive effect through cannabinoid system activation. Financial support and acknowledgments: CNPq (Nº 448283/2014-0), FAPEMIG and CAPES. Research approval by the Committee for Ethics in Animal Experimentation (CEUA, Brazil) under the protocol number 109/2011.
Introduction: Obesity consists in a multifactorial disease featured by unbalanced between metabolic and biochemical pathways and excessive accumulation of body fat in the individual. Obesity is often accompanied by debilitating comorbidities such as pain and depression. Clinical evidence suggests that the higher the body mass index, the greater risk of depression and the greater rate of self-reported pain. While obesity is more prevalent in men, chronic pain and depression are more reported by obese women, characterizing differences relative to the gender. Objective: In view of these facts, this study aimed to characterize the nociceptive and depressive-like responses in both male and female rats with obesity induced by neonatal administration of monosodium glutamate (MSG). Methods: Litters of Wistar rats (male and female) were subcutaneously treated with saline (SAL; control groups) or MSG (4 mg/g) from the first to the fifth day of extra-uterine life. Behavioral (mechanical hyperalgesia using the Randall Selitto test; depressive-like behaviors during the forced swimming test or locomotor activity in the open field test), biochemical (lipid profile and blood glucose) and biophysical (body mass, nasoanal length, abdominal circumference and visceral fat) parameters were evaluated at different time points (30, 60, 90 or 120 days) after SAL or MSG treatment. Results: Our data showed that, when compared to SAL group, the MSG rats had a lower rate of weight gain (19.8%), reduced nasoanal length (10.8%), and increased Lee Index (14.9%), abdominal circumference (21.1%) and visceral fat (158.8%), without significantly differences between the genders. Besides, the MSG treatment induced a significant change in the lipid profile, i.e. higher levels of triglycerides (67.9% in male and 115.1% in female, respectively), total cholesterol (37%, 26.4%) and VLDL cholesterol (67.8%, 57.9%), but no changes in HDL cholesterol neither in blood glucose. Regarding gender differences, the female MSG group had significantly higher total cholesterol levels when compared to male MSG rats. MSG rats of both genders manifested increased immobility time (16.6%) and reduction of the first immobility episode latency (92.7%) in the forced swimming test and a reduction of mechanical threshold (38.9%) in the Randall Selitto test. Finally, the treatment with MSG in male or female rats did not cause a significant change in the number of crossing in the open field test. Conclusion: In conclusion, our data evidenced that the mechanical hyperalgesia and the depressive-like behaviors are similarly observed in male and female rats treated with MSG, while some biophysical/biochemical parameters differ between genders. These findings may be relevant to future studies that target a better characterization of the pathophysiological mechanisms behind the interrelations among obesity, depression and pain. Acknowledgements: This study was supported by CNPq/Procad #552370/2011-8. Eliana R. Adami had a CNPq scholarship. This study was approved by the CEUA/BIO–UFPR (#779). [1] Department of Pharmacology, Biological Science Building, Federal University of Parana, Curitiba, Parana, Brazil.
05.006 Effect of a new Thiazolidine-2,4-Dione (TDZ) on the acute cold hypersensitivity induced by oxaliplatin in mice. Stoeberl LC, Quintão NLM, Silva GF, Kormann EC, Buzzi FC, Melato J Univali – Ciências Farmacêuticas

Introduction: Oxaliplatin, a third-generation platinum-based chemotherapeutic agent, is widely used to treat colorectal cancer. The development of sensory neuropathy is the most important, dose-limiting side effect. Analogic drugs used to treat oxaliplatin-induced pain are weakly effective. These symptoms are triggered or exacerbated by cold stimulus. Oxaliplatin neurotoxicity involves an oxidative stress in neuronal mitochondrias and peroxisome alterations. Aimed to evaluate the effect of a new TDZ (N-fenilbenzosulfonamida-4-[(Z)-3-benzil-2,4-dioxo-1,3-tiazolidin-5-ilideno)methyl]) on the acute oxaliplatin neurotoxicity, we studied the efficacy of this TDZ in decreasing the cold hypersensitivity induced by oxaliplatin in mice. Methods: All experiments were carried out with 4-6 week old male C57BL/6 mice housed on a 12/12 hour light/dark cycle. All procedures were previously approved by UNIVALI Ethical Committee, protocol number 043/15p. The behavioral tests were conducted in a cold planter assay consisted of a 6 mm thick borosilicate float glass. Mice, 10 in each experimental group, were acclimatized on the glass plate for 2 hours. The cold probe was made with powdered dry-ice packed into a modified 3 ml syringe. The open end of the syringe was held against the flat surface adjacent to the right hindpaw of each mouse, while pressure was applied to the plunger. The paw withdrawal latency, considered as an indicative of cold hyperalgesia, was measured with a chronometer. Withdrawal was defined as any action to move the paw vertically or horizontally away from the cold glass. An interval of 15 minutes was allowed between trials on any single paw and each animal was measured 3 times. The maximum time allowed for withdrawal was 20 seconds to avoid potential tissue damage and the value was recorded as 20 seconds. In experiment A there were three groups of 10 animals. The group 1 was naive, group 2 was treated with oxaliplatin 6mg/kg intraperitoneal and group 3 received oxaliplatin at the same dose concurrent with TDZ 1mg/kg intraperitoneal. In experiment B there were four groups of 10 animals. All the groups were treated with oxaliplatin 6mg/kg intraperitoneal, and groups 2, 3 and 4 also received 1mg/kg, 0,1mg/kg and 0,01mg/kg of TDZ intraperitoneal respectively. Results: Oxaliplatin induced significant cold hyperalgesia, observed from day 1 to day 14 ( p<0.001 ). Animals treated with TDZ (1 - 0,1 or 0,01mg/kg, i.p.) have the response latency significantly increased when compared with control group and naive group. The obtained inhibition rates were 20.5 ± 6.4%, 30.9 ± 7.8% and 98.1 ± 1.8% for the doses of 0,01, 0,1 and 1 mg/kg, respectively. Conclusion: The concurrent administration of TDZ with oxaliplatin was able to prevent the cold hypersensitivity induced by this chemotherapeutic agent in mice. This TDZ deserves further studies as a potential drug in preventing the acute neurotoxicity of oxaliplatin. Financial support: CNPQ, CAPES.
A study of peripheral antinociceptive mechanisms induced by serotonin. Diniz DA, Petrocchi JA, Navarro LC, Souza TC, Castor MGM, Perez AC, Duarte IDG, Romero TRL ICB-UFMG – Farmacologia e Ciências Biológicas

Serotonin (5-HT) is a biogenic amine with important functions in the central nervous system, such as behavior, mood, sleep and appetite, and is part of the descending inhibitory pain pathway. So far, only its pro-nociceptive role was described at the peripheral level. Therefore, we set out to verify that serotonin could be linked to a nociceptive effect at peripheral level, as well as the selectivity of its receptors in this event, and the participation of opioidergic and cannabinoidergic systems in this mechanism. The mouse paw pressure model was used to test in animals that had increased sensitivity to intraplantar injection of PGE₂ (2 μg), being used for statistical analysis to analysis of variance ANOVA followed by Bonferroni posttest. To check the selectivity of receptors in event, selective serotonin receptors antagonists (isamoltan 5-HT₁B, BRL 15572 5-HT₁D, ketanserin 5-HT₂A, ondansetron 5-HT₃ e SB-269970 5-HT₇) were used. Serotonin administered on right hind paw (62.5, 125, 250 and 500 ng and 1 μg) produced antinociceptive effect manifested in a dose-dependent. Selective antagonists for the 5-HT₁B, 5-HT₂A and 5-HT₃ receptors, in doses of 100 ng, 1 μg and 10 μg, reversed the antinociceptive effect induced by serotonin at the dose of 250 ng. Also, selective antagonists for the 5-HT₁D and 5-HT₇ receptors (10 μg) were unable to reverse the antinociceptive effect induced by serotonin. In order to evaluate the participation of the opioidergic system, use the non-selective antagonist for opioid receptors, naloxone, and the selective antagonist for opioid receptors μ, δ and κ clocinnamox (40 μg), naltindole (60 μg) and nor-binaltorfimina (200 μg) respectively, which reversed the antinociceptive effect induced by serotonin (250 ng). Bestatin (400 μg), an encefalinas inhibitor that degrade peptides, opioids increased the antinociceptive effect induced by serotonin (lowest dose 62.5 ng). To assess the participation of the cannabinoidergic system, selective antagonists for the CB₁ and CB₂ receptors, AM251 (80 μg) and AM630 (100 μg) respectively, were used and they reversed the antinociceptive effect induced by serotonin. In addition, MAFP (0.5 μg), an inhibitor of the FAAH enzyme which breaks down anandamide, JZL184 (3.75 μg), an inhibitor of MAGL enzyme that degrades the 2-AG, and the VDM11 (2.5 μg), an inhibitor of anandamide uptake, have strengthened the antinociceptive effect induced by serotonin (lowest dose 62.5 ng). Our data suggest, for the first time, the peripheral antinociceptive action induced by serotonin, and provide evidence that there is participation of the opioidergic and cannabinoidergic systems in the action. This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)
05.008 Static contraction-induced muscle pain is modulated by peripheral TRPV1 receptors and PKC epsilon, Jorge CO¹, Melo-Aquino B², Santos DFS¹, Bonfante R², Macedo CG², Clemente-Napimoga JT², Oliveira-Fusaro MCG¹ ¹LABEDI-Unicamp, ²FOP-Unicamp

Introduction: Muscle pain is an important health issue and frequently related to static force exertion. In spite of its clinical relevance, mechanisms underlying static contraction-induced muscle pain are unclear. We have previously demonstrated that muscle pain induced by static contraction in rats is modulated by peripheral inflammatory mechanisms, including bradykinin, sympathetic amines and prostanoids. There is a large literature that suggests numerous inflammatory mediators may enhance the activity of TRPV1 via PKC-dependent pathways. Therefore, the aim of this study was to evaluate the involvement of TRPV1 and PKC epsilon (PKCε) in the static contraction-induced muscle pain in rats. Methods: The static contraction-induced muscle pain was performed by an electrical stimulation. Two needle electrodes were inserted into the gastrocnemius muscle and an electrical stimulation (stimulator Grass, S88X) of 1.6V and 19 ms of pulse-width for 1 hour was used. Mechanical muscle hyperalgesia was quantified with a Randal Sellito pressure analgesimeter, applied to the gastrocnemius muscle, one hour after the end of contraction. To investigate the involvement of TRPV1 in the static contraction-induced muscle pain, the selective TRPV1 receptor antagonist, AMG9810, was injected into gastrocnemius muscle of rats 5 min. before static contraction. After evaluation of muscle pain, the gastrocnemius muscle was collected to analyze the expression of TRPV1 receptor and of PKCε by western blot. Male Wistar rats (200 - 250g), from CEMIB-UNICAMP, were used and all experimental procedures were previously approved by the Ethics Committee in Animal Research of the State University of Campinas. Results: Pre-treatment with AMG9810 (70 and 105µg/muscle, but not 35µg) prevented, in a dose-dependent manner, the static contraction-induced mechanical muscle hyperalgesia when administered in the ipsilateral (p>0.05, ANOVA, Tukey test, n=5) but not in the contralateral gastrocnemius muscle. Static contraction increased muscle expression of TRPV1 receptor and of PKCε (p<0.05, T test, n=4) when compared to sham group (no electrical stimulation). Conclusion: This study demonstrated that static contraction induced muscle pain by a mechanism dependent of TRPV1 receptors, and an up-regulation of muscle TRPV1 receptor and of PKCε. We speculate that static contraction induces release of inflammatory mediators, which in turn, enhance the activity of TRPV1 via PKC-dependent pathway to contribute to muscle pain. Financial Support: CNPq, 473790-2013-0. Animal Research Ethical Committee (protocol number 3277-1).
The selective TRPV4 channel antagonist HC-067047 reverted mechanical hypersensitivity in diabetic animals. Dias FC\textsuperscript{1,2}, Alves VS\textsuperscript{2}, Matias DO\textsuperscript{1,2}, Cruz JVR\textsuperscript{2}, Silva RV\textsuperscript{1,2}, Santos BLR\textsuperscript{3}, Lima CKF\textsuperscript{2}, Clarke JHR\textsuperscript{2}, Passos GF\textsuperscript{2}, Figueiredo CP\textsuperscript{1,2}, Miranda ALP\textsuperscript{1,2,3}, Costa R\textsuperscript{1,2} \textsuperscript{UFRJ} – Ciências Farmacêuticas, \textsuperscript{2}UFRJ – Farmácia, \textsuperscript{3}UFRJ – Farmacologia e Química Medicinal

**Introduction:** Painful diabetic neuropathy (PDN) is the most prevalent and debilitating complication associated to diabetes mellitus. PDN is a chronic neuropathic pain syndrome caused by metabolic damage to primary afferent neurons. The pharmacological management of PDN includes the use of antidepressants, anticonvulsants and opioids; however, these drugs have low efficiency and several side effects for most diabetic patients. Thus, the discovery of innovative therapies to control diabetic neuropathy is highly relevant. Several molecular targets have been claimed as alternatives to the development of more effective analgesics drugs; among them, we can highlight the transient receptor potential (TRP) vanilloid-4 (TRPV4) channel, which has been implicated in the maintenance of chronic pain in experimental models.

**Aim:** The present study aimed to determine the role of TRPV4 channel in PDN in a diabetes model induced by streptozotocin (STZ) in mice. **Methods:** Male Swiss mice were subjected to the type 1 diabetes model induced by intraperitoneal injection of STZ. PDN was assessed by plantar sensitivity of animals to thermal cold (acetone solution) and mechanical (von Frey filaments) stimuli. To assess the involvement of TRPV4 in the maintenance of mechanical hypersensitivity, animals were subcutaneously treated with the selective TRPV4 antagonist HC-067047 (10mg/kg) 6 weeks after diabetes induction. After treatment with HC-067047 diabetic animals were also submitted to open field and tail suspension tests to assess anxiety and depression behaviours, respectively. Finally, it was evaluated the sensitivity of diabetic animals to the selective TRPV4 agonist GSK1016790A by measuring nociceptive behaviour and paw edema, as well as the calcium influx in cultured DRG neurons. **Results:** The induction of diabetes by intraperitoneal injection of STZ produced thermal to cold and mechanical hypersensitivities in mice. The single or repeated treatment with HC-067047 reversed mechanical hypersensitivity in diabetic animals, without interfering with thermal cold hypersensitivity. Also, it was noted that repeated treatment with HC-067047 reduced the immobility time of animals in the tail suspension test, suggesting an antidepressant-like effect. However, this same treatment did not significantly interfere with the preference of animals for the peripheral area in the open field test, suggesting no anxiolytic activity. TRPV4 channels seem not to be hyperactivated in peripheral nervous system of diabetic animals, since the *in vivo* (nociception and paw edema) and *in vitro* (calcium influx in DRG neurons) responses generated by GSK1016790A did not differ between control and diabetic animals. **Conclusion:** Together, the results indicate that TRPV4 plays an important role in the maintenance of mechanical hypersensitivity in diabetic animals. Additional studies are in course to better understand the role of TRPV4 in this model. **Key-words:** diabetes, neuropathic pain and TRPV4 **Financial Support:** CAPES, CNPq and FAPERJ. **Ethics committee of the UFRJ protocol number:** 054/14.
05.010 HUF-101, a cannabidiol analog, decreases nociception in mice via facilitation of endocannabinoids receptors-mediated neurotransmission. Silva NR1, Gomes FV1, Fonseca MDM1, Zuardi AW2, Crippa JAS2, Hallak JEC2, Mechoulam R3, Cunha TM1, Guimarães FS1 1FMRP-USP – Farmacologia, 2FMRP-USP – Neurociências e Ciências do Comportamento, 3Universidade Hebraica de Jerusalém – Química Medicinal e Produtos Naturais

Introduction: Cannabidiol (CBD) is a phytocannabinoid with multiple pharmacological effects and several potential therapeutic properties. Its low oral bioavailability, however, could limit its clinical use. Preliminary results indicate that fluorination of the CBD molecule could increase its pharmacological potency. Here, we investigated whether HUF-101 (former HU-474), a fluorinated synthetic CBD analog, would induce antinociceptive effects. HUF-101 effects were compared to those induced by CBD. Methods: Swiss mice (30-40g) received intraperitoneal (i.p.) injection of vehicle (10 mL/kg), CBD (10, 30, 90 mg/kg) or HUF-101 (3, 10, or 30 mg/kg) and, 30 min later, were submitted to the hot plate, abdominal writhing induced by acetic acid and inflammatory hyperalgesia tests which are predictive of antinociceptive drugs. The effects produced by CBD and HUF-101 were compared to those induced by the CB1/2 receptor agonist WIN55,212-2 (1, 3 and 5mg/kg). To evaluate the involvement of CB1 and CB2 receptors in HUF-101 and CBD effects, mice received AM251 (a CB1 receptor antagonist, 1 or 3mg/kg) or AM630 (a CB2 receptor antagonist, 1 or 3 mg/kg) 30 min before CBD or HUF-101. Results: In the hot plate test, HUF-101 (30mg/kg) and WIN55,212-2 (5mg/kg) induced antinociceptive effects, which were attenuated by the pretreatment with AM251 and AM630. In the abdominal writhing test, CBD (30 and 90mg/kg), HUF-101 (30 mg/kg) and WIN55,212-2 (3 and 5mg/kg) induced antinociceptive effects indicated by a reduction in the number of writhing. While the pretreatment with AM251 attenuated only the effect caused by WIN55,212-2, AM630 did not mitigate the effect induced by any drug in this test. In the carrageenan-induced inflammatory hyperalgesia test, CBD (30 and 90mg/kg), HUF-101 (3, 10 and 30mg/kg) and WIN55,212-2 (1mg/kg) decreased the intensity of mechanical hyperalgesia measured by the electronic von Frey method. The effects of all compounds were attenuated by the pretreatment with AM251 and AM630. Conclusion: These results showed that HUF-101 induced antinociceptive effects at lower doses than CBD, indicating that the addition of fluoride improved its pharmacological profile. Furthermore, HUF-101 effects seem to involve the activation of CB1 and CB2 receptors. Thus, this new compound could be a therapeutical alternative for the treatment of acute pain at lower doses than CBD. Financial support: CAPES, CNPq, FAPESP, and FAEPa. The Institution’s Animal Ethics Committee approved housing conditions and experimental procedures (process number: 058/2013).
Environmental enrichment induced-analgesia after CCI injury involves endogenous opioids release in rats. Kimura LF, Sant’Anna MBM, Teixeira NB, Mattaraia VGM, Zambell VO, Picolo G, IBu – Dor e Sinalização, ICB-USP, IBu – Biotério Central

Introduction: Environmental enrichment (EE) can alter anxiety as well as the perception of nociceptive stimuli and the analgesic response induced by opioids, suggesting a relationship between well-being and analgesia. The aim of this work was to evaluate the role of animal welfare in pain sensitivity of rats against chronic noxious stimuli and the participation of opioid signaling in this effect. Methods: Male Wistar rats were used. Enriched animals were born in an enriched environment (with surgical cap, paper roll or tunnels - exchange one for another every week), and grouped in 5 animals/cage after weaning. Over 5 weeks of life, they were housed on larger cages (60 x 50 x 22 cm) and given three different objects at once (Ping-Pong balls, tunnels, huts, retreats, or surgical cap – exercise wheel was not added), being one of the three changed for another type every week. Control group remained in standard cages (49 x 34 x 16 cm) in groups of 5 animals/cage after weaning and did not receive objects. Within 7 weeks of life, under these conditions, chronic constriction of the sciatic nerve (CCI) was surgically performed. In order to validate EE protocol, animals anxiety was evaluated using elevated plus maze test. Effects of EE in mechanical and thermal hypernociception or tactile allodynia were analyzed before and 7 and 14 days after surgery, using rat paw pressure, Hargreaves and von Frey hair tests, respectively. After 14 days, animals were euthanized and serum was obtained to evaluate endogenous opioid levels using EIA kit. Results: EE protocol employed was effective in diminish anxiety and completely abolished pain behavior of enriched animals after 14 days of CCI, in tactile allodynia, and mechanical and thermal hyperalgesia parameters analyzed. Moreover, when CCI animals were treated with naloxone (1 mg/kg, s.c.), a non-selective antagonist of opioid receptors, the analgesic effect of EE on enriched animals was abolished. In addition, serum levels of endogenous opioids (beta-endorphin and met-enkephalin) were augmented in CCI enriched animals when compared to naïve EE animals or CCI non-enriched animals. Conclusion: The results presented herein showthat EE is able to abolish the chronic pain development, involving endogenous opioid pathway activation. This is the first time that the total control of chronic pain is obtained by an EE protocol without exercise wheel. The data presented herein demonstrate that the welfare per se is able to control chronic pain behavior using a non-cirurgical and non-pharmacological approach. Thus, this work can contribute for the understanding of endogenous mechanisms involved in pain control and in the diversity of responses to different treatments currently used for patients, helping in the search for appropriate handling of painful conditions. Financial support: CNPq and São Paulo Research Foundation (FAPESP) grant 2013/20795-8. Institutional Animal Care Committee of Butantan Institute (CEUAIB protocol number 1050/2013).

Introduction: Complex regional pain syndrome (CRPS) may be evoked by ischemia/reperfusion, characterized by an intractable and incapacitating pain of the affected limb, which is triggered by various injuries and is resistant to standard analgesics. Despite this, the underlying mechanism of chronic pain in this painful pathology has not been fully elucidated, but seems to involve the production of reactive compounds. Therefore, the aim of this study is to evaluate the involvement of transient receptor potential ankyrin 1 (TRPA1) channel, a chemosensor of inflammation and oxidative substances, in a mice model of chronic post-ischaemia pain (CPIP).

Methods: Male and female mice were subjected to 2h hind paw ischaemia/reperfusion (CPIP model). Different parameters of nociception, inflammation, ischemia, and oxidative stress were evaluated at 1 (acute) and 17 (chronic) days after CPIP.

Results: In the CPIP acute phase, we observed mechanical and cold allodynia; increased levels of lactate (serum); also increase the temperature and edema formation (hind paw and ankle). In the CPIP chronic phase, we detected mechanical and cold allodynia. In the chronic phase (17 days after CPIP), we observed antinociceptive effect after TRPA1 antagonists (HC-030031 and A-96) or TRPA1 antisense administration, as well as α-lipoic acid (antioxidant) and amitriptyline (an antidepressant) injection. However, no change in myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAGase) activities (hind paw and sciatic nerve tissue) were observed.

Conclusion: In conclusion, induction of CRPS/CPIP showed different mechanisms in acute and chronic phases and suggests that antagonism of TRPA1 receptor can reduce the development of neuropathic pain induced by CPIP in mice.

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05.013 A New Nβ-Alkanoyl-5-Hydroxytryptamide induces antinociceptive effect in mice. Giorno TBS¹, Moreira IGS², Rezende CM², Fernandes PD¹ ¹UFRJ – Farmacologia e Química Medicinal, ²UFRJ – Química

Introduction: The C18-5HT, a new Nβ-alkanoyl-5-hydroxytryptamide is naturally found in the surface wax of coffee beans (Speer et al., Braz J Plant Physiol. 18: 201, 2006). Some amides of the serotonin class demonstrated an anti-inflammatory effect by inhibiting the expression of caspases participants in inflammatory process (Meijerink et al., British J Pharmacol, 169: 772, 2013; Verhoecxx et al., Molec Cell Biol Lip 1811: 578, 2011). The aim of this study was to evaluate the antinociceptive activity of C18-5HT, a new fatty acid amide of serotonin. Methods: C18-5HT was diluted in tween 80 and administered orally to Swiss Webster mice (20-25g, n=6/group), at doses of 0.1, 1 or 10 mg/kg and evaluated in different nociceptive models (formalin-, capsaicin- and glutamate-induced licking response and hot plate model). Statistical analysis was performed by ANOVA and Bonferroni's post-test (*p<0.05). Results: C18-5HT showed significant activity in the peripheral nociception models as observed by the results obtained in formalin-(1st) phase: 1 and 10 mg/kg, 35.3±3.8* sec (30.1%) and 27.4±5.0*sec (45.7%), respectively, versus vehicle-treated group=50.5±1.4 sec and in the 2nd phase: 1 and 10 mg/kg, 142.6±12.6* sec (33.5%) and 97.6±4.2* sec (54.5%), respectively, versus vehicle-treated group= 214.3±3.1 sec; capsaicin-(vehicle-treated group=65.5±3.8 sec versus 45.8±2.8* sec (30.1%), 33.6±4.5* sec (48.7%) and 22.9±3.2* sec (65.0%) to 0.1, 1 and 10 mg/kg, respectively and glutamate-induced nociception (vehicle-treated group=32.9±5.8 sec versus 11.6±5.5* sec (64.7%), 7.9±2.6* sec (76.0%) and 18.9±4.6* sec (42.5%) to 0.1, 1 and 10 mg/kg, respectively. In the hot plate test, C18-5HT increased the area under the curve when compared with the vehicle group and in some cases was similar or higher than that value of morphine at all doses tested (vehicle-treated group=1,757.2±159.6; morphine-treated group=6,218.2±746.1; 0.1 mg/kg=8,022.2±673.7; 1 mg/kg=9,030.8±1,252.1; 10 mg/kg=10,804.0±1,544.3) In an attempt to characterize the possible mechanism by which C18-5HT exerted its effects we used several antagonists (naloxone, ondasentron and AM251) of receptors involved in the inhibitory control of pain. We observed that naloxone (nonselective opioid receptor antagonist), ondasentron (5-HT3 serotoninergic receptor antagonist) and AM251 (cannabinoid CB1 receptor antagonist) reverted the antinociceptive effect of C18-5HT suggesting that the opioid, serotonergic and cannabinoid pathways participate in its activity (vehicle-treated group=1,757.2±159.6; C18-5HT 10 mg/kg=10,804.0±1,544.3; Naloxone= 3,256.4±1,311.2; Ondasentron=3,365.2±955.5; AM251=4,780.2±1,997.2). In conclusion, C18-5HT produces peripheral and central antinociceptive effect. Data suggest that this substance could be used as new prototype forthe development of new antinociceptive drugs. Acknowledgements: Alan Minho for technical assistance, Instituto Vital Brazil (Niterói, Brazil) for donation of mice. Financial support: CAPES, CNPq and FAPERJ. Ethics committee of the CAUAP/UFRJ protocol number:DFBICB015-04/16.
Anti-hyperalgesic effect of N-(4Methyl-Phenyl)-4-Methylphthalimide – Adenyl Cyclase as main target.

Introduction: Preliminary data has shown the cyclic imide N-(4methyl-phenyl)-4-methylphthalimide (MFMFTAL) presented persistent anti-hyperalgesic effect in several models of mechanical hyperalgesia in mice. This study had the aim of investigating the mechanism involved in the MFMFTAL’s anti-hypersensitive effects using specific pharmacological tools.

Methodology: Female Swiss mice (25-35g, N=6-8) were used throughout this study (ethics committee nr: 033-13). The animals were intraperitoneally (i.p.) pre-treated with the compound MFMFTAL (1-10 mg/kg) or vehicle (10 mL/kg, i.p.), and, 30 minutes later, they received intraplantar (i.pl.) injection of carrageenan (300 mg/paw), CFA (20 uL/paw) or LPS (100 ng/paw). The animals’ mechanical hypersensitivity was assessed using von Frey monofilament 0.6g in different time points depending on the model. In an attempt to elucidate the compound’s mechanism of action, we also submitted mice to i.pl. injection of capsaicin (1.6 µg/paw; TRPV1 agonist), glutamate (30 mmol/paw), 8-Br-cAMP (10 nmol/paw; PKA activator) as well as PMA (30 ng/paw; PKC activator) and the nociceptive behavior was recorded in a dark room without the presence of the experimenter. The videos were analysed and the time spent licking or biting the injected paw was considered as indicative of nociception. The interference with adenyl cyclase activation was investigated using forskolin (1µmol/paw; i.pl.) to induce mechanical hyperalgesia.

Results: MFMFTAL showed a remarkable inhibition of mechanical hyperalgesia induced by i.pl injection of carrageenan, with inhibitions of 12.3 ± 3.0%, 20.4 ± 2.0%, 59.4 ± 3.0% and 73.8 ± 2.0% for the doses of 1-30 mg/kg respectively and ID50% of 17.0 (12.4 to 23.3) mg/kg. The compound was also able to inhibit mechanical hyperalgesia induced by bacterial components such as LPS and CFA, with inhibition of 45.0 ± 4.0% and 45.0 ± 3.0%, respectively. On the other hand, no effect was observed when mice were injected with TRPV1 and glutamate receptors agonists or with PKA and PKC selective activators. Eventually, when mice pre-treated with MFMFTAL were i.pl injected with forskolin (adenyl cyclase activator), they presented a prominent and persistent inhibition (62.0 ± 4%) of mechanical hypersensitivity, with significant inhibition for up to 48 h after the treatment. The behavior data was confirmed by in silico analysis that demonstrates that the MFMFTAL presents higher affinity that forskolin.

Conclusion: MFMFTAL could represent a promising pharmacological tool to treat painful diseases, with good bioavailability, absence of adverse effect and with adenyl cyclase enzyme as the main target for its anti-hypersensitive effects. Financial Support: CAPES, VRPPEC/UNIVALI, FAPESC, CNPq, FACEPE. CEP NUMBER: 033-13/UNIVALI.
05.015 Participation of opioid and cannabinoid endogenous systems in peripheral neuropathic pain modulation. Machado DPD, Ferreira RCM, Duarte IDG, Romero TRL, Duarte IDG ICB-UFMG – Fisiologia e Farmacologia

Introduction: Neuropathic pain, one of the signs of neuropathy, affects thousands of people. This type of pain is triggered by damage to the nervous system somatosensory causing structural and functional changes. Neuronal plasticity changes lead to the ectopic impulse generation and nervous impulse facilitation. These impulses are modulate by endogenous analgesic systems. Thus, the aim of this work was to evaluate, pharmacologically, the participation of endogenous opioid and cannabinoid systems in neuropathic pain during their inductions and when they are already consolidated. Methods: It was used male Wistar rats, 45 days, weighing 180 g, with n = 4 animals per group. To induce neuropathic pain, it was used the sciatic nerve constriction (CNC) technique which is the placement of four loose ligatures around the nerve. Nociceptive thresholds were measured, using the rat paw pressure test, which was done daily for 15 days to observe the neuropathic pain kinetic. Additionally, it was injected, via intraplantar, the non-selective opioid receptor antagonist (naloxone 50 μg/paw) and the selective μ (Clocinnamox 40 μg/paw), δ (naltrindole 60 μg/paw) and κ (nor-binaltorphimine 200 μg/paw) opioid receptor antagonists, besides the injection of the selective CB₁ (AM251, 80 μg/paw) and CB₂ (AM630 100 μg/paw) cannabinoid receptor antagonists. Posteriorly, it was administrated naloxone (50 μg/paw) with AM251 (80 μg/paw) and AM630 (100 μg/paw) simultaneously, to verify the participation of both systems on days 1, 6 and 12 after the CNC procedure. It was used variance analysis 2-way ANOVA, followed by Bonferroni test, with significance level of $p < 0.05$. Results: The kinetic curve of neuropathic pain development until the 15th day showed that on the 1st day there was hypalgesia while in the followed days there was a gradual reduction of nociceptive threshold. On the 6th day a moderate hyperalgesia was observed and finally from the 10th day an intense and persisted hyperalgesia until the last day of measurement. After opioid and cannabinoid receptor antagonists administration, on 1st, 5th, 6th and 12th days, the nociceptive threshold decreased. However, nor-binaltorphimine did not induce hyperalgesia intensification on the 12th day while AM630 did not induce it on the 1st and 12th days. When both endogenous cannabinoid and opioid systems are antagonized in the same time and in the same animal, the nociceptive threshold is lower than when the systems are antagonized separately on all days tested. Conclusion: The results suggest the participation of cannabinoid and opioid systems in the modulation of neuropathic pain and, possibly, those systems work together selectively controlling the pain during the neuropathic hyperalgesia development. Financial Support: CNPq, CAPEs e FAPEMIG (Protocol APQ-01112-15). This project was approved by the Ethics Committee on Animal Experiments of the Federal University of Minas Gerais (CEUA/UFMG) under the Protocol: 173/2014.
05.016 TRPA1 channel mediates Bothrops jararaca venom-induced nociception and oedema. Macedo-Júnior Sj1, Tonello R1, Silva LM1, Santos ARS1, Geppetti P3, Ferreira J1
1UFSC – Farmacologia, 2UFSC – Ciências Fisiológicas, 3University of Florence – Health Sciences, Clinical Pharmacology and Oncology

Introduction: Transient receptor potential channels (TRPs) are involved in the nociception induced by venoms and toxins from different animal species such as spiders, scorpions, bees and snakes. Therefore, we aimed to investigate the involvement of TRPs on Bothrops jararaca venom (BjV)-induced nociception and oedema. Methods: Male Swiss and C57/BL6-UFSC mice were used. Firstly, BjV (1 μg/site) or vehicle (20 μL/site) were intraplantarly injected (i.pl.) and the time mice spent licking the injected paw was recorded every 5 min. for 15 min., and used as an acute nociceptive parameter. Afterwards, 1, 3, 6 and 24 hours after BjV treatment were evaluated mechanical and cold hyperalgesia, guarding behavior and oedema. Finally, pharmacological and genetic approaches were used in order to investigate the involvement of TRPs in BjV-induced nociception. HC030031 (100 μg/site), a TRPA1 antagonist, SB366791 (1 nmol/site), a TRPV1 antagonist, or vehicle were i.pl. co-administrated with BjV (1 μg/site). The same behavioral tests at the same times mentioned above were used. TRPV1 and TPV4 knockout mice were used to further investigate their role in BjV-induced nociception. Unpaired t Test or Two-away repeated measures ANOVA followed by Bonferroni post hoc were used for statistical analysis. Results: Compared to saline injection, BjV significantly increased total licking time 15 minutes after administration (16±7 vs 90±12 s, respectively, p=0.001), presenting peak response between 5-10 min after injection (7±7 vs 45±8 s, p<0.001). BjV-induced mechanical and cold hyperalgesia and guarding behavior at 1 h and up to 6 h after treatment. BjV produced a significant paw oedema, with the same response profile observed in the nociceptive behavioral tests. HC030031 prevented BjV-induced nociception in the total licking time (61.50±7.44 vs 34.25±6.21, p=0.0307) and in the peak response time (5-10 min.) (43.25±3.37 vs 15.00±6.12, p<0.01). For VFT, HC030031 prevented BjV-induced paw withdrawal threshold reduction up to 1 h after co-administration (0.5±0.1 vs 1.3±0.2 grams, p=0.0106). HC030031 prevented BjV-induced mechanical (0.5±0.1 vs 1.3±0.2 grams, p=0.0106) and cold (2.8±0.4 vs 1.6±0.4 s, p=0.046) hyperalgesia 1h after co-treatment. In guarding behavior, HC030031 prevented BjV-induced nociception in the cumulative score parameter at 1 h (4.0±0.9 vs 1.3±0.7, p<0.01) and in the non-cumulative score parameter at 3 h after co-administration (0.8±0.2 vs 0.0±0.0, p<0.01). In addition, HC030031 significantly prevented BjV-induced oedema up to 1 h after co-administration (0.87±0.08 vs 0.04±0.12, p<0.001). On the other hand, SB366791 failed to prevent BjV-induced nociception and oedema. Similarly, BjV-induced nociception and oedema were similar in both TRPV1 or TRPV4 knockout mice when compared to respectively wild-type background. Conclusion: Our present results suggest that BjV-induced nociception and oedema is mediated, at least in part, by TRPA1 channel activation, but not by TRPV1 nor TRPV4 channels. Protocols approved by CEUA/UFSC (protocol n°: 00872). Financial support: CAPES, CNPq and UFSC.
05.017 Analgesic activity of betalain-rich dye of Beta vulgaris. Hohmann MSN¹, Martinez RM², Longhi-Balbinot DT¹, Zarpelon AC¹, Baracat MM², Georgetti SR², Sassonia R³, Verri Junior WA¹, Casagrande R²¹ UEL – Ciências Patológicas, ²UEL – Ciências Farmacêuticas, ³UFT – Ciências Integradas

Introduction: We have recently demonstrated that betalain rich-dye of Beta vulgaris (betalain) elicits pronounced anti-inflammatory effect observed as inhibition of carrageenan-induced paw edema and leukocyte recruitment by a mechanism dependent on the inhibition of superoxide anion production and regulation of cytokine production. However, it remains to be determined whether betalain presents analgesic effects and its mechanisms. Thus, the aim was to investigate the effect of betalain on inflammatory nociception and address its peripheral antinociceptive mechanisms of action focusing on cytokines and oxidative stress. Methods: The betalain-rich dye of Beta vulgaris (betalain) was produced by alcohol precipitation method. To assess the effect of betalain on abdominal writhing or paw licking and flinches, mice were treated with betalain (10-1000 mg/kg) or vehicle (saline, 10 ml/kg) by intraperitoneal (i.p.) route 30 min before i.p. injection of acetic acid (0.8% v/v) or Phenil-p-benzoquinone (PBQ, 1890 µg/kg) or intraplantar (i.pl.) injection of formalin or complete Freund’s adjuvant (CFA), respectively. The effect of the betalain (100 mg/kg) on mechanical hyperalgesia induced by carrageenan or prostaglandin E₂ (100 ng/paw) was also assessed. The effect of post-treatment with betalain (100 mg/kg) on the mechanical hyperalgesia induced by carrageenan or CFA was evaluated 30 min or 48.5 h, respectively, after stimulus. The efficacy of pre-treatment (30 min) with betalain via subcutaneous (s.c.) and p.o. routes of administration in inhibiting carrageenan-induced mechanical hyperalgesia was also evaluated. Lastly, the effect of betalain on IL-1ß, TNF-α, and GSH levels in the paw tissue following carrageenan stimulus and the in vitro antioxidant effect of betalain were determined. Results: Betalain (10-1000 mg/kg, i.p.) reduced abdominal writhing response induced by acetic acid and PBQ. Furthermore, betalain (100 mg/kg, i.p.) diminished the overt pain like behavior induced by CFA and formalin. Betalain treatment by i.p., s.c. or p.o. routes inhibited the carrageenan-induced hyperalgesia. Post-treatment with betalain also significantly inhibited carrageenan- and CFA-induced hyperalgesia. In vitro, betalain presented ability to scavenge the 2,2’-azino-di-(3-ethylbenzthiazoline-6-sulphonic acid) radical (ABTS⁺), 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH•), and hydroxyl radical, iron-chelation activity (bathophenantroline assay), and inhibited iron-independent and dependent lipid peroxidation. Furthermore, betalain significantly reduced carrageenan-induced increase in TNF-α and IL-1ß levels and depletion in reduced glutathione levels. Conclusions: This compound presents prominent analgesic effect against varied models of pain by reducing the production of hyperalgesic cytokines and inhibiting oxidative stress. Financial support: CNPq, CAPES, FAEPE, MCTI, SETI, Fundação Araucária. Approval by the Animal Research Ethical Committee of Universidade Estadual de Londrina: Process number 14013.2015.83
05.018 Lectin of *Abelmoschus esculentus* (OKRA) promotes antinociceptive and anti-inflammatory effects on formalin induced temporomandibular joint inflammatory hypernociception in rats. Pinto IR\(^1\), Vieira LV\(^1\), Assis EL\(^1\), Val DR\(^1\), Freitas RS\(^1\), Gadelha CAA\(^2\), Santi-Gadelha T\(^2\), Lacerda JTJG\(^2\), Napimoga JTC\(^3\), Pinto VPT\(^1\), Chaves HV\(^1\), Bezerra MM\(^1\)\(^1\) UFC-Sobral, \(^2\)UFPB, \(^3\)Unicamp

**Introduction:** *Abelmoschus esculentus* is largely cultivated in Northeastern Brazil for medicinal purposes, like in cases of pneumonia, bronchitis, and pulmonary tuberculosis, having it shown anti-inflammatory activity. We evaluated the antinociceptive and anti-inflammatory efficacy of *Abelmoschus esculentus* lectin (AEL) in reducing formalin-induced temporomandibular joint inflammatory hypernociception in rats. **Methods:** Wistar rats (180–240 g) were pretreated (i.v.) with saline or AEL (0.01, 0.1 or 1 mg / kg) and one hour after, they received intra-articular injection (i.art.) of formalin (1.5%) or saline (sham) in the left TMJ and were monitored for 45 minutes to observe the nociceptive behavioral response quantified by scratching the injected region and the act of raising his head reflexively, quantified in seconds. After behavioral tests, the animals were anesthetized and euthanized. TMJ tissue, trigeminal ganglion and caudal subnucleus collection was performed for TNF-\(\alpha\) dosage (ELISA). To investigate vascular permeability, animals received 50 mg / kg (i.v) of Evans Blue dye thirty minutes before administration of AEL 0.01 mg/kg (i.v) and, after one hour, they received intraarticular injection of formalin (1.5%, 50\(\mu\)L). After 45 minutes the animals were euthanized and TMJs removed for analysis. In addition, we elucidated the possible effect of opioids pathway on AEL efficacy, using the opioid antagonist naloxone (15 mg / 10 L), kappa(\(\kappa\))opioid receptor antagonist Norbinaltorphimine (15 or 90 \(\mu\)g / 10 \(\mu\)L) or delta(\(\delta\))opioid receptor antagonist, naltrindole (10 or 30 g / 10 L) via intrathecal application 15 minutes before AEL treatment. **Results:** AEL (0.01 mg/kg) was effective in reducing formalin-induced hypernociception (\(p<0.05\)) and Evans blue extravasation. AEL reduced TNF-\(\alpha\) levels (\(p<0.05\)) in TMJ tissue, trigeminal ganglion and caudal subnucleus. AEL effects, however, were not observed in the presence of the opioid antagonists. **Conclusion:** These findings suggest that AEL efficacy depends on TNF-\(\alpha\) inhibition and activation of \(\delta\) and \(k\) opioid receptors. **Funding Sources:** FUNCAP, CNPq, CAPES, and INCT-IBISAB. **Animal Research Ethical Committee (CEPA):** n\(^9\) 021502.1500. References: Torres-Chávez, K. E. et al., EUR. J. PAIN., 16, 204.2012.; Greene, C. S. et al., J. Am. Dent. Assoc., 141, 1086, 2010; Soares, G. D. S. F. et al., Protein J., 31 674, 2012.
Aromatase inhibitor-evoked pain is promoted by the enzyme substrate, androstenedione, via transient receptor potential ankyrin 1 (TRPA1) in mice. de Logu F, Monello R, Materazzi S, Nassini R, Fusi C, Coppi E, Li Puma S, Marone IM, Sadofsky L, Morire A, Susini T, Terreni A, Di Tommaso MR, Geppetti P, Benemei S. 1University of Florence, 2UFSM, 3University of Hull, 4Castle Hill Hospital, 5General Laboratory, Careggi University Hospital, Florence.

Aromatase inhibitors (AIs) are a mainstay in the treatment of estrogen-sensitive breast cancer in postmenopausal women. AIs block the activity of aromatase cytochrome P450, which, however, rather selectively transforms the androgens, androstenedione and testosterone into the estrogens, responsible for cancer cell replication and growth. Unfortunately, one-third of patients treated with AIs develop muscular and joint pain and inflammation (aromatase inhibitor-associated musculoskeletal symptoms, AIMSS), and also exhibit symptoms of neuropathic or mixed pain. AIMSS and the associated forms of pain represent a major medical problem because they affect the quality of life of the patients, thus limiting treatment adherence, and sometimes leading to therapy discontinuation. Furthermore, AIMSS respond poorly to current analgesic therapies, and the therapeutic needs of the patients remain unmet.

We reported that the transient receptor potential ankyrin 1 (TRPA1), a cation channel highly expressed by a subpopulation of primary sensory neurons of the dorsal root ganglia (DRG), mediates the entire constellation of pain-like behaviors evoked by AIs in mice. However, as aromatase inhibitor concentrations required to engage TRPA1 are higher than those found in patients’ plasma, we hypothesized that additional factors might cooperate with the anticancer drugs to promote AIMSS. Here, we report that the aromatase substrate, androstenedione (AIs), unique among several steroid hormones, targets TRPA1 in peptidergic primary sensory neurons in rodents and in human cells expressing the native or recombinant channel. By in vitro studies, we show that androstenedione selectively activates the recombinant and native human TRPA1 by targeting key electrophilic amino acid residues and excites DRG neurons by TRPA1.

Behavioral test (Von frey hair) was then used to study the mechanical hypersensitivity. We show that intraperitoneal administration of ASD (0.2-2 μg/kg, i.p.) induces a dose dependent mechanical allodynia via the activation of TRPA1. ASD (2 μg/kg, i.p.) also increases H2O2 levels in the sciatic nerve. To better understand the contribution of androstenedione and oxidative stress to the AIMSS-like behaviors, a low dose of ASD (0.2 μg/kg, i.p.) that failed to affect H2O2 generation, as well as mechanical allodynia, is used. Systemic ASD (0.2 μg/kg, i.p.) and letrozole (0.1 mg/kg, i.g.) that per se, or in combinations (letrazole/androstenedione) did not affect mechanical allodynia, when given simultaneously caused remarkable mechanical allodynia via the activation of TRPA1. The present study robustly underscores the role of TRPA1 in ASD-evoked AIMSS-like behaviors. Thus, TRPA1 blockade by both new compounds currently under clinical scrutiny for pain therapy and old medicines recently identified as TRPA1 antagonists may represent a new frontier to treat or even prevent AIMSS. Present studies were conducted under University of Florence research permits #204/2012-B and #194/2015-PR. This work was supported by: Istituto Toscano Tumori (ITT), grant 2014, (to P. Geppetti); Regione Toscana, grant Nutraceuticals 2014, ‘POFCAFD’ (to P. Geppetti); Associazione Italiana per la Ricerca sul Cancro (AIRC), My First Grant 2012 (to R. Nassini).
05.020 TRPV4 channel, in addition to TRPA1 mediates the oxidative stress-dependent peripheral painful neuropathy induced by vincristine. Marone IM¹, Trevisan G², de Logu F¹, Fusi C, Materazzi S¹, Benemei S, Nassini R², Geppetti P¹¹University of Florence, ²UNESC

Several anticancer medicines, including vincristine, evoke sensory adverse events, collectively referred to as chemotherapy-induced peripheral neuropathies (CIPN), which are represented by sensory symptoms (from paresthesias, allodynia and hyperalgesia to severe pain). In addition to impairing patient quality of life, CIPN may lead to dose-limitation or even discontinuation of anticancer treatment. Transient receptor potential (TRP) channels expressed by sensory neurons, including the A1 (TRPA1), V1 (TRPV1) and V4 (TRPV4) appear to contribute to CIPN (Nassini et al. 2014). Recently, vincristine has been found to produce CIPN-like symptoms via TRPA1 (Old et al. 2014), TRPV4 has been reported to contribute to mechanical hypersensitivity evoked by paclitaxel, and here we explored, the role of TRPV4 in a mouse model of vincristine-induced CIPN. Behavioral test (Von frey hair and acetone test) were used to study the mechanical and cold hypersensitivity, respectively. In vitro, calcium responses evoked in cultured primary sensory neurons from rat/mouse dorsal root ganglia were investigated. Vincristine-evoked (one single i.p. administration 0.2 mg/kg) CIPN-like behaviors were reduced but not abolished in TRPA1-deleted mice. Complete attenuation was obtained by treatment with the TRPV4 antagonist, HC-067047. Vice versa, CIPN-like behaviors evoked by vincristine were partially reduced in TRPV4-deleted mice and complete abolition was produced by the addition of the TRPA1 antagonist, HC-030031. The anti-oxidant alpha-lipoic acid or a combination of the two TRPA1 and TRPV4 antagonists also abated vincristine-evoked CIPN-like behaviors. In vitro, low concentrations of H2O2 evoked a selective TRPA1 calcium response. However, higher concentrations were able to engage the TRPV4 channel. While we confirmed that TRPA1 contributes to the oxidative stress-dependent mechanical and cold hypersensitivity evoked by vincristine in mice, we discovered that TRPV4 also play a major role. The contribution of TRPV4 is supported by the ability of reactive oxygen species to target this channel in primary sensory neurons. Nassini R, Materazzi S, Benemei S, Geppetti P. The TRPA1 channel in inflammatory and neuropathic pain and migraine. Rev Physiol Biochem Pharmacol. 2014;167:1-43. doi: 10.1007/112_2014_18. Old EA, Nadkarni S, Grist J, Gentry C, Bevan S, Kim K, Mogg AJ, Perretti M, Malcangio M. Monocytes expressing CX3CR1 orchestrate the development of vincristine-induced pain. J Clin Invest. 2014 May;124(5):2023-36. doi: 10.1172/JCI71389. Studies were conducted under University of Florence research permits #204/2012-B and #194/2015-PR Financial support "Associazione Italiana per la Ricerca sul Cancro (AIRC MFAG,13336, R.N.)"
05.021 Role of C5a/C5aR in the peripheral and spinal signaling for the development of neuropathic pain

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Introduction: Treatment and management of chronic, including neuropathic pain, is currently the core challenge of analgesic therapy. A growing body of evidence have been suggesting that neuro-immune interactions in the periphery and central nervous system have a crucial role in the long-term sensitization of pain pathways. In this context, emerging data indicate that C5a and its receptor, C5aR, participate in acute and chronic pain, although the related mechanisms are largely unknown. Thus, the present study aimed to further address the peripheral and spinal mechanisms by which C5a/C5aR signalling mediates neuropathic pain development.

Methods: Neuropathic pain was induced by PSNL (peripheral sciatic nerve ligation). Noceptive behaviour was evaluated by von Frey filaments (mechanical), Hargreaves (heat) and acetone test (cold). Spinal WDR neurons response was evaluated by in vivo extracellular single cell electrophysiology, in male Sprague-Dawley rats. Measure of cytokines was done by ELISA/Milliplex. Results: The noceptive effect of C5a/C5aR was firstly demonstrated by i.t. injection of recombinant (C5a 30, 100, 300 or 600 ng/μL) on male Balb/C mice. Mechanical threshold was reduced in a dose-dependent manner from 1 up to 24 hours after injection. Corroborating, directly spinal administration of 100 or 500 ng/50 μL C5a recombinant promoted facilitation of WDR neurons response after paw mechanical stimulation with both noxious and especially innocuous von Frey filaments. More important, the absence of C5aR in male and female mice submitted to PSNL resulted in less development and maintenance of mechanical, cold and heat noiceptive responses. Likewise, systemic (1 mg/kg orally) and i.t. (10, 30 or 100 μg/5 μL) treatment with DF2593A, an allosteric C5aR antagonist, in an established PSNL, reduced mechanical and cold allodynia. Corroborating, spinal administration of 400 ng/50 μL of PMX-53, a C5aR antagonist, reduced the excitability (wind-up) and the mechanical evoked response of spinal WDR neurons to noxious and mainly innocuous von Frey filaments in established PSNL (14th day). These effects seem to occur in both, peripheral and spinal sites. Indeed, by ELISA was demonstrated an increase in C5a release in sciatic nerve from 3rd hour to 3rd day after surgery, in DRG from 1st to 3rd day and in spinal cord at 14th day. Besides that, there is an important reduction in a wide panel of cytokines in the sciatic nerve (GM-CSF, INF-α, IL-1β, IL-2, 4, 5, 6, 7, 9, 12, 13, 15, 17, IP-10, KC, MCP-1, MIP-1α, MIP-1β, Rantes and TNF-α) 12 hours after surgery and spinal cord (G-CSF, IL-1α e -β, IL-2, 6, 15, IP-10, MIP-1α and MIP-2) 7 and/or 14 days after surgery, corroborating with the behavioral and electrophysiological data. Conclusion: Taken together, these results indicate that C5a/C5aR are clearly involved in both, genesis and maintenance of neuropathic pain, participating in response to polimodal stimulus, especially to innocuous, corroborating with the allodynia observed clinically. These effects seem to occur in peripheral and spinal sites, by among others, modulation in cytokines release at pain pathways. Financial support: FAPESP All mice experiments followed the rules of IASP and Animal Ethics Committee from USP (120/2014); and rats experiments followed the Project License 40/3647 from ASPA, United Kingdom.
05.022 Peptides participation in control opioids endogenous peripheral inflammatory pain induced different inflammatory mediators  Quintão JLD, Gonzaga ACR, Romero TRL, Duarte IDG UFMG – Farmacologia e Fisiologia

**Introduction:** Studies from our laboratory showed that administration of opioid antagonists intraplantar induced increase in carrageenan-induced hyperalgesia.

**Aim:** The objective of this study was to evaluate the inflammatory mediators which are responsible for inducing peripheral modulations of inflammatory pain through activation of the opioid system. **Methodology:** All drugs were administered via intraplantar in Swiss mice (n = 4). Hyperalgesia was induced in the animals by injection of the different mediators and measured using the paw pressure test. **Results:** The results across the induction of hyperalgesia by carrageenan (100 μg / paw) in mice were consistent with those previously found in rats. It was observed that nonspecific antagonist to opioid receptor Naloxone (12.5, 25 and 50 μg / paw), as well as injections specific antagonist μ-opioid receptor Clocinamox (10, 20 and 40 μg / paw), δ-opioid receptor, naltrindole (15, 30 and 60 μg / paw) and κ-opioid receptor Nor BNI (50, 100 and 200 μg / paw) induced an increase of hyperalgesia. Already in animals receiving opioid degradation inhibitor Bestatin (100, 200 and 400 μg / paw), the effect was observed antinociception. When we are using the release of inflammatory mediators cascade induced by carrageenan, TNF-α (100 pg / paw) CXCL-1 (10 pg / paw) IL-1β (0.1 pg / paw) and then the unspecific antagonist opioid receptors (Naloxone, 25 and 50 μg / paw) and specific antagonists for receptor μ-opioid receptors (Clocinamox; 20, 40 μg), δ-opioid (naltrindole, 30 and 60 μg) and κ-opioid (nor BNI ), there was an intensification of hyperalgesia. While the administration of aminopeptidase inhibitor Bestatin (200 and 400 μg / paw) induced significant reduction of the nociceptive threshold Δ. Naloxone the same protocol time and even using a double dose (100 μg / paw) further exacerbated hyperalgesia induced by mediators mentioned above, was unable to change the hyperalgesic effect induced by PGE2 (1 g / paw) and norepinephrine (62.5 μg / paw) in mice paw. **Conclusion:** The results provide evidence that activation of the opioid system and mobilization of its μ specific receptors, δ and κ is activated peripherally as inflammatory pain control mechanism induced by TNF-α mediators, CXCL-1, IL-1β. **Financial support:** CNPq, CAPES and FAPEMIG. This study was approved by CETEA-UFMG (the Ethics Committee for Animal Experimentation of the Federal University of Minas Gerais), 50/2013.
Neurotransmission Systems Involved in the Transcranial Direct Current Stimulation (tDCS) antiallodynic effect in mice. Ciocato SG¹, de Souza A², Martins D F³, Medeiros LF², Nucci C², Martins TC⁶, Siteneski A⁴, Caumo W⁴, Santos ARS², Torres ILS¹

Introduction: Despite the wide variety of pharmacological therapies for chronic neuropathic pain, the non-pharmacological therapies have been investigated as alternative treatment or an adjuvant management for treating this condition. Transcranial Direct Current Stimulation (tDCS) is a central neuromodulatory technique, however its action mechanism is not completely known. The objective of this study was to investigate the neurotransmission systems involved in the antiallodynic effect of tDCS in mice. Methods: NUMBER male Swiss mice (25-35g) were subjected to partial sciatic nerve ligation (PSNL); and allodynia was assessed by Von Frey filament (0.6g). After PSNL model mice were divided in tDCS (0.5mA/15 minutes) and tDCS sham, and then subdivided in saline (10ml/kg) or drug administrated intraperitoneally (i.p.) before the tDCS session: naloxone (1mg/kg), yohimbine (0.15 mg/ kg), a-methyl-p-tyrosine (100 mg/kg), q-chlorophenylalanine methyl ester (100 mg/kg), caffeine (10 mg/kg), AM281 (0.5 mg/kg), AM630 (3 mg/kg), flumazenil (3 mg/kg) and MK-801 (0.01 mg/kg). The data were analyzed by one-way ANOVA or repeated measures ANOVA followed by Tukey test, when indicated. P<0.05 was considered a significant difference. Data were expressed as mean ± S.E.M. (Standard Error of Mean). Results: We observed an antiallodynic effect after 15 minutes and 4 hours after tDCS session (repeated measures ANOVA/Tukey P<0.05). The tDCS antiallodynic effect was involved with all systems evaluated, such as opiodergic (one way ANOVA/Tukey, P< 0.01), adenosinergic, serotonergic, noradrenergic, cannabinoid, gabaergic and glutamatergic (one way ANOVA/Tukey, P<0.01 for all system analyzed). Conclusion: tDCS presented long-lasting antiallodynic effect after PSNL in mice (until 4 hours), that is related to tDCS mechanism associated to different neurotransmitters systems involved in the pain modulation evaluated in this study. Financial Support: CAPES (L.F. Medeiros); CNPq (I.L.S. Torres, W.Caumo); FIPE-HCPA (Dr. I.L.S. Torres, grant No. 14 0038); MCT/FINEP – COENG/2013. Approved by the Institutional Animal Care and Use Committee GPPG-HCPA protocol No. #140078. Keywords: tDCS, chronic pain, neuropathy, antiallodynic, Von Frey test.
05.024 Diabetes mellitus hastens the establishment of oxaliplatin-related experimental peripheral sensory neuropathy. Silva CMS¹, Pereira LMS¹, Pereira AF¹, Silva CMP¹, Silva KO¹, Aguiar LA¹, Pereira AC¹, Almeida PRC², Pontes RB², Lima-Júnior RCP¹, Vale ML¹ ¹UFC – Farmacologia e Fisiologia, ²UFC – Patologia, ³UFC – Morfologia

Introduction: Oxaliplatin is an anticancer agent used for clinical management of advanced colorectal cancer. Acute and chronic peripheral sensory neuropathies are common side effects associated with oxaliplatin. Interestingly, neuropathy is also observed in diabetic patients with uncontrolled glycaemia. However, there is no consensus whether diabetes worsens peripheral neuropathy in patients submitted to cancer treatment with oxaliplatin based regimens. Then, this study aimed to investigate whether diabetes would change the course of oxaliplatin-related experimental peripheral sensory neuropathy. Methods: Swiss male mice (n=6/group) were injected saline (5 mL/kg), alloxan (single injection at doses of 25, 50 or 75 mg/kg, iv.), oxaliplatin (4 mg/kg, iv. twice a week for 4.5 weeks) or alloxan (50 mg/kg) + oxaliplatin (4 mg/kg). Blood glucose levels were determined on days 0, 15, 30, 45 and 60. Von Frey and rota rod test respectively investigated the intensity of hyperalgesia and motor coordination at different times (0, 7, 14, 21, 28, 35 42, 49 and 56 days). At day 60, the animals were euthanized for histological evaluation of the pancreas (number of cells/field at x200 magnification). One-way ANOVA or Two-way ANOVA/Bonferroni’s or Kruskal-Wallis/Dunn’s tests were used for statistical analysis. P<0.05 was accepted. Results: Alloxan (50 and 75 mg/kg) induced a significant hyperglycaemia. Since animals injected with alloxan (50 mg/kg) did not show signs of neuropathy, that dose was used in combination with oxaliplatin for subsequent studies. The oxaliplatin injection induced hyperalgesic responses starting at day 28, which was maintained over the experimental period versus the saline group. In addition, hyperalgesia was significantly hastened (P<0.05) in one week (starting at day 21) when compared to oxaliplatin group. Furthermore, groups that received alloxan showed a pronounced loss of islets of Langerhans (alloxan: 0.0 ± 0.0; and alloxan-oxaliplatin: 0.0 ± 0.0) versus saline (1.2 ± 0.2) and oxaliplatin (1.0 ± 0.0) groups. Conclusions: Diabetes seems to accelerate the establishment of oxaliplatin-related peripheral neuropathy. More studies are needed in order to investigate the molecular mechanisms involved. Financial support: CNPq, CAPES and FUNCAP. This study was approved by local ethics committee (protocol number 27/12).
05.025 Essential oil from *Piper aleyreanum* C.DC. (Piperaceae) reduces chronic pain induced by paracel sciatic nerve ligation in mice. Nascimento LF^1, Nucci-Martins C^2, Tizziani T^3, Pizzolatti MG^3, Facundo VA^3, Santos ARS^1^UFSC, ^2Unicamp, ^3UNIR

Introduction: Neuropathic chronic pain is a disabling condition which treatment is a major challenge for science once the therapies are less effective. These therapies cause important side effects or make patients refractory to the treatment. Otherwise, *Piper aleyreanum* C.DC. is a specie used as immunomodulator, antidepressant and analgesic in the folk medicine. Aim: To investigate the antinociceptive effect of the Essential Oil from *Piper aleyreanum* (EOPa) in a chronic pain model. Methods: Chemical analysis of the EOPa was done by GC-MS. Experiments were performed using adult female Swiss mice (25-35g, 2 months-old, Protocol Number PP00745) and the antinociceptive effect of EOPa (10-100 mg/kg, i.g.) was assessed on the mechanical (von Frey test) and thermal (hot/cold plate test) hyperalgesia induced by partial sciatic nerve ligation (PSNL) and on the nociception caused by intrathecal administration (i.t.) of both ionotropic (NMDA, AMPA and Kainate) and metabotropic (trans-ACPD) glutamatergic agonists. In addition, were evaluated neuropathic animals on the locomotor activity in the open field test. The evaluation of mechanical hyperalgesia was performed using analysis of variance two-way ANOVA followed by Bonferroni post hoc test for multiple comparisons. Results: Chemical analysis of the EOPa identified 42 compounds, representing 95.6% of the total oil content. Camphene (4.1%), β-pinene (5.1%), p-cymene (3.8%), β-elenene (8.6%), (E)-caryophyllene (6.6%), cis-β-guaiene (29.3%), trans-calamene (6.4%) were the major constituents. Eight days after the PSNL animals presented severe mechanical hyperalgesia on ipsilateral side of the lesion, which was significantly reduced by acute treatment with EOPa (100 mg/kg, i.g.) with inhibitions of 68 ± 12% and 59 ± 13%, from 1^st^ to 4^th^ hour, respectively. Prolonged treatment (from 8^th^ up to 13^th^ day post lesion) reduced the mechanical hyperalgesia with average inhibition of 68 ± 12% up to 100%. On the 14^th^ day it was observed a cumulative effect which lasted until 48h (53 ± 3%). On the 16^th^ day, animals were treated again and caused 100% hyperalgesia inhibition, which was maintained for up to 36h. In the hot/cold test the EOPa increased the latency time (withdrawal latency) without affect the locomotor activity (open field test). Furthermore, EOPa (100 mg/kg, i.g.) decreased the nociceptive behavior (licking and biting) provoked by glutamate (94 ± 5%), AMPA (60 ± 12%) and kainate (92 ± 4%), but not by NMDA or trans-ACPD. Conclusion: These results demonstrate that EOPa presents an important antinociceptive effect on neuropathic pain, by the blockade of a mechanism dependent on glutamatergic system, in particular of the ionotropic AMPA and kainate receptors. Financial Support: CNPq, CAPES, FAPESC, UFSC
05.026 A1 Adenosine Receptor (A1R) agonist ameliorate tactile allodynia and thermal hyperalgesia in STZ-induced diabetic neuropathy. Santos BLR¹, Lima CKF², Jesus CHA³, Calcutt NA⁴, Miranda ALP⁵, PPGCF-LEFEx-FF-ICB-UFRJ,²LEFEx-FF-UFRJ – Biotecnologia Farmacêutica,³UFSC – Farmacologia,⁴UCSD – Pathology

Introduction: Diabetic neuropathy is one of the most common chronic complications of Diabetes Mellitus, affecting about 50% of diabetic patients (Callaghan et al, Lancet Neurol, 11, 521, 2012). Among the diverse range of symptoms involved in diabetic neuropathy (DN), spontaneous and evoked pain manifestations are one of the most disabling and poorly understood of them. The knowledge limitation of painful DN pathogenesis make the treatment options ineffectual and inespecific, showing the necessity of new therapeutic targets identification. Adenosine plays important analgesic effects, specially by A1R activation (Jacobson et al, Nat Commun, 5, 247, 2006). In view of that, the aim of this study was to investigate the A1R agonist (CCPA) effects in the painful symptoms of DN, understanding the mechanisms behind that. Methods: Experimental type 1 diabetes was induced (Calcutt, Methods Mol Med, 99, 55, 2004) in Sprague-Dawley female rats by a single i.p. injection of streptozotocin (STZ), 60mg/kg. Rats with blood glucose ≥300mg/dl were considered diabetic and divided in two groups: vehicle (n=10) and A1R agonist (n=10). The treatment started just after diabetes confirmation and during seven weeks. Vehicle and A1 agonist group received one daily i.p. injection of saline-DMSO 3% or CCPA 0.01mg/kg respectively. Weight, blood glucose and tactile response (Chaplan et al, J Neurosci Methods, 53, 55, 1994) were checked weekly. Locomotor activity (Jones et al, Naunyn Schmiedebergs Arch Exp Pathol Pharmacol, 259, 211, 1968) and thermal (Hargreaves et al, Pain, 32, 77, 1988) tests were performed every two weeks. We also evaluate the spinal inhibition, by rate-dependent depression (RDD) test (Calcutt et al, Pain, 155, 250, 2014), A1R and adenosine desaminase (ADA) spinal cord levels by western blot on week 8. All animal studies were approved by the Institutional Animal Care and Use Committee of the UCSD, S02059R. Results: Rats developed neuropathic pain signals four weeks after diabetes induction. CCPA treatment was able to control tactile allodynia and thermal heat hyperalgesia of diabetic neuropathy rats, without affect the locomotor activity. In order to understand the central contribution to the A1R agonist antinociceptive effects, the spinal inhibition was evaluated by RDD test. RDD is a measure of the decline in amplitude of the spinal Hoffman reflex (H-reflex) over consecutive stimulations that provides an indicative of the function of spinal inhibitory systems. STZ-diabetic rats showed a significant RDD decrease (Control=49.97±10.14% versus Vehicle=6.23±11.64%, results expressed in % of H-reflex reduction, One-way ANOVA, p<0.05). CCPA treatment partially recovery the spinal inhibition of diabetic rats (CCPA=28.80±8.02%). Trying to understand the adenosinergic system contribution to the painful symptoms of diabetic neuropathy, the spinal levels of A1R and ADA (the main enzyme responsible for adenosine degradation) were quantified. There was no significant difference on A1R and ADA spinal levels in diabetic rats. Conclusion: This study showed that A1R selective agonist chronic treatment is effective in alleviating different symptoms of painful diabetic neuropathy. Furthermore, our results suggest that antinociceptive activity of this agonist involves spinal inhibitory system modulation. Financial support: CAPES, CNPq, FAPERJ.
05.027 Quercetin inhibited Granulocyte-Colony Stimulating Factor (G-CSF)-induced mechanical hyperalgesia in mice: effect on cytokine production and NO-Cyclic GMP- Protein Kinase G-ATP-sensitive potassium channel signaling pathway and NFkB activation

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Introduction: Quercetin is a flavonoid that has demonstrated anti-inflammatory activity in several models. Filgrastim (Granulocyte-colony stimulating factor [G-CSF]) is used as a therapeutic approach to increase peripheral neutrophil counts after anti-tumor therapies although its usage can cause pain as a major side effect. Intraplantar administration of G-CSF induces mechanical hyperalgesia in mice. However, the peripheral mechanisms involved in this effect were not completely elucidated. In this study we investigated the effect of quercetin in G-CSF-induced mechanical hyperalgesia in mice. Methods: Male Swiss mice (n= 5/group) were injected with G-CSF (100 ng/i.pl.) or vehicle (saline, i.pl., 20 µL) and the mechanical hyperalgesia was measured at 1, 3, 5, and 7 h after stimulation, cytokine production was evaluated 2 h and NFkB activation 3 h after G-CSF injection. The groups that received G-CSF were also treated with quercetin (10, 30 or 100 mg/kg, i.p., 30 min before G-CSF), indomethacin (0.5 mg/kg, i.p., 45 min before G-CSF), morphine (2 µg, i.pl., 1 or 4 h after G-CSF), L-NAME (90 mg/kg, i.p., 1 h before quercetin), glibenclamide (0.3 mg/kg, p.o., 45 min before quercetin), 1H-(1,2,4)-oxadiazolol-(4,3-a)-quinoxalin-1-one (ODQ, 0.3 mg/kg, i.p., 30 min before quercetin), KT5823 (0.5 µg, i.p., 5 min before quercetin), or vehicle (2 % DMSO in saline or Tris/HCl pH 8.0, i.p., 100 µL; saline, i.pl., 20 µL). Results: G-CSF injection induced mechanical hyperalgesia that was inhibited by quercetin (100 mg/kg). In agreement, G-CSF injection induced significant TNFα, IL-1β and IL-10 production in paw tissue and treatment with quercetin inhibited G-CSF-induced cytokine production. The combined treatment of quercetin with indomethacin or morphine, at doses that are ineffective as single treatment, diminished G-CSF-induced hyperalgesia. Furthermore, quercetin inhibited the G-CSF-induced mechanical hyperalgesia significantly at 3, 5, and 7 h after the stimulus, and treatment with L-NAME, ODQ, KT5823, or glibenclamide inhibited the analgesic effect of quercetin significantly. Moreover, G-CSF stimulus induced NFkB activation in paw tissue and treatment with quercetin inhibited this activation. In addition, systemic treatment with quercetin and indomethacin, and local treatment with morphine did not alter G-CSF-induced mobilization of neutrophils to peripheral blood. Conclusion: These results demonstrate that quercetin reduced the G-CSF-induced mechanical hyperalgesia through inhibition of TNFα, IL-1β, and IL-10 production and NFkB activation, also the analgesic effect of quercetin depends on NO-Cyclic GMP-Protein Kinase G-ATP-sensitive potassium channel signaling pathway activation in mice. Therefore, this study advances in the understanding of G-CSF-induced hyperalgesia and suggests quercetin as therapeutic approach for its control. Financial support: CNPq, CAPES, MCTI, SETI/Fundação Araucária and Paraná State Government, Brazil. Ethics Committee Approval: Male Swiss mice were used in this study with the approval of the Ethics Committee for Animal Use from UEL, process n° 11654.2015.81.
05.028 Antihyperalgesic synergistic effect of celecoxib associated with terpinolene in inflammatory pain in rats. de Macedo EMA, Santos WC, Araujo JM, Lopes EM, Reis Filho AC, de Sousa DP, Oliveira FA, Almeida FRC UFPI – Bioquímica e Farmacologia

**Introduction:** Inflammatory pain is characterized by an increased sensitivity of the injured tissue generated by the release of inflammatory mediators. Pharmacological treatment of inflammatory pain is usually done with non-steroidal anti-inflammatory agents (NSAIDs) to produce good efficacy, but cause side effects, especially gastrointestinal lesions. Given the difficulty of finding an effective drug with minimal side effects for the treatment of pain syndromes, the researchers look for other pharmacological strategies such as drug combinations. The monoterpenes terpinolene (TPL) is a chemical constituent of the essential oil of many plant species that exhibit various pharmacological activities, among them, analgesic and anti-inflammatory. The aim of this study was to investigate the anti-hyperalgesic effect of celecoxib associated with TPL in inflammatory pain models. **Methodology.** Female Wistar rats (170-230 g/ n=6-9) (Animal Ethics Committee/UFPI, no. 82/2014) received 50 uL of Complete Freund’s Adjuvant (CFA) in the intraplantar region. After 24 hours, the rats were treated orally with TPL (3.125; 6.25; 12.5 and 25 mg/kg); celecoxib (3; 10 and 30 mg/kg); combination of celecoxib (3 mg/kg) with TPL (3.125 mg/kg) or vehicle (2% Tween 80 in saline 0.9%), followed by mechanical compression test (Randall Selitto) at the times (0, 1, 2, 3, 4, 5 and 6 hours), and during the subchronic phase (10 days), the rats were treated and assessed their pain threshold daily. In order to elucidate the mechanisms of the anti-hyperalgesic effect, mice were pretreated with naloxone to investigate the involvement of opioid receptors in the association, 24 h after CFA injection the animals were pretreated (30 min before) with Naloxone (3 mg/kg, sc, opioid antagonist) or vehicle, and then the association and the isolated substances were administered. The animals were submitted to Randall Selitto test every hour after this administration until the sixth hour. Statistical analyzes were performed using ANOVA (two way) followed by Bonferroni Test, p<0.05. **Results:** Animals that received the lowest doses of celecoxib (3 mg/kg) and TPL (3.125 mg/kg) did not alter the mechanical threshold; but the 10 and 30 mg/kg celecoxib group increased threshold the first to fifth time of the acute phase, when compared to control (p<0.05), and this effect was kept in sub-chronic phase. TPL (6.25; 12.5 and 25 mg/kg) increased the threshold. The combination of ineffective doses of celecoxib and TPL showed anti-hyperalgesic effect the second to fifth hour, similar to the positive control, celecoxib 30 mg/kg, and this effect was kept in sub-chronic phase. The pre-treatment with naloxone completely reversed the anti-hyperalgesic effect of TPL, celecoxib and of association. **Conclusion:** Celecoxib associated with TPL showed synergistic anti-hyperalgesic effect in acute and subchronic inflammatory nociception and one of the possible mechanisms of action involves the system opioid. **Financial support:** UFPI/CAPES
Resolution of inflammatory response is not associated with reduction of hypernociceptive response during antigen-induced arthritis in mice. Gonçalves WA\textsuperscript{1}, Rezende BM\textsuperscript{1}, Ribeiro LS\textsuperscript{2}, Amaral FA\textsuperscript{2}, Souza DG\textsuperscript{3}, Teixeira MM\textsuperscript{4}, Cunha TM\textsuperscript{4}, Pinto V\textsuperscript{1}  
\textsuperscript{1}ICB-UFMG – Morfologia, \textsuperscript{2}ICB-UFMG – Bioquímica e Imunologia, \textsuperscript{3}ICB-UFMG – Microbiologia, \textsuperscript{4}FMRP-USP – Farmacologia

Introduction: Rheumatoid arthritis (RA) is characterized by persistent synovitis and pain. Several therapeutic agents reduce synovitis but are not effective to treat pain. These findings suggest that RA trigger neuronal changes that remain even if the inflammation has gone. In the current study, we evaluated the expression of TNF-\(\alpha\) and IL-1\(\beta\) in the dorsal root ganglion (DRG) during inflammatory response in a murine model of antigen-induced arthritis (AIA).

Methods: The mice were immunized with 500 \(\mu\)g of mBSA. Fourteen days later, each mouse received an injection of 10 \(\mu\)g of mBSA in the knee joint. The knee cavity was washed and total and differential number of leukocyte was determined. Mechanical hypernociception was evaluated using an electronic von Frey. Expression of TNF-\(\alpha\) and IL-1\(\beta\) were assessed in the ipsilateral DRGs (L2, 3 and 4) by real-time PCR. Results and discussion: We have observed a neutrophil accumulation between 12 and 24 hours after challenge. This time point (24 hours after challenge) indicates the early stage of the resolution process. The mechanical hyperalgesia was markedly higher in the AIA group than in the control group and it was persistent at day 20 after challenge. No change was observed in the IL-1\(\beta\) transcription of DGR. However, we observed an increase of TNF-\(\alpha\) transcript 72 hours after challenge. This increase remained up to 144 hours (six days) after AIA induction. Conclusion: Our study reveals potential role of TNF-\(\alpha\) at persistent nociception observed after resolution in AIA. Number of approval ethics committee (CETEA/UFMG): 86/2014. Financial support: CNPQ, FAPEMIG, CAPES.
05.030 Investigation of the protective role of interleukin 27 (IL-27) on the genesis and maintenance of neuropathic pain. Fonseca MD¹, Santa-Cecília FV, Ferreira DW, Oliveira FFB, Kuzuda R, Ferreira-Davoli M, Cunha FO, Cunha TM – FMRP-USP – Farmacologia

Introduction: Although there has been significant progress in the understanding of pain processes, neuropathic pain remains a prevalent, persistent, and debilitating problem. Attempts to elucidate its mechanisms have demonstrated that neuro-immune interactions are important players in the genesis of this type of pain, including peripheral immune system cells and glial cells. These cells can induce the production of pro- as well as anti-inflammatory components, such as cytokines. In some inflammatory conditions in the central nervous system (CNS), interleukin IL-27, which is formed by two subunits (p28 and EBI3), is produced and promotes the protection of the CNS by regulating the exacerbated inflammatory response, suggesting a new interesting target for neuropathic pain treatment. Methods: The experiments were carried out on male C57BL/6, IL-10⁻/⁻ and IL-27EBI⁻/⁻ mice. Mice were submitted to the Spared Nerve Injury (SNI) neuropathy model. Mechanical thresholds and cold allodynia were determined respectively by application of von Frey filaments and acetone test. Recombinant IL-27 (IL-27R) was administered intrathecally (i.t) before or after SNI. mRNA expression was determined by RT-PCR. Results: After SNI, it was observed an increase in the IL-27p28, IL-27EBI3 and WSX-1 gene expression in spinal cord (SC) and dorsal root ganglia (DRGs). IL-27R had no effect on the genesis of neuropathic pain. On the 7th day after SNI induction, mice treated with 100 ng IL-27R showed increased mechanical threshold 24 h after treatment. Furthermore, mice injected twice daily with IL-27R (100ng/animal) showed an increase in mechanical threshold after the second treatment at 10th and 14th days after SNI. In order to evaluate the possible IL-27 antinociceptive mechanisms, mice were daily treated with 100 ng of IL-27R at 10th to 14th days after SNI. Also, IL-27R induced an increase in IBA-1, GFAP, IL-10, CD39, CD73 and SOCS3 gene expression in SC as well as IL-10, CD39 and IBA-1 in DRGs, compared to SNI group treated with vehicle. The mechanical hypersensitivity was not reduced in IL-10⁻/⁻ mice treated with IL-27R after SNI surgery. On the other hand, IL-27EBI⁻/⁻ mice showed a higher frequency response in mechanical sensitivity testing and increased cold sensitivity when compared to wild type (WT) mice. These effects were associated with a reduction in the expression of IL-10 anti-inflammatory cytokine and a increase in the expression of IL-1β and TNF-α pro-inflammatory cytokines in both SC and DRGs at 7th day after SNI when compared to WT mice. Conclusion: The results point the IL-27 interleukin as a new interesting target to treat the neuropathic pain. The treatment with IL-27R in intermediate stages of neuropathic process can induce the activation of anti-inflammatory and/or antinociceptive pathways that could be acting adversely to control the neuropathic pain. Such effects occur possibly by increased IL-10 and/or adenosine synthesis or by increased ectonucleotidases (CD39 and CD73) expression and/or activity. Together, all these factors can culminate in nociceptive process control and neuroinflammation regulation. Financial Support: FAPESP. Animal Research Ethical Committee: protocol n° 211/2014.
05.031 Hypoalgesia is not modulated by peripheral opioid receptors in high-fat diet-induced obese mice. Silva NP¹, Aquino BM¹, Santos DF¹, Torsoni AS², Oliveira-Fusaro MCG¹
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Introduction: Obesity and pain present serious public health concerns in our society and a number of associations between obesity and chronic pain have been displayed in several studies. Recently, we demonstrated that in high-fat diet-induced obese mice there is a decrease in sensitivity to inflammatory pain stimuli (hypoalgesia), while the development of nociception is not affected. Therefore, the aim of this study was to evaluate whether peripheral opioid receptors modulate the hypoalgesia in high-fat diet-induced obese mice. Methods: Inflammatory pain was induced by injection of carrageenan into subcutaneous tissue of mice’s hind paw after three, six or nine weeks of treatment with normal or high-fat diet. The nociceptive behavioral responses characterized by flinching and licking the treated paw, were quantified three hours after carrageenan injection, for 60 minutes. Male Swiss mice were used and methods were approved by the Ethics Committee in Animal Research of the UNICAMP. To investigate the involvement of peripheral opioid receptors, naloxone, a non-selective inhibitor of opioid system, was injected into subcutaneous tissue 30 minutes before the injection of carrageenan. Body weight and blood glucose were monitored for nine weeks. Results: Carrageenan (100µg/paw) did not induce hyperalgesia in mice treated with high-fat diet over the nine weeks of treatment (p>0.05, Two Way ANOVA, n=6) when compared to mice treated with normal-fat diet. Pretreatment with naloxone (1.0, 2.5 and 5.0µg/paw, s.c.) did not reverse (p>0.05, Tukey test, n=5) the hypoalgesia after three, six or nine weeks of high-fat diet when compared to hyperalgesia induced by carrageenan in mice treated with normal-fat diet. It is important to point out that treatment with high-fat diet induced significant weight gain and but not an increase in blood glucose over the nine weeks of treatment. Conclusions: These data demonstrated that peripheral opioid receptors do not modulate the hypoalgesia in high-fat diet-induced obese mice. We cannot exclude the involvement of opioid receptors of the central nervous systems in this hypoalgesic response. Financial Support: CNPQ (473790/2013-0). Animal Research Ethical Committee (protocol number 3877-1).
05.032 Spinal Cord CXCL1/CXCR2 signaling mediates the development of paclitaxel-induced peripheral neuropathy in mice. Manjavachi MN¹, Matias DO², Trevisan G², Costa R³, Calixto JB⁴ ¹UFSC – Farmacologia e Ciências Biológicas, ²UFRJ – Ciências Farmacêuticas, ³UFSC – Farmacologia, ⁴UFRJ – Farmácia, ⁵Centro de Inovação e Ensaios Pré-Clinicos.

Introduction: Peripheral neuropathy is a dose-limiting side effect caused by paclitaxel (PTX) treatment, a widely used chemotherapeutic agent. Besides interfering with patient adherence to treatment, peripheral neuropathy can become chronic in some individuals. Despite much effort, the available pharmacotherapies fail to provide satisfactory pain relief for patients with chronic pain. Recent studies provide compelling evidence that neuroinflammation plays a key role in the pathogenesis of chronic pain. Importantly, it was recently showed that the interaction between CXCL1 chemokine and its receptor CXCR2 mediates glial-neuronal communication in the spinal cord, driving neuropathic pain in the mouse model of spinal nerve ligation (Zhang ZJ. Pain, 154: 2185, 2013). In this study we sought to evaluate the involvement of CXCL1/CXCR2 signalling in the pathogenesis of neuropathic pain induced by PTX in mice. Methods: Male Swiss mice (30-40 g, n = 6-8 per group) were treated with PTX (2 mg/Kg, i.p.) or its vehicle (0.9% NaCl) once a day for 5 consecutive days (days 0 to 4 of the protocol). Peripheral neuropathy was assessed by plantar sensitivity of animals to mechanical (von Frey filaments) stimuli. Spinal cord levels of CXCL1 chemokine and its mRNA were determined at different time-points after neuropathy induction by ELISA and RT-qPCR, respectively. The role of CXCL1 and CXCR2 in the maintenance or development of PTX-induced peripheral neuropathy was assessed by the treatment with anti-CXCL1 antibody or selective CXCR2 antagonist (SB225002), respectively. Results: The 5-days treatment with PTX significantly reduced the plantar withdraw threshold to mechanical stimuli from 7 days of the experimental protocol. PTX treatments significantly increased the spinal cord levels of both CXCL1 mRNA and protein from 4 hours up to 3 days after its first injection. Intrathecal (i.t.) single treatment with either anti-CXCL1 antibody (1 ng/site) or SB225002 (10 µg/site) slightly reduced installed PTX-induced mechanical hypersensitivity at 7th day. However, both drugs were able to significantly delay the beginning of mechanical hypersensitivity when given once a day for 7 days, starting at the moment of the first PTX treatment (p<0.001; Two-way ANOVA). Conclusion: The results of this study have suggested that CXCL1 and CXCR2 play an important role in the establishment of PTX-induced peripheral neuropathy in mice. Additional studies are in course to better understand the role of CXCL1/CXCR2 signaling in this model. Financial support: CNPq, CAPES, FAPESC. Animal Research Ethical Committee Protocol Number: PP00811 (UFSC). Key-words: paclitaxel, neuropathic pain, spinal cord, CXCL1 and CXCR2.
05.033 Pregabalin attenuates tactile hypersensitivity and anxiety-like behavior in a model of facial carcinoma in rats. Gambeta E, Kopruszinski CM, dos Reis RC, Zanoveli JM, Chichorro JG UFPR- Farmacologia

Introduction: Head and neck cancer is highly incident in the world population. Pain and anxiety are common symptoms in this condition, and both significantly impair the quality of life of cancer-suffering patients. Pharmacological pain management in cancer is considered unsatisfactory and associated with frequent and severe side effects. Moreover, clinical studies indicate that anxiety disorders are under diagnosed and undertreated in these patients population. For this reason, studies are warranted to better understand the relationship between cancer pain and anxiety to provide new strategies that can help to control both symptoms. Thus, the aim of this study was to investigate the relationship between tactile hypersensitivity and anxiety-like behavior in rats with facial carcinoma. Furthermore, to investigate the influence of the anticonvulsant pregabalin, a drug with potential analgesic and anxiolytic-like effect, in both aspects. Methods: Facial carcinoma was induced by subcutaneous inoculation of tumor Walker-256 cells into the right vibrissal pad of male Wistar rats. On the third and sixth day after inoculation, facial mechanical hypersensitivity was assessed by the application of a series of Von Frey filaments in the vibrissal pad, followed by animals evaluation on elevated plus maze (EPM) test. In a different set of experiments, the effect of pregabalin treatment (30 mg/kg, p.o.) was evaluated in all tests. Results: Tumor-bearing rats showed facial mechanical hypersensitivity 3 days after tumor cells inoculation, but the anxiety-like behavior was not detected. On the other hand, on day 6 after tumor cells inoculation, tumor-bearing rats displayed facial mechanical hypersensitivity and anxiety-like behavior. In addition, systemic treatment with pregabalin was able to reduce facial mechanical hypersensitivity for 2 to 4 hours after its administration, and produced an anxiolytic-like effect when assessed in the EPM 2 hours after its administration. Conclusion: Our data indicate that facial tumor-bearing rats develop early mechanical hypersensitivity, which precedes the development of the anxiety-like behavior. Pregabalin may represent a useful drug in the treatment of both conditions. Financial support: We thank CAPES for the financial support. All experimental procedures were previously approved by UFPR’s Committee on the Ethical Use of Animals (#938).
05.034 Evaluation of antinociceptive activity and possible mechanisms of action of isoxazoline-acylhydrazone derivatives. Carvalho VMF¹, Silva NM¹, Melo MCS¹, Rios ACM¹, Correia JCA¹, Carvalho JA¹, Neto PPM¹, Mota FVB¹, Faria AR², Silva TG¹.¹ UFPE – Antibióticos, ²UFPE – Ciências Farmacêuticas

Introduction: compounds containing isoxazolic ring and acylhydrazone function have been reported as anti-inflammatory and analgesic agents. Studies conducted by our group found that isoxazoline-acylhydrazone derivatives are active against several inflammatory models in vivo. This study aimed to evaluate the anti-nociceptive activity of isoxazoline-acylhydrazone derivatives (R-99 and R-123) using the formalin-induced paw licking test (HUNSKAAR, Pain 30:103, 1987) and acid-induced abdominal constriction test (KOSTER, Fed Proc 18:412, 1959), with investigations of the possible mechanisms of action (DE SOUZA, Pharmacol Biochem Behav 93:40, 2009).

Methods: R-99 and R-123 were provided by the Laboratório de Síntese Orgânica Aplicada a Fármacos from UFPE. Male swiss mice (Mus musculus) (n=6) were used for the experiments. Acetic acid-induced abdominal constriction test: mice were pretreated orally (p.o.) with R-99 or R-123 (15 mg / kg), indomethacin (10 mg / kg), or saline 60 min prior algesic stimulation. After that, abdominal writhes were induced by intraperitoneal (i.p.) injection of acetic acid (1%, 0.1 mL / 10 g). Abdominal writhes were observed between 10-30 min after the injection of the nociceptive agent. Formalin-induced paw licking test: mice were pretreated 60 min prior the observation with R-99 and R-123 (15 mg / kg, p.o.), saline (p.o.), or 45 min prior morphine (5 mg / kg, s.c.). An intraplantar injection of 2.5% formalin solution (20 μL) was injected into the right hindpaw of the mouse. The time that the animal spent licking/biting its paw was measured during the neurogenic phase (first phase) (0–5 min) and the inflammatory phase (second phase) (15–30 min) of the test. Further experiments were carried out to elucidate the possible mechanisms by which the different isoxazoline-acylhydrazone derivatives exert their antinociceptive action, using acetic acid-induced abdominal constriction test. Mice were pretreated with the following antagonists agents (i.p.): (a) opioid antagonist, naloxone 5 mg / kg; (b) α1-adrenergic receptor antagonist, prazosin 0.15 mg / kg; (c) α2-adrenergic receptor antagonist, yohimbine 0.5 mg / kg; (d) cholinergic antagonist, atropine 5 mg / kg. After 15 min, R-99 and R-123 were administered orally. The antinociceptive response was obtained 1 h after the treatment with R-99 and R-123, using the acetic acid-induced abdominal constriction test. The results are shown as mean ± SD. Multiple comparisons were performed by one-way ANOVA followed by Turkey test (p < 0.01). Results: R-99 and R-123 inhibited 68% and 69% the number of writhes, respectively. They also reduced both first (43% and 28%), and second (38% and 55%) phases of the formalin-induced nociception test, respectively. Regarding of the mechanism of action, both derivatives have had their antinociceptive effects reversed by naloxone or yohimbine, but only R-99 has had the analgesic effect reversed by atropine. The R-99 and R-123 did not change the nociceptive action when the α1-adrenergic system was blocked. Conclusion: the results showed that the compounds have antinociceptive activity due to its action on opioids and α2-adrenergic receptors. Committee for Ethics in Animal Research n°23076.017767/2016-65. Financial support: FACEPE, CAPES and CNPq.
05.035 Chronic administration of fish oil is capable of preventing inflammatory and neuropathic pain in mice. Melat J¹, da Silva GF¹, Stoeberl LC¹, Costa R², Quintão NLM¹
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Introduction: Recent studies have demonstrated that polyunsaturated fatty acids and their derivatives present potent anti-hyperalgesic effects in acute pain models in rodents. It was observed that polyunsaturated fatty acids enriched-diets have prophylactic effect on the development of thermal hypersensitivity after spinal cord injury, visceral pain caused by dysmenorrhea, back pain and headaches, possibly due to a higher bioavailability of lipid anti-inflammatory mediators. The aim of this study was to investigate e effect of chronic administration of fish oil [OMACOR®; combination of ethyl esters of eicosapentaenoic acid (EPA) 465 mg/g and docosahexaenoic acid (DHA) 375 mg/g] on hypersensitivity induced by carrageenan (inflammatory hypersensitivity) or by oxaliplatin (chemotherapy-induced neuropathy) in mice. Methods: We used C57BL/6 male mice (8 weeks old; 25-30 g, N = 6-10). The animals were orally pre-treated for 30 days with fish oil Omacor® (23 mg/kg of ethyl esters) or vehicle (saline + gum arabic 5%). Then, they were submitted to mechanical hypersensitivity assay induced by carrageenan (300 µg/paw). The mechanical withdrawal threshold was assessed in different time points by the use of von Frey filaments (up and down method). In the model of chemotherapy-induced neuropathy, the animals were also orally pre-treated for 30 days with fish oil Omacor® (23mg/kg) or vehicle (saline + gum arabic 5%). After that, they received an i.p. injection of oxaliplatin (6 mg/kg) and the cold hypersensitivity was assessed 24 hours later using a cold probe made with powdered dry-ice packed into a modified 3 mL syringe. The open end of the syringe was held against the flat surface (Pyrex 6 mm borosilicate float glass) while pressure was applied to the plunger. Results: The fish oil chronic treatment was able to reduce the mechanical hypersensitivity induced by carrageenan with inhibition of 84.7 ± 3.0%, which was better than the anti-inflammatory indomethacin (5 mg/kg, p.o.; positive-control drug) which inhibited 81.5 ± 4%. In oxaliplatin-induced neuropathy model, animals treated with fish oil Omacor® showed significant reduction of cold hypersensitivity, with inhibition of 76.6 ± 13.2%. Conclusion: These results allow us to suggest that supplementation with fatty acids DHA and EPA (ω-3) can attenuate the effects of the inflammatory process maintained by arachidonic acid derivatives synthesis (such as prostaglandins, thromboxanes and leukotrienes); besides acting as a powerful antioxidant, since the main mechanism of oxaliplatin-induced neuropathy involves the production of free radicals. UNIVALI Ethical Committee of Laboratory Animals Use approval protocol number = 017/15. Financial Support: CNPq, VRPPEC/UNIVALI, CAPES.
Role of bradykinin receptors B1 and B2 in the paclitaxel-associated acute pain syndrome. Zanata GC¹, Silva RL¹, Oliveira FFB², Fonseca MD¹, Cunha TM¹ ¹FMRP-USP – Farmacologia, ²UFC – Farmacologia e Fisiologia

Introduction: Paclitaxel (PCX) is the first line of choice to treat several types of cancer, such as breast cancer, ovary, lung and head and neck. However, patients who receive treatment with PCX develop pathological pain, which can occur immediately after the treatment, known as PCX-associated acute pain syndrome (P-APS). Bradykinin receptors, called B1 and B2, have an important role in the modulation of physiological and pathological pain processes. The objective of this work was to evaluate the involvement of B1 and B2 receptors in P-APS in mice.

Materials and methods: The study was approved by the Ethics Committee on the use of Animals in the Faculty of Medicine of Ribeirão Preto (42/2015). C57BL/6 wild type (WT) or knockouts for B1 receptor (B1−/−) and B2 (B2−/−) male mice were used for the experiments. Behavior tests were performed on the right hind leg of the animals. Evaluation of mechanical hyperalgesia was performed Von Frey filament testing, and cold sensitivity test was performed using acetone applied externally to the paw. Tests were evaluated before or after 2, 4 or 24 hours after administration of PCX (4 mg/kg). WT animals were pretreated with vehicle or captopril (5 mg/kg) 1 hour before administration of PCX, followed by the subsequent evaluation of the thermal and mechanical hyperalgesia 2 and 4 h after administration of PCX. Results: Animals treated with PCX developed mechanical and cold hypersensitivity. However, B1−/− and B2−/− mice had lower levels of hypersensitivity to mechanical and cold stimulation compared to WT mice. Additionally, pretreatment with captopril potentiated the hyperalgesia induced by PCX 2 h after treatment. Conclusion: Our results suggest that bradykinin contributes to the P-APS, and that inhibition of the B1 and B2 receptors could be an alternative to reduce this side effect. Notwithstanding, the use of captopril in patients who use paclitaxel possibly can increase the intensity of P-APS.
05.037 Analysis of astrocyte activation in the amygdala succeeding cfa-induced chronic tooth pulp inflammation in rats. Scalzilli PA, Freitas RDS, Costa KM, Filippini HF, Campos MM PUCRS

Introduction: It has been demonstrated that glial cells display a pivotal role in either inflammatory or neuropathic orofacial painful conditions, including tooth pulp pain (Chiang et al., Neuroscientist 17:303, 2011). Functional and anatomical alterations in both central and peripheral nervous system are likely involved in pain transmission related to tooth pulp inflammation (Zhang et al., Neuroscience 142:833, 2006; Adachi et al., Mol Pain 6:59, 2010), although the participation of specific brain regions remain to be elucidated. Our group recently demonstrated that chronic inflammation induced by complete Freund's adjuvant (CFA) application into the rat tooth pulp led to marked behavioral changes in the open-field arena, which was associated to an increased activation of GFAP-positive satellite cells in the trigeminal ganglion. Present study aimed at analyzing the astrocyte activation in an amygdala-rich region, after induction of long-term tooth pulp inflammation by CFA in rats. Methods: Male Wistar rats were used (N=6/group). The local Animal Ethics Committee (CEUA/PUCRS 13/00362) approved all the protocols. The pulps of the upper left first molars were surgically exposed with a 1/2 size drill in low-speed rotation, under irrigation. In the group A, rats without surgical pulp exposure were used as negative controls. In the group B, the pulps were left exposed to the oral cavity. For the group C, the pulps were exposed and the teeth were immediately sealed with temporary dental filling. In the group D, the pulps were exposed, and 0.2-ml of CFA were applied to the pulp tissue for 1 min, followed by dental sealing. At each time point (1, 2, 3 and 8 days after pulp exposure), a separate group of animals was euthanized. The ipsilateral amygdala-rich region was removed for immunohistochemistry analysis, to determine astrocyte GFAP-positive cells. The maxillae were collected for hematoxylin-eosin (H&E) staining, to confirm the induction of pulp inflammation. Results: The analysis of H&E-stained maxillae sections confirmed the induction of chronic pulpitis in the groups C and D, with a marked inflammatory response in the group D that received CFA. Pulp tissue underwent necrosis in the group B. The immunohistochemical analysis revealed similar numbers of GFAP-positive cells in all the experimental groups, according to evaluation of ipsilateral amygdala-rich region. The mean ± SEM values for GFAP-positive cells were: on the 1st day after pulp exposure, 17 ± 6 (A), 27 ± 9 (B), 41 ± 8 (C) and 20 ± 9; at the 2nd day, 24 ± 9 (A), 30 ± 5 (B), 50 ± 17 (C), 39 ± 8 (D); at the 3rd day, 31 ± 8 (A), 28 ± 6 (B), 40 ± 12 (C), 41 ± 9 (D); at the 8th day, 33 ± 16 (A), 31 ± 6 (B), 55 ± 13 (C), 31 ± 8 (D). Conclusion: Taken together, the present data suggest that nociceptive behavior alterations secondary to CFA-induced tooth pulp inflammation do not appear to rely on the activation of astrocytes in amygdala. Additional experiments are in progress to evaluate the astrocyte activation in other brain regions such as pre-frontal cortex, medullary dorsal horn and pons. Financial support: BPA/PUCRS, CAPES, CNPq, FINEP. Animal Research Ethics Committee/PUCRS: CEUA/PUCRS; protocol 13/00362.
05.038 Evaluation of Antinociceptive Activity of Methanolic Fractions of Sugarcane Juice (Saccharum officinarum L.) Soares MA¹, Silva NLC², Gomes ACC², Simas NK³, Kuster RM³, Miranda ALP¹, Tributino JLM¹ UFRJ, ²FAPERJ, ³IFRJ, ⁴UFES

Introduction: Different sugarcane (Saccharum officinarum L.) segments are popularly used for the treatment of pathological conditions such as anemia, infections, and hypertension. Some components identified in the sugarcane stalk juice are flavonoids derived from apigenin, luteolin and tricine, for which there are important biological actions described, such as anti-inflammatory activity. Furthermore, some flavonoids are described as ligand of opioid receptors, key pharmacological targets for analgesia. The methanol fraction of natural (SJ) and fermented (FSJ) sugarcane juice were obtained from reverse chromatography of the sugarcane stalks juice, variety SP71-1406, which has high concentration of flavonoids. These fractions are rich in tricin-7-O-(2''-α-L-rhamnopyranosyl)-α-D-galacturonicide, and our previous results showed antinociceptive activity through modulation of the opioid system. This effect was observed in mice who orally received the fractions at 100 mg/kg in neurogenic phase of formalin test, and it was reversed by naloxone. Considering the results showed by the fractions in nociception models and flavonoid interaction with opioid receptors, this study aims to evaluate the pharmacological profile of 30 and 300 mg/kg of SJ and FSJ in formalin assay. Given that opioids analgesics affect spontaneous locomotion in the open field test, we evaluate the action of SJ in this model, and the influence of the fractions on cell viability. Methods: the effects of orally administration of 30 and 300 mg/kg of SJ or FSJ were evaluated in nociception model induced by formalin 2,5% (i.pl.). For assessment of the locomotor activity, animals orally received morphine 10 mg/kg or SJ 100 mg/kg and were subjected to the open field test. Cellular viability was studied by the MTT assay, with the addition of 100 µg/mL of each fraction in cultured murine peritoneal macrophages. Results: both SJ and FSJ at 30 and 300 mg/kg showed no antinociceptive activity in the formalin test, observed only at 100 mg/kg. This dose reduced the number of squares crossed in the open field test by 37.8%, similar to that described for morphine. Macrophages treated with SJ and FSJ remained viable, equivalently to untreated cells. Conclusion: only the fractions at 100 mg/kg were effective in reducing nociception through modulation of the opioid system. SJ reduced locomotor activity of animals similarly to morphine, supporting the hypothesis that there is interaction of components of fractions with opioid system, possibly with low toxicity in cellular systems. As perspective of this work we will evaluate the isolated major flavonoid activity in formalin and open field tests. Financial support: FAPERJ, CAPES, PIBIC/UFRJ. Ethical committee approval:CEUA/UFRJ FARMACIA02.
Evaluation of antinociceptive activity of the essential oil of *Stevia Serrata*. Cordeiro MS¹, Simas DLR¹, Taracena E¹, Reyes MM², Wug MM², Oliva B², Martínez JV¹, Silva AJR¹, Fernandes PD¹, Giorno TBS¹ ¹UFRJ, ²Universidad de San Carlos de Guatemala

**Introduction:** *Stevia serrata* is a plant from Asteraceae family that grows in Central America and Mexico and in northern South America (1). The aim of this study was to evaluate the antinociceptive activity of the essential oil (EO) from *Stevia serrata*. **Methods:** EO was obtained by hydrodistillation using a Clevenger-type apparatus for 2 h. Swiss Webster mice (20-25g, n=6) were orally pretreated with 10, 30 or 100 mg/kg doses and evaluated in formalin-, capsaicin- or glutamate-induced licking response and hot plate test. One hour after treatment mice received intraplantar injection (20 μL) of formalin (2.5%), capsaicin (1.6μg/paw) or glutamate (3.7ng/paw). The time (in seconds, sec) that animal spent licking the injected paw was counted with a stopwatch during the 1st 5 minutes (min) (1st phase) and between 15 and 30 min (2nd phase), during 5 min or during 15 min after formalin, capsaicin or glutamate injection, respectively. In hot plate test, animals were placed on a plate (Insight Equipment, Brazil) set at 55±1°C. At 30 min intervals between 30 and 180 min, after oral administration of EO or vehicle the reaction time was recorded when the animals licked their fore- and hind-paws and jumped. Antinociception was calculated by the area under the curve (AUC) of responses between 30 and 180 min after drug administration. **Results:** EO significantly reduced 1st and 2nd phases of formalin-induced licking, 1st phase: vehicle-treated group= 42.4±7.7 sec versus 23±10.4 sec (45.7%); 18.1±3.6 sec (57.3%) and 16.2±7.1 sec (61.9%) to 10, 30 and 100 mg/kg, respectively; and 2nd phase: vehicle-treated group=224.7±25.7 sec versus 195.5±16.6 (12.1%); 127.7±31.3 sec (43.2%) and 77.6±26.3 sec (65.5%) to 10, 30 and 100 mg/kg, respectively. Higher doses of EO also reduced capsaicin-induced licking: vehicle-treated group=57.8±7.8 sec versus 41.6±9.9 sec (28%); 25±10.2 sec (56.8%) and 18.1±6.2 sec (68.7%). Only 100mg/kg dose reduced glutamate-induced licking: vehicle-treated group=77.9±27.6 sec versus 26.9±12.6 (65.5%); EO also demonstrated significant effect at the hot plate model. Doses of EO significantly increased the AUC values when compared with vehicle-treated group. Vehicle-treated group=986.5±193.1; 10mg/kg=1,725.5±370 (74.9% increase); 30mg/kg=1,229.5±152.2 (24.6% increase); 100 mg/kg=1,868.5±339.1 (89.4% increase).

**Conclusions:** Our results are the first evidence that EO from *S. serrata* produces peripheral and central antinociceptive effects. The mechanism of antinociception and antinociceptive pathways involved in *S. serrata* effect are under investigation. 1. QUATTROCCHI, U. 2012. CRC World Dictionary of Medicinal and Poisonous Plants, 5: 594, 2012. Acknowledgements: Alan Minho for technical assistance, Institute Vital Brazil (Niterói, Brazil) for donation of mice. **Financial support:** CAPES, CNPq and FAPERJ. Ethics committee of the CAUAP/UFRJ protocol number:DFBCICB015-04/16.
05.040 Differential contribution of TRP channels in antinociceptive and nociceptive effects of jambu. Dallazen JL, Maria-Ferreira D, Nascimento AM, Cipriani TR, de Souza LM, Geppetti P, Werner MF, UFPR. Farmacologia, UFPR. Bioquímica e Biologia Molecular, Instituto de Pesquisa Pelé Pequeno Principe, Universidade de Florença

Introduction: Acmella oleracea popularly known as "jambu", is used in Amazon culinary and in folk medicine to treat toothache, causing numbing or tingling sensations on the mouth. The anesthetic properties from the yellow flower heads of jambu are attributed to alkylamides, mainly spilanthol. Thus, our aim is investigate, in pain models, the involvement of TRP channels in jambu effects. Methods: Hexanic Fraction (HF) was obtained from jambu flowers and through phytochemical analysis was detected several alkylamides, including spilanthol. Male Swiss mice (~30 g) received intraplantar injections of HF (0.01; 0.03; 0.1; 0.3; 1; 3; 10; 30 and 100 μg/20 μL) and the time that mice spent licking and guarding the hindpaw were recorded (s). A synthetic isobutylalkenyl amide (IBA) was used as control. Antinociceptive effect of local injection of HF and IBA (0.1 and 30 μg/20μL, i.pl.) were evaluated 15 min prior 20μL i.pl injection of 2.5% formalin, 1 nmol capsaicin (TRPV1 agonist) or 200 nmol cinnamaldehyde (TRPA1 agonist). We also evaluated the prior treatment with the TRPV1 antagonist capsazepine (10 nmol/20μL, i.pl., 15 min) and the TRPA1 antagonist HC-030031 (100 mg/kg, i.p, 30 min) on nociceptive behavior induced by i.pl. injection of 30 μg/20μL of HF and IBA. To further explore the role of TRP-positive sensory fibres, mice received resiniferatoxin (RTX, 50 μg/kg, s.c.) 7 days prior i.pl. injection of HF and IBA (30 μg/20 μL), and desensitisation was confirmed by the reduction of the number of eye wipes induced by 0.1% capsaicin instillation (CEUA/BIO-UFPR; 970). Results: As observed with IBA (30 μg), only higher doses of HF (3-100 μg) induces nociceptive-like behaviors 5 min after injection (HF 0.1: 1.0 ± 0.7 s and 30 μg: 46.0 ± 6.0 s). In the formalin test, HF and IBA (0.1 μg) reduced the first phase by 39 and 45% (C: 102.6 ± 2.0 s), and second phase by 79 and 77%, respectively, whereas at 30 μg HF partially reduced in 29% and IBA blocked in 91% the second phase of formalina (C: 175.2 ± 6.5). HF and IBA (0.1 μg) significantly reduced nociception induced by capsaicin in 67 and 70%, respectively (C: 50.0 ± 5.0 s). However, HF and IBA (0.1 μg) had opposite effects on cinnamaldehyde-induced nociception, potentiating the nociceptive response in 203 and 95%, respectively (C: 56.0 ± 8.2 s). Interestingly, nociceptive behavior induced by HF 30 μg was unchanged by capsazepine (63.0 ± 6.0 s), being reduced only by HC-030031 in 56% (C: 61.0 ± 4.0 s). In comparison, both capsazepine and HC-030031 significantly decreased the nociceptive response induced by IBA 30 μg in 33 and 87%, respectively (C: 56.0 ±10.0 s). Finally, the desensitization with RTX largely reduced the nociceptive behavior induced by 30 μg of HF and IBA in 81 and 63%, respectively.Conclusion: Our data is the first to try explain the complex analgesic and aversive sensations related to medicinal folk use of jambu. We hypothesized that HF could acts as antagonist via TRPV1 and as agonist via TRPA1 channels, and that differences with IBA may due to different alkylamides, such as spilanthol. Further studies will be conducted for understanding the HF and spilanthol effects.
05.041 Dimetil fumarate treatment failed to reduce hyperalgesia in a model of HIV-related neuropathy. Ferreira AM, Luckenmeyer DD, Tonello R, Prudente AS, Ferreira J UFSC — Farmacologia

Introduction: A distal polyneuropathy is the most common neurologic manifestations associated with HIV infection. With the global introduction of the antiretroviral therapy, the long-term survival with HIV infection has improved dramatically, but it brought within a higher incidence of the HIV-associated sensory neuropathy (HIV-SN). HIV-SN is associated with the use of some antiretrovirals (such as stavudine) and with the presence of viral proteins at spinal cord, immune activation and oxidative stress. The pain arising from HIV-SN is very difficult to manage, and the drugs commonly used for neuropathic pain are usually not effective. Ziconotide has its intrathecal use approved in patients with HIV-associated pain, but produces serious adverse effects. Thus, novel effective and safe treatments for neuropathic pain are urgently needed. Drug repositioning, the identification and development of new uses for existing drugs, could be an interesting approach to achieve this goal. The immune modulator and antioxidant dimethyl fumarate (DMF) is effective in treating multiple sclerosis and psoriasis and also could have a potential application in HIV-SN. Thus, the goal of our study is detect the possible analgesic-like effect of DMF in model of HIV-SN in mice. Methods: Female C57Bl/6-UFSC mice were used and the experiments followed the ARRIVE guideline. To induce HIV-SN, mice were injected with the HIV envelop protein gp120 (100 ng/site, intrathecal-i.t., three injection on days 0, 3 and 6) and with the antiretroviral drug stavudine (50 mg/Kg, intravenous, two injection on days 0 and 4). The control group received the same injections of boiled gp120 plus the stavudine vehicle (saline). Before and several days after treatment, mice were subjected to the von Frey test to detect the hindpaw withdrawal thresholds (g). A reduction of the threshold greater than 50% was considered as hyperalgesia. As a positive control, a different group of animals were treated once with ziconotide (100 pmol/site, i.t.). Moreover, another group of animals were treated with DMF (15 mg/kg, orally, twice a day for 7 days) or vehicle. Hyperalgesia was detected several times after ziconotide or DMF injection. Results: When compared to boiled gp120+vehicle, the animals injected with gp120+stavudine developed a marked hyperalgesia, which as detected as early as 3 days, peaked at 13 days (thresholds of 0.69±0.07 and 0.04±0.01 g, respectively) and lasted up to 28 days after the first injection of the drugs. Thus, the drug treatment was carried out 13 days after the first injection of gp120+stavudine. As expected, ziconotide administration almost fully reversed gp120+stavudine-induced hyperalgesia, from 0.5 (thresholds of 0.03±0.01 and 0.70±0.05 g, for vehicle or ziconotide, respectively) to 4 hours after its injection. On the other hand, DMF treatment was not able to significantly alter gp120+stavudine-induced hyperalgesia, even after 7 days of treatment (thresholds of 0.28±0.11 and 0.49±0.20 g for vehicle and DMF treated animals). Conclusion: Despite the model of HIV-SN induced by gp120+stavudine in mice has predictive validity to detect analgesic-like drugs, DMF treatment seems not have an potential to treat the pain related to HIV-SN. Acknowledgments: Financial support by CNPq and CAPES. The project was approved by the Ethics Committee on Animal Use of UFSC (117/CEUA/PROPESQ/2013).
05.042 The role of the transient receptor potential vanilloid-1 in the induction of herpetic neuralgia. Pereira JA¹, Silva CR¹,², Cunha TM¹ ¹USP – Farmacologia e Inflamação, ²UFU – Genética e Bioquímica

Introduction: Herpetic neuralgia (HN) is characterized by a painful vesicular rash resulting from Varicella Zoster virus (VZV) reactivation in dorsal root ganglia (DRGs) or the cranial nerves, decades after the primary infection. In rodents, the infection with herpes simplex virus type-1 (HSV-1) is used as the animal model of HN. HSV-1 (and VZV) is able to reach the sensory neurons cell body, which also appears to have an important role during the infection. Interestingly, a subpopulation of these sensory neurons, responsible for pain transmission, express the Transient Receptor Potential V1 (TRPV1). TRPV1 activation is involved in inflammatory and neuropathic pain modulation, and TRPV1 antagonism shows as a promise analgesic tool against different forms of acute and chronic pain. Although some studies suggest a possible involvement of TRPV1 receptors in herpetic neuralgia, little progress has been achieved in order to determine the mechanisms involved in the induction and maintenance of these painful conditions. Aim: The aim of this study is to verify the possible role of TRPV1 in pain caused by HSV-1 infection. Methods: Adult male Wild-type (WT), C57BL/6, and TRPV1KO mice (18-25g) were anesthetized and the right midflank depilated for inoculation with HSV-1 (1x10⁶ plaque-forming units/20μl) for HN induction. Articular nociception (Weight Bearing - incapacitance and acetone - cold allodynia) were analyzed from 1-14 days post-infection (dpi) and compared with non-infected and naive animals. Levels of TRPV1 gene expression on DRGs were determined by RT-PCR in WT infected animals different days after HSV-1 infection. Results: Compared with WT HSV-1 infected animals, TRPV1KO-infected animals had significative less pain from 7-14 dpi, when incapacitance was observed, and from 5-14 dpi, when cold allodynia was analyzed. Additionally, there was a decrease in TRPV1 RNA levels on DRGs 7-21 dpi. Conclusions: TRPV1 activation seems to be involved in herpetic neuralgia induction and could be a target for the development of new treatments for this painful condition. However, more studies are required to clarify the mechanisms involved in these effects. Research support: This study is supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP). Research approval by the Human or Animal Research Ethical Committee: 208/2014
**05.043 Ethanol extract of Leonurus sibiricus reduces oxidative stress and nociception.**

Santos-Oliveira A, Cercato LM, Santana MT, Biano LS, Melo AJO, Duarte MC, Silva AM, Camargo EA ¹ UFS – Fisiologia, ²UFS – Farmácia, ³UFS – Nutrição

Inflammation is a limiting condition mainly because of pain, a common complaint reported by patients affected by many types of inflammatory conditions. The production of reactive oxygen species can be an important feature in this process. The great need for new compounds to treat this condition is imminent. Medicinal plants represent a viable alternative for this purpose. *Leonurus sibiricus* L. is a plant traditionally used to treat inflammation and scarce studies have described the effect of this plant. This study aimed to investigate the antioxidant and antinociceptive effects of the ethanol extract of arterial parts of *L. sibiricus* (EELs). Total phenolic and total flavonoid contents, as well as the antioxidant capacity by elimination of DPPH radicals, inhibition of lipid peroxidation and the ferric reducing antioxidant power (FRAP) were measured for EELs (in triplicates). Formalin (1%)-induced nociception in the mouse paw was performed in male Swiss mice (20-30 g, n = 8/group) which were treated with vehicle (saline solution 0.9% + Tween 80 0.2%), EELs (100, 200 and 400 mg/kg, v.o.), AAS (300 mg/kg, i.p.) or morphine (3 mg/kg, i.p.). Results were expressed as mean ± SEM, and analyzed by ANOVA followed by Tukey’s test. P values <0.05 were considered as significant. The experiments were performed after the approval of the protocol by the Institutional Ethics Committee (CEPA/UFS 28/16). Total phenol and total flavonoid contents in the extract were 60.1 ± 0.1 mg/g of gallic acid equivalents and 15.4 ± 0.1 mg/g of quercetin equivalents, respectively. A significant decrease of DPPH radical (relative to the reaction system) was observed at concentrations of 50, 100, 250 and 500 µg/mL of EELs (8.9 ± 0.7, 18.3 ± 0.2, 46.2 ± 0.2 and 64.2 ± 0.8% respectively). Trolox (100 µg/mL) also produced antioxidant capacity as it reduced (p<0.001) the DPPH radical (77.5 ± 3.5%). A significant increase in the reducing potential was detected through FRAP assay for the concentrations of 50, 100, 250 and 500 µg/mL of EELs (52.7 ± 9.4, 103.4 ± 5.3, 236.7 ± 1.1 and 431.2 ± 3.4 ferrous sulfate equivalent (FSE) respectively). Trolox (100 µg/mL) also increased (p<0.05) this parameter (721.4 ± 1.4 FSE). The basal lipid peroxidation was inhibited at 100, 250, 500, 1000 and 1500 µg/mL of EELs (16.4 ± 0.8, 35.0 ± 0.7, 48.0 ± 2.4, 70.6 ± 0.5 and 84.0 ± 0.7% respectively). These concentrations of EELs also inhibited the lipid peroxidation induced by FeSO₄ (19.1 ± 1.9, 29.8 ± 0.8, 33.1 ± 1.0, 46.7 ± 1.3 and 52.3 ± 0.6% respectively). Trolox (100 µg/mL) also reduced (p<0.05) the basal and induced lipid peroxidation (55.9 ± 1.7 and 42.6 ± 0.6% respectively). In the formalin-induced nociception in mouse paw, EELs (400 mg/kg) inhibited (p<0.05) licking/biting time in the second phase (34.0 ± 22.2 s) in comparison to the group of animals pretreated with vehicle (169.0 ± 14.6 s). As expected, morphine and aspirin also reduced this parameter (16.3 ± 7.7 and 34.0 ± 22.3 s; p<0.05). Morphine, but not EELs and aspirin, decreased the licking/biting time in the first phase of formalin test. The present study showed that EELs possesses antinociceptive properties, by possible inhibition of inflammatory mediators production, and antioxidant activity, mainly by neutralizing free radicals and inhibiting lipid peroxidation.
05.044 Subcutaneous injection of IFN-β causes pain-like behaviors and edema in mice.
Silva ML, Tonello R, Ferreira J UFSC

Introduction: IFN-β is a cytokine used by subcutaneous or intramuscular route for the treatment of multiple sclerosis. One of the most common adverse effects of IFN-β therapy are the injection-site reactions (ISR, specially burning or stabbing pain, erythema and edema), which may affect the treatment adherence and reduce the patients quality of life. The precise causes of ISR still remain unknown, hindering the enhancement of treatment adherence and patient satisfaction. Since there is no pre-clinical model for the study of ISR mechanisms, we aimed to standardize a model of IFN-β-induced ISR in mice. Methods: Male C57Bl/6-UFSC mice were used and the experiments followed the ARRIVE guidelines. Mice were subcutaneously (s.c.) injected into the right hind paw with murine IFN-β (1x10³, 3.2x10² or 1x10³ IU/site), appropriate vehicle or saline (10 μL/site). Before and after treatment, mice were subjected to behavioral nociceptive evaluation verifying spontaneous guarding behavior score (sGB), paw withdrawal threshold using von Frey test (VFT), evoked guarding behavior (VFGB), acetone drop test (ADT), and the Hargreaves test (HT). Injected paw thickness was also measured as an index of edema. Results: The highest tested dose of IFN-β (1x10³ IU/site) induced rapid and long-lasting nociceptive behavior, 0.5-24h post-injection, in all nociceptive parameters evaluated as well as edema when compared to vehicle. The peak effect was usually 2 h after injection when compared to vehicle: sGB (1.0±0.26 vs 1.7±0.3); VFT (0.53±0.19 vs. 0.018±0.004 g); VFGB (2.3±0.6 vs 6.7±0.2); ADT (1.14±0.1 vs 2.6±0.2 s); HT (73.3±4 vs 44.9±2%). Compared to vehicle, the intermediary dose used (3.2x10² IU/site) produced nociception only in VFT (0.53±0.19 vs 0.018±0.004 g), VFGB (2.3±0.6 vs 5.8±0.37) and HT (73±4 vs 45.7±5.4%), without causing edema. The lowest dose tested (1x10² IU/site) was just able to elicit a significant increase in VFGB (2.3±0.6 vs 4.0±0.5). Excluding a possible vehicle influence in the effects observed, no significant differences between vehicle and saline were found for the evaluated parameters. Conclusion: Our results demonstrate that IFN-β can cause rapid and long-lasting edema, spontaneous nociception as well as mechanical, heat and cold hyperalgesia, suggesting that our model might be useful to study the mechanisms related to IFN-β_ISR. Financial support: CAPES, CNPq and UFSC. Protocols approved by CEUA/UFSC (protocol nº: 00872).
05.045 Muscle hypoalgesia induced by chronic exercise is dependent of peripheral PPARy receptors. de Azambuja G1, Botasso-Gomez B1, Messias LHD2, Manchado-Gobatto FB2, Oliveira-Fusaro MCG1 1LABEDI-Unicamp 2LAFAE-Unicamp

Introduction: Exercise is an effective non-pharmacological treatment to muscle pain. It is well known that exercise increases the expression of peroxisome proliferator-activated receptor gamma (PPARγ) receptors in skeletal muscle. PPAR family of nuclear receptors plays a major regulatory role in energy homeostasis and metabolic function. Recently, PPARγ receptors are emerging as a promising target for the treatment of painful process, since PPARγ agonists suppress inflammatory and neuropathic pain. Therefore, we hypothesized that peripheral PPARγ receptors were activated to produce hypoalgesia in response to chronic exercise.

Methods: Male Wistar rats were used and methods were approved by the Ethics Committee in Animal Research of the UNICAMP. Two swimming protocols were used: classical (loads at 4% of body weight) or individualized (loads at 80% of the Maximum Lactate Steady State (MLSS)). The animals included in exercise groups were submitted to a water adaptation protocol and the MLSS test. The exercise protocol consisted of 40 min. of swimming, 5 days a week, during 10 weeks. After 5 weeks of exercise, the loads of the individualized group were adjusted according to a new MLSS test. Every 2 weeks of exercise, the nociceptive threshold of rat's gastrocnemius muscle was performed by the analgesimeter Randall Selitto. To test the hypoalgesia induced by exercise, 72 hours after the end of both protocols of chronic exercise, carrageenan was injected into gastrocnemius muscle of rats. Three hours later, the mechanical muscle hyperalgesia was quantified by the analgesimeter. To investigate the contribution of PPARγ receptors to exercise-induced hypoalgesia, GW9662, a selective PPARγ antagonist, was injected into gastrocnemius muscle 30 min. before carrageenan. Results: Carrageenan (100µg) induced muscle hypoalgesia in rats submitted to classical or individualized swimming protocol (p<0.05, Tukey test) when compared to control group (without exercise). Pretreatment with GW9662 (9ng, but not 3 and 6ng) reversed, in a dose dependent manner, the exercise-induced hypoalgesia in both protocols when injected in the ipsilateral (p<0.05, Tukey test) but not in the contralateral muscle (p>0.05). GW9662 didn’t alter the nociceptive threshold when injected alone (p>0.05, T test). Conclusions: These data demonstrated that classical and individualized protocols of chronic exercise induce muscle hypoalgesia and this effect is dependent on activation of peripheral PPARγ receptors. We speculate that exercise increases the expression of PPARγ receptors and their activation contributes to exercise-induced muscle hypoalgesia. Therefore, activation of PPARγ receptors by indirect mechanisms, as chronic exercises, could be a target to control the muscle pain experienced by patients with chronic musculoskeletal pain. Financial support: São Paulo Research Foundation – FAPESP (2015/20738-0); Animal Research Ethical Committee (protocol number 3869-1).
05.046 Muscle hypoalgesia induced by chronic exercise is dependent of peripheral PPARγ receptors. 
Almeida D¹, Freitas Lima LC, Valadares WCP, Quintão JL², Silva JF³, Romero TRL², Santos SHS ¹ICB-UFMG - Fisiologia e Farmacologia, ²ICB-UFMG - Farmacologia, ³ICB-UFMG - Fisiologia e Biofísica

Introduction: Diabetic Neuropathy is a chronic complication associated with late stages of Diabetes Mellitus and is the cause of morbidity and mortality in patients that suffer from this condition. Painful symptoms are associated with alterations in conduction properties of the nerves, especially in C fibers that conduct nociceptive stimuli, and these alterations have an inflammatory and oxidative stress basis due to high glycemia levels in obese diabetic mice. Endogenous pain modulation systems are involved in processing of pain stimuli that ascends from the periphery, and these systems work in a central level and in a peripheral level. The aim of this study was to evaluate the role of the cannabinoid and opioid systems in this modulation.

Methods: This study was sent to the CEUA/UFMG-ICB ethics committee under the protocol number of 0045/2016. Two groups, Normal Diet (n=8) and High Fat Diet (n=8) fed mice; Confirmation of the Type II Diabetes like phenotype by the Intraperitoneal Glucose Tolerance and Insulin Resistance Tests (n=8); Randall and Sellito mechanical threshold test (n=8); Intraplantar drug injection (AM251 - 50, 100, 200 µg (n=4); AM630 - 50, 100, 200 µg (n=4); JZL184 - 10, 20, 40 µg (n=4); Bestatin - 100, 200, 400 µg (n=4); Naloxone - 25, 50, 100 µg (n=4); Clocinamox - 20, 40, 80 µg (n=4); Naltrindole - 30, 60, 120 µg (n=4); NORBNI - 50, 100, 200 µg (n=4)); Western Blott analysis of CB1 and CB2 cannabinoid receptors; µ, κ, and δ opioid receptors; total and phospho p38 (n=4).

Results: High fat diet was able to increase body weight, induce glucose tolerance and insulin resistance in mice. After 12 weeks of treatment with the diet, mice started showing hyperalgesia confirmed by the reduction in the nociceptive threshold in the Randall and Sellito test. The endogenous pain modulation was accessed by antagonism of cannabinoid and opioid systems in the 14th week. AM630 was able to reduce the nociceptive threshold in obese mice but not AM251, suggesting the participation of the CB2 cannabinoid receptor in this model of neuropathic pain. JZL induced antihyperalgesia with the three doses used. The antagonism of opioid receptors showed similar responses in control and in obese mice. Higher doses of all the antagonists in this study induced decreases in the nociceptive threshold in both groups. Bestatin induced antihyperalgesia in control and in obese mice. Western blott evaluation of cannabinoid CB1 expression in the footpad showed reduced expression of this receptor in obese mice. Only µ opioid receptor showed reduction in its expression in obese mice, while δ and κ opioid receptors did not show significant alterations. Total p38 and p38 phosphorylation was not significantly altered in obese mice. Conclusion: Activation of the cannabinoid and opioid systems are involved in positive pain modulation in a model of diabetic neuropathy by high fat diet feeding in mice. The hyperalgesia observed in obese mice might be caused by a reduction in CB1 cannabinoid receptor and in µ opioid receptor expression. Financial Support: FAPEMIG (PPM-00474-15), CAPES, CNPq.
05.047 Exercise does not reverse the hyperalgesia induced by neonatal morphine exposure, instead it decreases the nociceptive threshold in naïve rats. Freitas JS, Nunes EA, Macedo IC, Kuo J, Rozisky JR, Medeiros LF, Caumo W, Torres ILS UFRGS – Pharmacology of Pain and Neuromodulation: Pre-Clinical Investigations

**Introduction:** The administration of analgesics has increased in the Neonatal Intensive Care Unit (NICU) over the past decades as a result of changes and advances in the understanding, identification and treatment of pain in neonates. Studies in animals have shown that exposure of infants to noxious stimuli and / or pharmacologic manipulation may induce behavioral changes in nociceptive and long term may trigger a hyperalgesic response. Additionally, clinical studies and preclinical demonstrate that aerobic exercise provides relief of pain related to opioid system, a phenomenon called exercise-induced-analgesia. **Methods:** 64 Male Wistar rats of 7 days (P7) were divided into 4 groups: control, exercise, morphine and morphine+exercise. The groups received saline or morphine 5µg, midi-scapular, P8 to P14. The nociceptive response was assessed at baseline, 1 hour and 24 after exercise in hot plate test (thermal threshold) and von Frey (mechanical allodynia). At P30 and P60 the animals were subjected to a single exercise session in the treadmill speed 12m/min for 20 minutes. The data were analyzed by repeated measures ANOVA / SNK and was considered significant with P<0.05. **Results:** In the hot plate test was interaction between time and group at P30 and P60 (F(3,28) = 12.363 P <0.001 and F(6,54) = 3.345 P <0.005, respectively, n = 8). In the von Frey test was interaction between time and group at P60 (F(3,27) = 10,542 P <0.001, n = 8), but it not at P30 (F(1,23) = 1.406 P>0.05, n = 8). Conclusion: Our results corroborated previous data of research group that showed hyperalgesic mechanical and thermal response in medium (P30) and long term (P60) induced by neonatal exposure to morphine. Also we showed that a single exercise session was not effective in reversing the hyperalgesia induced by morphine, but decreased the nociceptive threshold in the control animals. We can suggested that a single exposure to exercise induces the liberation pro-nociceptive mediators that activate primary afferent neurons directly or indirectly enhancing the nociceptive signal transmission to the central nervous system, such as glutamate, cytokines and tropic factors. **Financial support:** FIFE/HCPA, PROPESQ/UFRGS, CNPq, FAPERGS, CAPES. This project was approved by Ethics Committee on Animal Use in the Hospital de Clínicas de Porto Alegre (CEUA- HCPA)/ accepted protocol project: No. 140425.
05.048 Pharmacological standardization of hypersensitivity response induced by *Physalia physalys*’ venom (MLU_080047) in mice. M Anjos, da Silva, GF, Quintão NLM

Univali – Ciências Farmacêuticas

Thousands of cases involving stingrays and jellyfish accidents are reported per year. The number increased 2.000 % in Santa Catarina only in 2015. Cnidarian-induced injury implicates not only in health problems but also compromise the local tourism. The most common symptoms experienced after the contact with a jellyfish involves high-intensity pain local pain, burning sensation, red spots on the skin and, in some cases, nausea, vomiting, blood pressure decreasing, headache and breathing difficulties. Any specific treatment has been reported for this kind of tissue injury. This study had the aim of evaluating the hypersensitivity response induced by MLU_080047 (*Physalia physalys’* venom) in mice. We used C57BL/6 male mice (2 months old, 25-30 g; N=6-10). All experiments were approved by the Ethics Committee for the Use of Laboratory Animals from UNIVALI (protocol number = 011/14). Mice were injected with different doses of MLU_080047 or vehicle (PBS; 10 μL/paw) into the plantar surface of right hindpaw. Then, the mechanical hypersensitivity was evaluated at different times after the venom injection. In all experiments, the animals were habituated to the apparatus for 15 days before the test in a reduced visible-light room. The basal withdrawal threshold response was determined 24 h before the test using the von Frey filaments (up and down method). There was no evidence of spontaneous nociception (licking or biting the injected paw). The mechanism involved in the MLU_080047-induced hypersensitivity was investigated using the dose of 0.01 ng/paw (which presented the highest hypersensitivity index). For this purpose, mice were previously treated with indomethacin [100 μg/paw; i.pl.; non-selective cyclooxygenase (COX)1/2 inhibitor] or dexamethasone (0.5 mg/kg, s.c., corticosteroid). Animals injected with *P. physalis’* venom presented mechanical hypersensitivity at doses of 0.01, 0.03 and 0.1 ng/paw, with 245.1 ± 62.2%, 202.2 ± 19.8% and 188.9 ± 47.1% of enhance in hypersensitivity, respectively. This mechanism sensitization seems to involve de novo synthesis of chemical mediators important for the maintenance of hypersensitivity, once the treatment with dexamethasone reduced significantly the painful-behavior (21.4% ± 7.2%) observed after 3 h of experiment. No effect was observed with the treatment with indomethacin, suggesting that the COX products do not interfere with the neuron sensitization induced *P. physalis’* venom. **Financial Support:** CNPq, VRPPEC/UNIVALI, CAPES.
**05.049 Fish oil concentrate treatment alleviates neuropathic pain behavior in mice after peripheral nerve injury**

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**Introduction:** Neuropathic pain is a multifactorial condition arising from injury or malfunction of peripheral or central nervous system (TREEDÉ, Neurology, 18, 1630, 2008). Neuroinflammation initially established in peripheral nerve injury-induced neuropathic pain is a crucial process to its physiopathology. Neuro-immune interaction drives the process of peripheral and central sensitization, essential phenomena in neuropathic pain (Jl. Nat Rev Drug Discov, 13(7), 533-48, 2014). Omega 3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are well known for their anti-inflammatory activity displayed by their endogenous conversion to resolvins and protectins as lipid mediators (SERHAN, J Exp Med., 196, 1025, 2002). This study aimed to investigate the therapeutic potential of fish oil concentrate (FOC), rich in EPA and DHA, in a model of neuropathic pain induce by peripheral nerve injury. **Methodology:** Swiss mice were submitted to a model of neuropathic pain induced by partial sciatic nerve ligation (PSNL) (SLETZ, Pain, 43, 205, 1990). Briefly, anesthetized animals underwent a procedure allowing a ligation from 1/2 to 1/3 of their sciatic nerve on the left paw. Before and after surgery thermal (Hargreaves test) and mechanical (von Frey hairs) stimuli assessed paw withdrawal threshold. Two treatment protocols were assessed: 1) Animals received daily oral treatment for 5 days beginning 5 days after surgery (Therapeutic protocol); 2) Or received daily oral treatment for 10 days beginning on the day of the surgery (Preventive protocol). The treatment was performed with vehicle (gum arabic 5%), FOC (2.3 or 4.6 g/kg) or Gabapentin (100 mg/kg). Thermal or mechanical hypersensitivity was assessed on 5th, 7th and 9th day after nerve injury. On 9th day animals were euthanized and dorsal root ganglia (DRG), spinal cord and sciatic nerve were collected for further analysis. ATF-3 expression in dorsal root ganglia (DRG) was quantified by western blot. TNF production was measured in spinal cord. MPO activity was evaluated on sciatic nerve samples. All animal experiments were approved by the animal ethics committee (CEUA-UFRJ), protocol nº 011/16. **Results:** FOC treatment, at the highest dose, reversed mechanical allodynia and thermal hypernociception when administered for 5 days, following the therapeutic protocol. Similarly, FOC treatment, at the dose of 2.3 mg/Kg, also prevented neuropathic pain establishment with reduced allodynia and hypernocicepition when animals were the animal were submitted to preventive protocol. TNF levels in spinal cord were diminished by FOC treatment in both cases. However, the difference was statistically significant only for the lower dose (2.3 g/kg) with preventive treatment protocol, suggesting reduced microglial activation. ATF-3 expression diminished in the DRG of animals under the preventive treatment protocol with FOC, indicating reduced neuronal activation. MPO activity showed a slight but not statistically significant reduction on sciatic nerve for both treatments protocols. **Conclusion:** FOC oral treatment reverses and prevents the development of mechanical and thermal hypersensitivity after peripheral nerve injury. The molecular mechanisms involved seem to be related to reduction of neuroinflammation and neuronal activation. Furthermore, FOC arises as a safe and efficacious therapeutic alternative for the treatment of neuropathic pain.
05.050 Evaluation of antinociceptive effect of coumarins umbelliferone and mammeisine in mice. Vieira L¹, Saldanha AA¹, Pedro LP¹, Melo CM¹, Marcondes HC², Taylor JG², Araújo MGF¹, Souza ACS¹ ¹UFSJ- Centro-Oeste, ²UFOP

**Introduction:** Mammeisine is a 4-phenylcoumarin present in some plant species of genera Kielmeyera¹ and umbelliferone (UMB), a derivative of coumarin (7-hydroxycoumarin), is a benzopyrone in nature and it is present in the edible fruits like apple and orange. Plant derived phenolic coumarins might play an important role in dietary due their consumption in the human diet in fruits and vegetables². Antinociceptive activity has been described about plants rich in coumarins³ and besides the coumarins have demonstrated several biological properties, such as antifungal, antibacterial, and anti-inflammatory activities³.⁴ Methods: Writhing test was performed to screening the antinociceptive effects of mammeisine and UMB. Swiss mice (n=7, 28-30 g) were treated orally with compounds at different concentration (1, 10, 50, 100 and 200 mg/kg) 60 min before acetic acid (1%) intra-peritoneal injection. Acetilsalicilic acid (ASA, 200 mg/kg) was used as positive control. The number of abdominal muscle contractions, which occurred between 10 and 30 min after chemical stimulus, was registered⁵. The results were expressed in mean value and the analyses were performed using the software GraphPad Prism 5.01 software (San Diego, CA, USA). Effects were considered significant at level of p<0.05.

**Results:** The mammeisine significantly inhibited the writhing with the percent inhibition value 53.45% and 57.76% at the dose of 100 and 200 mg/kg, respectively. The UMB produced a significant dose-related inhibition of the abdominal constrictions (37.18%, 35.74%, 48.01% and 37.18% at dose of 10, 50, 100 and 200 mg/kg respectively). Conclusion: The discovery of the new active compounds to treat the pain conditions, with less adverse reaction, is constantly under debate and research. In summary, our results showed that mammeisine and UMB possesses similar analgesic effect of ASA, which appear to be related to COX inhibition.

05.051 Involvement of muscarinic receptors, opioid system/K\textsuperscript{+}\text{ATP} and L-arginine/NO/cGMP pathway in the isopulegol acute antinociceptive effect in mice. Próspero DFA\textsuperscript{1}, Piaulillo CA\textsuperscript{1}, Libâno LL\textsuperscript{1}, Fontenele RV\textsuperscript{1}, Reis Filho AC\textsuperscript{1}, Alcântara AEL\textsuperscript{1}, Lopes EM\textsuperscript{2}, Sousa DP\textsuperscript{2}, Ameida FRC\textsuperscript{1} \textsuperscript{1}UFPI – Bioquímica e Farmacologia, \textsuperscript{2}UFPB – Ciências Farmacêuticas

Introduction: The ISO-isopulegol is a monoterpenic alcohol, present in essential oils of various herbs. The monoterpenes usually have antinociceptive property. Studies have shown that the ISO-isopulegol has anxiolytic activity, antiepileptic, gastroprotective and antioxidant in rodents. The objective of this study was to evaluate the antinociceptive effect of the ISO and possible mechanisms involved in rodents. Methods: Female and male Swiss mice (n=5-7, 20-30g) were treated with ISO (0.78; 1.56; 3.12; 6.25; 12.5 and 25 mg/kg, p.o.) to formalin test, ISO (0.78 to 12.5 mg/kg, p.o.) to capsaicin test and ISO (1.56-6.25 mg/kg, p.o.) to glutamate test, and other groups received vehicle (5% Tween 80 in 0.9% NaCl) or morphine (5 mg/kg, s.c.), 60 and 30 min before the stimuli. The right hind paw was injected with formalin 2% (20 μL); capsaicin (2 μg/20 μL) or glutamate (20 μmol). Nociception was evaluated by quantifying paw licking time after formalin (0-5 e 15–30 min), capsaicin (5 min) and glutamate (15 min). To investigate some mechanisms of action in the glutamate test, the animals were pretreated i.p. (20 or 15 min) before ISO (6.25 mg/kg), with naloxone (2 mg/kg); glibenclamide (3 mg/kg); atropine (1 mg/kg); L-arginine (600 mg/kg) and methylene blue (20 mg/kg). All experimental protocols were approved by Animal Experimentation Ethics Committee, CEEA/UFPI n° 82/14. Statistical analyzes were performed using ANOVA (one way) followed by Tukey test, p<0.05. Results and Discussion: ISO (1.56-25 mg/kg) significantly decreased the paw licking time in both phases of the formalin test, neurogenic (**p<0.05) and inflammatory (**p<0.001), when compared to the control group. ISO (0.78-12.5 mg/kg p.o.; ***p<0.001) significantly reduced paw licking time in the capsicain test, confirming the formalin test Results: ISO (3.12 and 6.25 mg/kg) also inhibited in a dose-dependent manner the glutamate-induced nociception (**p<0.001). The naloxone pretreatment (142.3±19.13) reversed ISO-6.25 antinociception (28.45±3.33), as well as glibenclamide-GLIB (ISO 6.25=23.41±3.01;GLIB+ISO=76.29±12.93;***p<0.001). The ISO-6.25 antinociception (22.25±2.64; ***p<0.001) was also reduced by atropine-ATR (ATR+ISO=71.04±4.67; ***p<0.001, at the same way of the pilocarpine (PILO=1.56±0.14; ATR+PILO=59.11±4.30; ***p<0.001). The participation of L-arginine/NO/cGMP pathway was investigated, and L-arginine reversed the ISO-6.25 response (55.96±8.01) when compared to ISO-6.25 (17.79±2.56; ***p<0.001), what was confirmed by the treatment with methylene blue+ISO (65.23±7.94) in comparison to ISO-6.25 (19.18±2.75) and the control group (107.67±8.75). In conclusion, these results suggest an isopulegol acute antinociceptive effect in mice, with the involvement of opioid system, K\textsuperscript{+}\text{ATP} channels, muscarinic receptors and L-arginine/NO/cGMP pathway inhibition. Financial support: UFPI/CAPES.
05.052 Hydrogen sulfide (H₂S) donors alleviate pruritus induced by activation of type-2 protease activated receptors (PAR-2) in mice. Coavoy-Sánchez SA¹, Rodrigues L¹; Teixeira SA¹, Soares AG¹, Wood M², Whiteman M², Costa SKP¹, Muscará MN¹ ¹ICB-USP – Pharmacology, ²University of Exeter Medical School

Introduction: Pruritus is the most common symptom of cutaneous diseases and anti-histamines are the usual treatment; however, anti-histamine-resistant pruritus is very common in some clinical settings, thus reflecting the need to target alternative pathways. The intradermal (i.d.) injection of the peptidic PAR-2 agonist SLIGRL-NH₂ evokes a scratching behavior in mice (Shimada et al. Eur J Pharmacol. 2006;530(3):281-3). On the other hand, H₂S donors can inhibit histamine-mediated itching (Rodrigues et al. Nitric Oxide. 2013;31 Suppl 2:54). We thus decided to investigate the effects of H₂S donors on the acute scratching behavior mediated by the activation of PAR-2 in mice, as well as some of the potentially underlying mechanisms.

Methods: Male C57BL/6 mice (7-10 wk-old) received an i.d. injection of SLIGRL-NH₂ (40 nmol/site) into the dorsal neck region. The itching response was quantified by the number of scratching bouts during the 20 min following SLIGRL-NH₂ injection. To assess the participation of histamine H1 receptors in this response, pyrilamine (a histamine H1 receptor antagonist) was injected i.p. 30 min before SLIGRL-NH₂. In order to assess the effects of H₂S, GYY4137 or NaHS (a slow-release and a spontaneous H₂S donor, respectively) were injected concomitantly with SLIGRL-NH₂. The participation of K<sub>ATP</sub> channels, the NO/cGMP pathway and transient receptor potential ankrin-1 (TRPA1) receptor were evaluated by treating the animals with glibenclamide (a K<sub>ATP</sub> channel blocker), sodium nitroprusside (SNP, a NO donor), ODQ (a soluble guanylyl cyclase inhibitor), HC-030031 (a TRPA1 antagonist) and allyl isothiocyanate (AITC; a TRPA1 agonist).

Results: The intradermal injection of SLIGRL-NH₂ (8-80 nmol/site) caused a dose-dependent scratching, which peaked at 10 min and returned to the basal response along the next 30 min; pre-treatment with pyrilamine (30 mg/kg, i.p.) did not inhibit this behavior but significantly inhibited the one induced by histamine (300 nmol/site). Co-injection of SLIGRL-NH₂ (40 nmol/site) with either GYY4137 (1 and 3 nmol) or NaHS (1 and 0.3 nmol) significantly reduced pruritus responses (57% and 72% respectively; P<0.05), and co-treatment with glibenclamide (200 nmol) abolished these H₂S effects. The simultaneous injection of SNP (10 nmol) significantly reversed (64%; P<0.05) the antipruritic effect of H₂S; however, ODQ (30 μg) had no significant effects. Western blot analysis revealed that both PAR-2 and TRPA1 are constitutively expressed in the skin of mice. HC-030031 (20 μg) significantly reduced (70%; P<0.05) SLIGRL-NH₂-induced pruritus; however pruritus induced by AITC (1000 nmol/site) was unaffected by NaHS.

Conclusions: Our data show that pruritus secondary to PAR-2 activation can be alleviated by H₂S, which acts through K<sub>ATP</sub> channel opening and also involves NO in a cGMP-independent manner. Furthermore, TRPA1 receptors mediate the pruritus induced by activation of PAR-2, but H₂S does not interfere with this pathway. In conclusion, these results provide support for the development of new treatments for pruritus, mainly those aiming situations where anti-histamines are devoid of significant efficacy. Financial Support: CAPES, CNPq, FAPESP. Animal Research Ethical Committee: CEUA-ICB-USP 100-09-03/2013
Introduction: Trigeminal neuralgia (TN) is an intense orofacial neuropathic pain that can involve one, two or three branches of trigeminal nerve. TN is a disorder difficult to control, often shows insufficient response to classic analgesics and surgical approaches. Thus, the TN management remains a challenge and there is a general lack of knowledge about the mechanisms involved in the generation of pain and its complex characteristics. Thus, a better knowledge of mechanisms of this condition can be the key to improve the treatment of this pathology. The objective of this study was to evaluate the involvement of GABAergic and glutamatergic pathways in the pathogenesis of trigeminal neuralgia model in rats. Methods: 64 male Wistar rats (55-65 days old, ≥ 250g) were divided in 7 groups: control-vehicle (CV), sham neuralgia-vehicle (SV), sham neuralgia- GABAergic agonist (SGa), sham neuralgia-glutamatergic agonist (SGlu), neuralgia (NV), neuralgia-GABAergic agonist (NGa), neuralgia- glutamatergic agonist (NGlu). The trigeminal neuralgia model was induced through infraorbital nerve constriction according the technique described by Imamura et al. (1997) under anesthesia with ketamine (50 mg/kg), xylazine (5mg/kg), and maintained isoflurane (2-3%). In the sham neuralgia groups, the nerve was exposed similarly, but the constriction was not performed. The immediate post-operative was treated with tramadol hydrochloride (10mg/kg 12/12h for 48h). The mechanical hyperalgesia was assessed by Von Frey test on baseline, 7 and 14 days after TN model; immediately before treatment, 15, 30 and 60 minutes after treatment. The treatment consists in one dose of vehicle (saline 10ml/kg), GABAergic agonist (diazepam 2 mg/kg) or glutamatergic antagonist (MK-801 0,25mg/Kg). Data were expressed as the mean±standard error of the mean (S.E.M). Generalized Estimating Equation (GEE) followed by Bonferroni was performed to compare all groups in different times of nociceptive tests. Results: In nociceptive response we observed interaction between group and time in the Von Frey test at 14th day after infraorbital nerve constriction (Wald χ²=15.81; 2, P<0.05), confirming the establishment of trigeminal neuralgia. After administration of treatment, we observed interaction between group and time in the Von Frey test (Wald χ²=175.74;18, P<0.01). NGa group showed an increased nociceptive threshold 15 minutes after agonist administration, that is sustained for up to 60 minutes. NGlu group presented an increased nociceptive threshold only 60 minutes after the treatment. Conclusion: Our data suggests the involvement of GABAergic and glutamatergic pathways in orofacial neuropathic pain processing. According with nociceptive test, the glutamatergic route was able to promote an early response compared to GABAergic pathway. We suggest that the delayed response of glutamatergic route can be associated with glutamatergic receptors located in demyelinated fibers in peripheral areas as described in previously studies. It is important to highlight the importance of expression and quantification of GABA and glutamatergic receptors to elucidate these mechanisms.
05.054 Effects of binge-like ethanol exposure during adolescence on hyperalgesia during sickness syndrome. Oliveira BMT¹, Telles TMBB², Correia D², Zampronio AR¹ ¹UFPR-Farmacologia, ²UFMG – Biologia Geral

Introduction: During illnesses caused by infectious disease an adaptive brain-mediated response called sickness syndrome occurs, which includes hyperalgesia and fever. Changes in this response may be related to susceptibility to infections. Considering the harmful use of alcohol starts at the young age where the nervous system is still vulnerable, this study aimed to assess the effects of ethanol exposure, in a binge-like pattern, during adolescence, on hyperalgesia induced by systemic administration of lipopolysaccharide (LPS). Methods: Naïve adult (180 g) male Wistar rats received different doses of intraperitoneal LPS (0.5 to 50 µg/kg) and the mechanical hyperalgesia in the hind paw was evaluated using an electronic Von Frey anesthesiometer. In a second series of experiments, ethanol 3 g/kg (25% w/v in saline, intraperitoneally) was administered to the rats on postnatal day 25 (pre-treated group with ethanol) or saline at equivalent volume (control group). On postnatal days 26, 29, 30, 33, 34, 37, and 38 animals received the same treatment. To evaluate late mechanical hyperalgesia response, the animals were divided in two groups to receive an additional dose of ethanol, by oral route: 1) Twelve days after binge-like exposure (postnatal day 50) or 2) twenty-five days after binge-like exposure (postnatal day 63). Hyperalgesia was evaluated on the postnatal day 51 or 63, respectively, when animals were treated with saline, LPS (50 or 5 µg/kg, i.p.). Results and conclusion: LPS administration at doses of 0.5, 5 and 50 µg/kg in naïve animals induced a reduction in the mechanical threshold in a dose-dependent manner 3 h (7%, 11% and 16%) and 6 h (11%, 15% and 25%), respectively. Binge-like ethanol exposure did not change the mechanical hyperalgesia LPS 5 µg/kg evaluated on postnatal day 51. However, binge-like ethanol exposure during adolescence increased the intensity of hyperalgesia induced by LPS by 41%, 21% and 22% at 3, 6 and 24 h, respectively. In contrast, binge-like ethanol exposure seems no longer influence this response on postnatal day 63 since it did not change the mechanical hyperalgesia induced by both doses of LPS. The acute ethanol exposure also did not change the mechanical hyperalgesia induced by LPS 50 µg/kg when compared to saline-exposed animals. These findings suggest that binge-like ethanol exposure during adolescence can increase mechanical hyperalgesia. These effects were evident for days after ethanol exposure and dissipated thereafter. Financial support: CNPq. All procedures were approved by the Institution Ethics Committee of UFPR (protocol # 813).
05.055 Isopulegol anti-inflammatory activity involves inhibition of the histamine-serotonin and prostaglandin E2 induced edema, leukocytes migration and myeloperoxidase activity Prósito DF, Leite LCTF, Pires LF, Araújo JM, Lima MPD, Sousa Neto BP, Oliveira FA, Sousa DP, Almeida FRC UFPI

Introduction: The isopulegol (ISO) (p-Menth-8-en-3-ol) is a monoterpenic alcohol, present in essential oils of various aromatic plants such as Melissa officinalis L. Previous studies have shown that ISO has anxiolytic, anticonvulsant, gastroprotective and antioxidant properties, but so far there are no studies showing an activity in inflammation models. Methods: Female Wistar rats (180-250g, n= 5-8) and female Swiss mice (25-30g, n=5) were used in the tests under authorization of the Animal Ethics Committee (CEEA/UFPI N° 82/2014). Mice were treated with ISO (3.12, 6.25 and 12.5mg/kg, po), vehicle (5% Tween 80 in 0.9% NaCl) and indomethacin (10mg/kg, po), 60 min before administration of carrageenin 1% (0.1mL/paw). Paw volumes were measured by digital caliper and expressed in mm at time zero (t₀) and after treatment from the 1st to 6th hour. The mice received orally ISO (12.5 mg/kg), vehicle and indomethacin (10 mg/kg), 60 min before being treated with dextran and PGE₂ (1%, 50 µL) on the right hind paw (RHP) and saline (50µL) in the left hind paw (LHP). After 2 h the animals were euthanized, both hind paws were cut and weighed to record the exudate (RHP-LHP=edema in mg). In another protocol, we have induced air pouch in the rats dorsal region and they were orally treated with ISO (3.12, 6.25 and 12.5mg/kg, po), vehicle and indomethacin (10mg/kg) before the injection of carrageenan 1% in the air pouch. After 4 h, air pouch exudate was collected for the total white cell count and myeloperoxidase assay. For differential leukocytes count, mice received orally ISO (12.5 mg/kg), vehicle and indomethacin (10 mg/kg), 60 min before receiving the intraperitoneal injection of 1% carrageenan, and after 4 h the exudate was obtained to quantify the different leukocytes types. Statistical analyzes were performed using ANOVA (two way) followed by Bonferroni test, p<0.05. Results and Discussion: The ISO (12.5mg/kg, po) inhibited significantly (**p<0.001) carrageenan-induced edema from the 1st to 6th hour. ISO (12.5mg/kg, po) attenuated the paw edema induced by dextran (14.50±3.27; ***p<0.001) compared to control (36.80±3.05). As well as reduced paw edema induced by PGE₂ (13.33±3.64; control= 44.66±4.41;***p<0.001). ISO (6.25 and 12.5mg/kg, po) decreased total leucocytes migration to the air pouch, 65.98 and 55.68%, respectively (**p<0.001). On the other hand, ISO (12.5mg/kg) also attenuated the migration of polymorphonuclear-PMN (12.4±1.74) and mononuclear-MONO (3.00±0.51) leukocytes compared to control (46.50±2.77), an inhibition percentage of 73.3% and 74.3%, respectively (**p<0.001). Indomethacin (10mg/kg) produced values of 56.1% and 72.9%, respectively. ISO (12.5mg/kg) reduced MPO enzyme activity (13.58±0.30) in the exudate from the carrageenan-activated air pouch compared to the control group (33.85±3.15)(**p<0.001). The ISO anti-inflammatory action appears to be related to the inhibition of the inflammatory mediators actions, such as histamine, serotonin and PGE₂ as well as PMN/MONO leukocyte migration. Financial support: UFPI/CAPES.
Antinociceptive effects of *Condalia Buxifolia* Reissek in a mouse model of postoperative pain. Simões RR¹, Coelho IS¹, Zambenedetti A², Morel A F², Zanchet EM², Santos ARS¹ UFSC, ²UFSM

**Introduction**: The infusion of root bark from *Condalia buxifolia* is used in traditional medicine in Brazil as an antipyretic, anti-inflammatory and against dysentery, and is known popularly as coronilha-folha-de-buxo or espinhillo. Acute pain is amongst the most common outcome after surgery. Therefore, this study evaluated the antinociceptive effect of the methanolic extract of *Condalia buxifolia* (MECb) in a model of postoperative pain (PP) induced by plantar incision surgery (PIS). **Methods**: Female Swiss mice (n=50) were used (26-30g). Root bark of *C. buxifolia* were collected in Lavras do Sul and were dried at 50 °C, ground into a fine powder and then was extracted with methanol. The resultant extract was filtered and the methanol was removed under reduced pressure to obtain MECb (yield 980 g; 28%). In PIS model were evaluated mechanical and thermal (heat and cold) hyperalgesia. In the mechanical hyperalgesia, the right hind paw was stimulated with a constant pressure of 0.4 g von Frey filaments. The frequency of response to 10 applications was taken as the nociceptive behavior. One day before surgery was performed the baseline response of the animals and 24 h after PIS was investigated the time-course (1, 2, 3 and 4 h) of the antinociceptive effect of MECb (30-100 mg/kg, i.g.). Control animals received vehicle (10 mL/kg, i.g.). To investigate the effects of long-term with MECb (100 mg/kg, i.g.), the treatment was repeated for 6 days after PIS. To analyze cold and heat hyperalgesia mice were placed in the cold plate (10±1°C, cut-off 120 s) and in the hot plate (48±1°C, cut-off 60 s). Mice were pretreated with MECb (100 mg/kg, i.g.) or vehicle (10 mL/kg, i.g.) 1 h before the tests. The nociceptive behavior was detected by von Frey filaments and the vehicle-treated group. The nociceptive behavior was tested 48 h (heat) and 72 h (cold) after PIS. The statistical significance between groups was determined by One-way or Two-way Anova followed by post hoc test of Newman-Keuls or Bonferroni’s test, respectively. **Results**: MECb caused an inhibition of frequency of response at 100 mg/kg as compared to the vehicle-treated group. The effect was maintained by 3 h. With a prolonged administration (once a day) for six days, MECb (100 mg/kg, i.g.) significantly reduced the mechanical hyperalgesia until the fifth day. The PIS induced a decrease of the paw withdrawal latency for thermal heat and cold stimulus in comparison to non-injured mice. MECb (100 mg/kg) reduced the heat hyperalgesia and the latency to paw withdrawal was increased. MECb treatment was not able to reduce the cold hyperalgesia. **Conclusions**: These data show that MECb presented significant antinociceptive effect in mice in a model that mimics an important clinical condition of pain. Such findings are of interest because they support the use of *C. buxifolia* in popular medicine. **Financial support**: FAPESC, CAPES. All protocols used were approved by CEUA-UFSC (protocol number PP0745).

Introduction: Activation of A_2A_ and A_3 adenosine receptors (ARs) reduces inflammation in murine models. LASSBio-1027 was previously described to activate both A_2A_ and A_3_ receptors using molecular docking studies and binding assays (Leal et al., 2012). Aims. The aim of the present work was to investigate the beneficial effects of LASSBio-1027, a ligand to A_2A_ and A_3_ receptors, in murine model of acute and chronic inflammation. Methods and Results: Protocols were approved by the Animal Care and Use Committee at Universidade Federal Rio de Janeiro (113/14). Male Swiss mice were used (25-30 g) and treated (8 animals per group) by oral gavage with vehicle (DMSO), LASSBio-1027 (25, 50 and 100 mg/kg), thalidomide (100 mg/kg) and acetyl salicylic acid (ASA, 300 mg/kg). Two typical phases of nociceptive behavior induced after formalin injection (20μL i.pl.) were evaluated after administration of LASSBio-1027. Chronic inflammation was observed in the monoarthritis, which was induced in mice under 2% sevoflurane anesthesia after subcutaneous injection of complete Freund adjuvant around the tibio-tarsal joint. After seven days, the animals were treated orally (gavage) with LASSBio-1027 and the thermal and mechanical hyperalgesia and paw edema were analyzed using the paw immersion test, pressure test and plethysmometer device, respectively. LASSBio-1027 (100 mg/kg) reduced the time of liking/biting from 240.9 ± 26.8 to 117.2 ± 17.9 s in the inflammatory phase of the formalin test. The antinociceptive action of LASSBio-1027 was reversed after pre-treatment with MRE 3008F20, an adenosine A_3_ antagonist, which increased the time of response to 286.0 ± 32.8 s. However, pre-treatment with ZM 241385, an adenosine A_2A_ antagonist, did not alter the response in the inflammatory phase. CFA-induced monoarthritis reduced the paw withdrawal threshold in the thermal and mechanical stimulation from 14.8 ± 0.1 to 7.4 ± 0.5 s and from 250.0 ± 0.1 to 78.3 ± 5.2 g. Oral administration of LASSBio-1027 (100 mg/kg) improved the thermal and mechanical hyperalgesia and decreased paw edema. TNF-α expression increased in animal with monoarthritis, and was recovered after treatment with LASSBio-1027. Conclusions: LASSBio-1027 which is a ligand of adenosine receptor promoted antinociceptive effect in the chronic inflammatory pain indicating a new alternative for the treatment of monoarthritis. References: Leal, CM, Eur J Med Chem. 55: 49, 2012. Financial Support: CNPq, FAPERJ, CAPES, INCT, PRONEX. Keywords: A_3_ adenosine agonist, LASSBio-1027, monoarthritis, antinociceptive and anti-inflammatory actions, developmental pharmacology.
05.058 TRPA1 channel mediates the analgesic action of dipyrone and pyrazolone derivatives. Nassini R¹, Materazzi S¹, de Logu F¹, Marone IM¹, Coppi E¹, Fusi C¹, Preti D², Tonello R³, Patacchini R³, Chairugi A¹, Geppetti P¹, Benemei S¹ ¹University of Florence, ²University of Ferrara, ³UFSM, ⁴Chiesi Farmaceutici Spa

Since the seminal discovery of antipyrine (AntiP) by Ludwig Knorr in 1883, pyrazolone derivatives (PDs) have been the most successful classes of drugs in pain pharmacotherapy. However, although still used by hundreds of millions of people worldwide, the mechanism of the analgesic action of PDs, such as dipyrone (Dip), propyphenazone (PPh) and AntiP, remains unknown. PDs, like NSAIDs/coxibs, have been proposed to act through prostaglandin synthesis inhibition (Hinz et al., 2007). However, pain models responsive to Dip are clearly distinct from those inhibited by classical COX-inhibitors (Brune et al., 1983; Lorenzetti et al., 1985). In addition, COX inhibition by PDs is, however, weak, resulting in poor anti-inflammatory effects, which do not match the remarkable analgesic action. Higher efficacy of Dip vs. NSAIDs in reducing pain with respect to prostaglandin-dependent inflammation and neuropathic pain conditions, further discriminate PDs from NSAIDs with regard to their pharmacological activities.

The transient receptor potential ankyrin 1 (TRPA1) channel, expressed by a subset of capsaicin-sensitive primary sensory neurons, represents a major target for pain transduction. Emerging evidence indicates that oxidative and nitroative stress and the ensuing lipid peroxidation byproducts produce nociception and hyperalgesia by TRPA1 targeting (Bautista et al., 2006; Taylor-Clark et al., 2009; Trevisani et al., 2007). This novel pathway has been reported to contribute to models of both inflammatory and neuropathic pain (McNamara et al., 2007; Nassini et al., 2014; Trevisan et al., 2013). We hypothesized that PDs, selectively inhibit TRPA1 channel expressed in nociceptors and via this mechanism produce analgesia. Through calcium imaging and electrophysiology we demonstrated that PDs selectively target the TRPA1 channel, inhibiting calcium responses and currents in both transected and constitutive TRPA1-expressing cells (human embryonic lung fibroblasts and rodent dorsal root ganglia primary neurons). In addition, the two most largely used PDs, Dip and PPh, reduce TRPA1-mediated nociception and mechanical allodynia in models of inflammatory and neuropathic pain (formalin, carrageenan, partial sciatic nerve ligation, and the peripheral neuropathy evoked by chemotherapeutic drug, bortezomib). Notably, Dip and PPh attenuate carrageenan-evoked mechanical allodynia, without affecting prostaglandin E₂ levels and edema. present data suggest that attenuation of the pain-producing TRPA1-dependent pathway activated by oxidative stress by-products might also contribute to the analgesic action of PDs in various types of pain in humans. Moreover, present findings strongly support the rationale for the development of TRPA1 antagonists as new analgesics for the treatment of both inflammatory and neuropathic pain. The new chemical entities with TRPA1 antagonistic properties, while maintaining good efficacy in pain treatment and general safety profile of Dip or PPh, should be devoid of the life-threatening hematologic adverse reactions, presumably associative to the chemical structure of PDs. This work was supported by Progetto Impatto-Ministero Dello Sviluppo Economico (2012-2013); Associazione Italiana per la Ricerca sul Cancro (AIRC); PRIN-2010Y4WMCR-007. Studies were conducted under University of Florence research permits #204/2012-B and #194/2015-PR.
Native and Recombinant Pho1β Toxin Produce Anti-hyperalgesic Effect in a Model of Bortezomib-induced Neuropathy in Mice


Introduction: Bortezomib is a proteasome inhibitor used in different types of cancer. Chemotherapeutic-induced peripheral neuropathy (CIPN) has emerged as a major complication of bortezomib therapy, which usually appears in the first courses of therapy with a number of sensory and painful symptoms, including mechanical and cold hyperalgesia. No effective therapy is currently available to treat or prevent CIPN, most likely because the underlying mechanisms are poorly understood. Previously, we demonstrated that systemic antagonism or gene deletion on TRPA1 receptor, an ion channel expressed in sensory neurons, reversed bortezomib-induced hyperalgesia in mice, indicating that TRPA1 is a potential target for the development of new analgesic drugs to treat CIPN-related pain. The toxin Pho1β, a peptide purified from the army worm venom, and its recombinant form CTK01512-2 (CTK) are prototypes of analgesic drugs, which produce anti-hyperalgesic effect in several models of pain in rodents. Besides neuronal voltage-sensitive calcium channels (VSCC), we recently demonstrated that Pho1β is also a potent antagonist of the TRPA1 receptor. To further evaluate the analgesic potential and mechanism of action of Pho1β toxin, the objective of this study was to evaluate if peripheral or spinal administration of Pho1β, as well as selective of VGCC and TRPA1 blockers would present antinociceptive effects in a model of bortezomib-induced neuropathy in mice. Methods: C57BL/6 mice (male, 20–25 g) were used. The CIPN was induced by an intraperitoneal injection of bortezomib (1 mg/kg) and drug effects were tested 7 days after. Pho1β (10-300 pmol/site or pmol/paw), its recombinant form CTK-01512 (CTK, 100 pmol/site or 300 pmol/paw, subcutaneous), TRPA1 antagonist HC-030031 (HC, 30 pmol/site or 300 pmol/paw), neuronal VSCC blocker ω-conotoxin MVIIA (100 pmol/site or 300 pmol/paw) were administered through intrathecal (5 μL/site) or intraplantar (10 μL/paw) route. Mechanical (withdrawal threshold with von Frey filaments) or cold (response to acetone droplet) hyperalgesia was determined in the right hind paw before (baseline threshold) and after treatments. Results: Intrathecal Pho1β (30-100 pmol/site) markedly reduced mechanical and cold hyperalgesia (92% and 100% inhibition for 100 nmol/site 1 h after injection) induced by bortezomib. Intrathecal CTK and HC, but not ω-conotoxin MVIIA, also almost abolished mechanical and cold hyperalgesia induced by intraplantar bortezomib. Similarly, intraplantar injection of CTK and HC, but not ω-conotoxin MVIIA, largely reverted bortezomib-induced hyperalgesia. Conclusion: Pho1β and CTK, probably antagonizing TRPA1, have now been identified as antinociceptive in a relevant model of neuropathic pain, then they could be suggested as novel strategies for the treatment of pain diseases. Financial support: This work was supported by CAPES (AUX-PE Toxinologia, Process 2622-14-9 and Science without Borders). Research approval by Ethical Committee: Italian National Committee for Animal Research (204/2012-B and 194/2015-PR, UniFI).
Kynurenine metabolic pathway links peripheral immune response to central sensitization that account for the development of neuropathic pain. Souza GR¹, Fonseca MD¹, Dagostin ALA¹, Lemos H², Huang L², Pacholczyk G², Santana DA, Talbot J¹, Sant'Anna MB¹, Leão RM³, Alves-Filho JC¹, Cunha FQ¹, Mellor AL², Cunha TM¹ ¹FMRP-USP – Farmacologia, ²Georgia Regents University, ³FMRP-USP – Fisiologia

Introduction: Chronic pain is a common problem in health care management. It can be divided in two main types: inflammatory and neuropathic. Neuropathic pain (NP) is related to a dysfunction in the nervous system. However, the exact mechanism of NP development is not well understood, therefore, limiting the treatment options. The aim of this study was to test whether the kynurenine pathway (represented mainly by idoleamine-2,3 dioxigenase 1 (IDO1) and kynurenine 3-monooxygenase (KMO) contributes to genesis of neuropathic pain and the mechanisms triggering this pathway. Methods: Spared nerve injury (SNI) model was induced in B6 or IDO KO mice to induce neuropathic pain. Mechanical allodynia was tested using filaments of von Frey. mRNA and protein expressions were evaluated by RT-PCR and western blotting, respectively. Furthermore, HPLC was employed to determine IDO enzymatic activity. Results: Neuropathic pain is abrogated when kynurenine metabolic pathway is pharmacologically or genetically inhibited. A peripheral activation of kynurenine pathway leads to an increase in plasmatic levels of indoleamine 2,3-dioxigenase-1-derived L-kynurenine L-kynurenine is transported to the spinal cord where they are metabolized into quinolinic acid (QA) in a kynurenine-3-monooxygenase-dependent way. In the later, QA is responsible for the enhancement of NMDA glutamatergic transmission and consequently to the maintenance of pain hypersensitivity. Conclusion: Our findings reveal that the kynurenine pathway is important for the genesis of neuropathic pain, being an important target for chronic pain therapy. The animal care and handling procedures were in accordance with Committee for Ethics in Animal Research of the Ribeirão Preto Medical School-USP (Process n° 097/2011). Supported by: FAPESP, CNPq and FAEPA.
05.061 P2X4 Receptors modulate fatigue-enhanced muscle pain. Oliveira-Fusaro MCG, Gregory N, Kolker S, Wilson S, Sluka KA. Labeled-4, University of Iowa; 2University of South Carolina – Pharmacology

Introduction: Exercise is an effective non-pharmacological treatment to chronic muscle pain. However, acutely, exercise can exacerbate pain, limiting the involvement of those with muscle pain-clinical problems. It was recently demonstrated depletion of muscle macrophages prevents the hyperalgesia induced by combining a fatiguing exercise task with muscle insult. ATP is released from fatiguing muscle and can activate purinergic receptors, like P2X4. P2X4 receptors are expressed on macrophages and have previously shown to play a role in nociception. Therefore, we hypothesized that P2X4 receptors on muscle macrophages were activated to produce hyperalgesia in response to muscle fatigue. Methods: Our model of exercise-enhanced muscle pain was used. It consisted of two injections of pH 5.0 saline into gastrocnemius muscle (5 days apart) in combination with an electrically induced fatigue of the same muscle (just prior to the second pH 5.0 injection). Needle electrodes connected to a Stimulator (Grass S88) were inserted into the muscle. Baseline maximum force was established by applying three 100-Hz trains at 7V. To induce fatigue, mice were given 6 min of submaximal contractions using 7V stimulations at 40 Hz for 3.75 s with 4.25 s of rest between contractions. Three additional maximum force contractions were then elicited to determine the decline in force after fatiguing contractions. Force was measured by attaching the plantar surface of the foot to a force plate connected to an iWORX FT-302 force transducer. Muscle pain was measured by squeezing the muscle with force-sensitive tweezers. We pharmacologically blocked P2X4 receptors in muscle with 5-BDBD, a potent antagonist, and genetically depleting P2X4 expression in macrophages with a lentivirus expressing an artificial P2X4 miRNA injected into the muscle. Immunohistochemistry and quantitative PCR were used to analyze expression of P2X4 receptors in muscle macrophages. Male and female C57BL6/J mice were used and experiments were approved by the Institutional Animal Care and Use committee. Results: Muscle fatigue increased the number of muscle macrophages expressing P2X4 receptor protein (t test, p<0.05), and the lentivirus expressing miR_P2X4 significantly reduced P2X4 expression in muscle macrophages (t test, p<0.05). Pharmacological blockade of peripheral P2X4 receptors by 5-BDBD (50 and 500µM, but not 5 µM) and pre-treatment with the Lentivirus expressing miRNA_P2X4 prevented the decrease in ipsilateral muscle withdrawal thresholds normally observed in male and female (p<0.05, Tukey’s test). The force of contraction decreased significantly across time in all groups and there were no significant differences in the post fatigue force (p>0.05). Conclusion: These data show that P2X4 receptors expressed on muscle macrophages are activated in the fatigue-enhanced muscle pain model to induce muscle hyperalgesia. Therefore, peripheral P2X4 receptors could be a target to control the exercise-induced muscle pain experienced by patients with chronic musculoskeletal pain. Financial Support: FAPESP (2014/01119-4), Institutional Animal Care and Use committee (protocol number 4081135).
05.062 α-Spinasterol: A dual TRPV1 Antagonist and cyclooxygenase inhibitor presents antinociceptive effects in pathological pain models in mice. Oliveira SM1, Brusco I1, Trevisan G2, Ferreira J3 1UFSM – Bioquímica e Biologia Molecular, 2UFSM – Fisiologia e Farmacologia, 3UFSC – Farmacologia

Introduction: α-Spinasterol is a phytosterol found in a variety of plant with biological activities. α-Spinasterol is a TRPV1 antagonist and presents antinociceptive and anti-edematogenic effects in an arthritic pain model. Here, we investigated another possible target for spinasterol, as cyclooxygenase 1 and 2 (COX-1/2) inhibitor to characterize it as a multitarget compound to treat acute (postoperative pain) and chronic (neuropathic pain) pain. Methods: Adult male Swiss mice (30-35 g) were used (Approved by Committee of Ethics in Animal Use- UFSM; License number 3652150416/2016). The antinociceptive effect (mechanical allodynia and spontaneous nociception) of α-spinasterol or its positive control (indomethacin, celecoxib and acetaminophen), all oral route, was evaluated in a postoperative pain model induced by plantar incision and neuropathic pain caused by partial sciatic nerve ligation (PSNL) or by chemotherapy paclitaxel (single injection or four repeated injections of paclitaxel in alternate days- 1 mg/kg, i.p.). We also evaluated the possible development of adverse effects caused by α-spinasterol [gastric lesions, urea and creatinine levels (renal dysfunction markers), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (liver injury indicators)]. The effect of α-spinasterol on COX activity was evaluated in vitro. Results: Plantar incision caused mechanical allodynia and spontaneous nociception in the animals. α-spinasterol (0.3 mg/kg) and indomethacin (10 mg/kg), administered before (0.5h; pretreatment) or after (0.5h; post-treatment) plantar incision prevented and reversed the mechanical allodynia from 0.5 to 6 h after treatments with maximal inhibition (Iₘₐₓ) of 60±9% and 61±5% to pretreatment and 76±5% and 82±3% to post-treatment, respectively, at 2h after its administrations. Moreover, the pretreatment with α-spinasterol (0.1-1 mg/kg) or indomethacin (1-10 mg/kg) prevented the mechanical allodynia at 2h with an effective dose 50% (ED₅₀) of 2.03 (0.19-26.93) mg/kg and 6.53 (3.16-13.49) mg/kg, respectively. Indomethacin was able to reduce the spontaneous nociception induced by plantar incision. Neuropathic animals submitted to PSNL presented mechanical alldynia which was reverse by α-spinasterol (0.3 mg/kg) or celecoxib (100 mg/kg) with Iₘₐₓ of 50±9% and 63±11%, respectively at 1h after its administrations. The single or repeated paclitaxel injection promoted mechanical alldynia at 1 or 21 days after its first injection. α-Spinasterol (0.3 mg/kg) or acetaminophen (100 mg/kg) reversed the acute mechanical alldynia from 1 up to 4h after treatments with Iₘₐₓ of 39±9% and 51±5%, respectively. Moreover, α-spinasterol and acetaminophen reversed the chronic mechanical alldynia from 1 up to 4h after treatments with Iₘₐₓ of 38±4% and 44±9% at 2h after treatments, respectively. α-Spinasterol neither cause gastric lesions, nor altered the urea and creatinine levels or ALT and AST activities. α-spinasterol inhibited COX-1 and COX-2 activities with inhibitory concentration 50% (IC₅₀) of 7.76 (1.27-47.52) μM and 16.17 (15.12-17.30) μM, respectively. Conclusion: As a multitarget compound, α-spinasterol is orally efficacious in pathological pain models without cause adverse effects supporting its potential as a new analgesic molecule.
**05.063 The role of pattern recognition receptors like toll-like receptors 4 in herpetic and post-herpetic neuralgia.** Silva CR¹,², Pereira JA², Raymondi J², Cecílio NT², Cunha FQ², Cunha TM² ¹UFU – Bioquímica e Farmacologia, ²FMRP-USP – Farmacologia

**Introduction:** Herpetic neuralgia (HN) is a painful vesicular rash resulting from Varicella-Zoster virus reactivation in the dorsal root ganglia (DRGs) or cranial nerves. However, even after the rash resolution, pain may persist for months or even years, defining the post-herpetic neuralgia (PHN). It is believed that pain during HN and PHN is due to the release of inflammatory mediators on the DRGs, as a result from glial cells activation, cells from the immune response which infiltrate the DRGs or sensory neurons sensitization. First, these cells can release different danger-associated molecular patterns such as the MRP14 protein, which can act as a Toll-like receptor 4 (TLR4) agonist, leading to the release of that inflammatory mediators. Additionally, TLR4 are involved in hyperalgesia induction during some neuropathic pain models, and are implicated in triggering pro-inflammatory immune system signaling events which may be a target for pain during the HN and PHN. **Aim:** The aim of this study is to verify the possible role of TLR4 activation by MRP14 protein released on DRGs during herpetic and post-herpetic neuralgia. **Methods:** Adult male Wild-type (WT), C57BL/6, TLR4KO and MRP14KO mice (25-35g) were anesthetized and the right midflank depilated for inoculation with HSV-1 (1x10⁸ plaque-forming units/20μl) for HN and PHN induction. Pain (mechanical allodynia) was monitored from 1-42 days post-infection (DPI). Levels of TLR4 and MRP14 (analyzed from 1-42DPI), different interleukins (IL-1β, TNFα and IL-6 analyzed on 7, 14 and 21 DPI) and glial cells activation markers (Iba and GFAP analyzed on 7, 14 and 21 DPI) gene expression were determined by RT-PCR in DRGs and spinal cord (L4-L6) from infected animals. MRP14 gene silencing on DRGs was induced by three alternate intrathecal injections (on 3, 5 and 7DPI) of MRP14 shRNA and pain development was analyzed until 9 DPI. **Results:** TLR4KO- and MRP14KO-infected animals had significant less pain (from 5-30 and 5-18 DPI, respectively) if compared with WT HSV-1 infected animals, suggesting the involvement of these targets on herpetic neuralgia induction. There was an increase in MRP14 and TLR4 RNA levels on DRGs 7-21 DPI but not in spinal cord, suggesting that these effects were restricted to peripheral system. Additionally, MRP14 gene silencing prevented mechanical hypersensitivity induced by HSV-1 infection from 6-9 DPI. RNA levels of TNFα were increased 7 and 21 DPI in WT-HSV-1 infected animals and this increase was prevented by TLR4 and MRP14 ablation. RNA levels of GFAP and Iba were also increased 7 and 14 DPI in WT-HSV-1 infected animals and GFAP increase was prevented by TLR4 ablation, whereas Iba increases was prevented by TLR4 and MRP14 ablation. **Conclusions:** TLR4 activation seems to be involved in herpetic and post-herpetic neuralgia and the MRP14 protein seems to be, at least one of the triggers for this activation. Research support: This study is supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico (CNPq). Animal Research Ethical Committee's number: 208/2014 UFU.
Introduction - Venoms and secretions of Bufonidae anurans are abundant sources of potentially useful bioactive molecules in public health. However, little is known about the antinociceptive and anti-inflammatory propriety of venom these anurans. Thus, the purpose of this study was to evaluate the antinociceptive and anti-inflammatory activity of venom of two anurans species: *Rhinella marina* and *Rhinella jimi*. Methods - In the study we used swiss mice provided by the facility of Fiocruz RO. These animals were previously pretreated with venom of each species and, after 1 hour, evaluated in formalin, carrageenan or open field test tests which evaluate, respectively, the antinociceptive and anti-inflammatory effect and locomotor activity these venoms. The venoms were administered subcutaneously in the dorsum at doses of 60, 180 or 360 µg in 300 µL. Results - As result, it was observed that pretreatment with *Rhinella marina* and *Rhinella jimi* venoms have significant antinociceptive activity in both phases of the nociceptive test. At the dose of 60 and 180 µg / 300 µL, *Rhinella marina* venom did not significantly change the 1st phase of nociceptive responses but significantly reduced the intensity and duration of nociceptive responses in the 2nd phase of the test. At the dose of 360 µg / 300 µL, the *Rhinella marina* venom significantly reduced the 1st phase and significantly abolished the nociceptive responses in the 2nd phase of the test. In the three doses tested, the *Rhinella jimi* venom significantly attenuated both 1st as the 2nd phase of nociceptive responses induced by formalin. The results also demonstrate that both venoms significantly attenuated the intensity of edema induced by carrageenan 1% intraplantar injection. The anti-inflammatory effect of Rhinella marina venom was observed with doses of 180 and 360 µg / 300 µL, but not 60 µg / 300 µL, while the anti-inflammatory effect of *Rhinella jimi* venom was observed in all doses. Finally, none of the venoms changed significantly the locomotor activity when assessed in the open field test. Conclusion - Conclude that both venoms have antinociceptive and discrete anti-inflammatory activity and that these effects do not result from motor impairment. Therefore, the results support the proposition of use of Bufonidae anurans venoms as a source for bioprospecting products useful to public health. Financial support: CNPq, CAPES Research approval by Institutional Animal Research Ethical Committee, Process Number 2014/09
Quinolinic acid (QA) is a final product of the kynurenine pathway (represented mainly by idoleamine-2,3-dioxigenase 1 (IDO1) and kynurenine 3-monooxygenase (KMO)). Previous results from our group suggest the importance of the kynurenine pathway in the maintenance of neuropathic pain. Pharmacological and molecular results suggest that QA is responsible for the enhancement of NMDA glutamatergic transition and consequently can contribute to the maintenance of pain hypersensitivity. To test the effects of Quinoline over nociceptive neurons in the dorsal medulla, we compared the actions of quinoline and NMDA on neurons from the spinal cord dorsal horn (layers I and II) from adult mice through electrophysiological experiments using the whole cell patch clamp technique. For this, we anesthetized the animals with Isoflurane and decapitated them right away. The dorsal spine was quickly removed, the meninges peeled off and the portion containing the lumbar intumescence carefully dissected and cut in 500µM thick slices containing the layers I and II dorsal horn neurons. Following the cutting process, the slices were kept in 34°C recovery solution for 12 – 15 min and left to rest in room temperature cutting solution for at least 1 hour prior to recording. For recording, the slices were put in artificial cerebrospinal fluid (aCSF) containing picrotoxin (20 µM) and strychnine (1µM) to block inhibitory currents (GABAergic and glycineric respectively). The recording electrodes were pulled from borosilicate capillaries, with a resistance ranging from 4 – 6 MΩ. In voltage-clamp (-70mV holding potential, no Mg2+), we observed a baseline NMDA-induced inward current decay when NMDA (10 to 50µM) was applied. Due to an increased probability of the NMDA receptor opening on the presence of its agonist, another effect observed was an increase in the baseline peak to peak amplitude following control levels, i.e. the most the baseline decayed, the greater the peak to peak amplitude. Also, the spontaneous glutamatergic excitatory post synaptic currents (EPSCs) had their amplitude and frequency augmented during NMDA application. On the other hand, when quinolinic acid (5µM) was applied, we did not observe any change in the baseline current, but we did observe, as with NMDA, an increase in EPSCs amplitude and frequency. To verify if the NMDA-induced current is due to an increased spilled-over-glutamate-like effect, we repeated the recordings in the presence of DNQX (10µM) and CdCl₂ (100µM) to block the AMPA/kainate currents and the neurotransmission. After NMDA application, the phenomenon observed was the same as the NMDA-induced currents proving that neurons in the substantia gelatinosa of the dorsal horn respond directly to NMDA receptors stimulation. Thus, conclude, that while all neurons tested in the dorsal horn of the spinal chord respond pre- and post-synaptically to NMDA, quinolinic acid, at this concentration, was only able affect them pre-synaptically, increasing the glutamatergic neurotransmission on dorsal horn neurons. Our results suggest that quinolinic acid is acting by potentiating the glutamatergic neurotransmission on dorsal horn neurons, rather than acting post-synaptically on NDMA receptors. Financial support: FAPESP, CAPES, FAEPA. Ethics committee approval protocol CETEA FMRP/USP: 097/2011
05.066 Nociceptive Alterations in the Offspring of Diabetic Rats. Campos-Lima T, Guimarães BV, Lotufo CMC ICB-UFU

Introduction: Glycemic control during gestational period is challenging for diabetic patients or for woman that might develop gestational diabetes during pregnancy. Peripheral sensory neurons can be affected by hyperglycemia or insulin alterations, resulting in diabetic neuropathy. The effects of the in utero diabetic environment to the development of the sensory system of the offspring were not established. Therefore the aim of the present study was to evaluate whether the morphology and function of nociceptive primary neurons are affected by maternal diabetes induced by alloxan in rats. Methods: Maternal diabetes was induced in female Wistar rats by alloxan injection (100 mg/kg, i.p.) in the first gestational day. Morphological analysis of primary sensory neurons was performed by immunofluorescence of frozen sections of dorsal root ganglia (L5) from the adult male offspring (300 g) of diabetic and control rats. Images were obtained through confocal microscopy (Zeiss, LSM510 Meta) and analyzed using Image J (NIH). Neurons were categorized according to IB4, NFH, TRPV1 and Substance P labels. The function of the nociceptive system was tested in adult male rats (300 g) born from diabetic, control and rats that were injected with alloxan but did not develop hyperglycemia. Mechanical sensitivity was evaluated before and after carrageenan injection (100 µg/pata, 50 µl, i.pl.) using the electronic von Frey test. Thermal sensitivity was evaluated using the hot plate test (55°C). Nociceptive behavior was also accessed using the formalin test (2.5%, 50 µl, i.pl.). Results: Percentage of non-nociceptive neurons, positive for NFH, was lower in the offspring of diabetic rats compared to controls (p=0.23, n=3, t test) while percentage of nociceptive neurons positive for IB4 and Substance P was higher in the offspring of diabetic mothers (p<0.05, n=3, t test). Basal thermal sensitivity was enhanced in the offspring of diabetic rats compared to controls (p<0.001, n=10, t test) while no alteration was observed in mechanical sensitivity. Carrageenan induced hypernociception was reduced only after 6 hours of carrageenan injection in the offspring of diabetic rats (p=0.045, n=6, two way ANOVA, Bonferroni test). In the formalin test, the offspring of diabetic rats exhibited a higher nociceptive response only at 15 minutes after formalin injections (p=0.02, n=9, two way ANOVA, Bonferroni test). Conclusion: Maternal diabetes affects the development of the nociceptive system resulting in altered sensations in the adult offspring. Acknowledgment: Marian Borges Franco for technical assistance. Financial support: FAPEMIG, CNPq, UFU. Ethics committee approval (CEUA/UFU protocol 093/11).
05.067 Alterations in BDNF and NGF brainstem levels of rats submitted to orofacial pain model treated with melatonin. Scarabelot VL\(^1\), Medeiros LF\(^2\), Oliveira C\(^3\), Cioato SG\(^3\), Adachi LS\(^2\), Macedo IC\(^4\), de Souza A\(^5\), Caumo W\(^2\), Torres ILS\(^3\), UFRGS – Farmacologia, \(^2\)UFRGS – Ciências Médicas, \(^3\)UFRGS – Farmacologia e Terapêutica, \(^4\)UFRGS – Fisiologia, \(^5\)Unilasalle – Saúde e Desenvolvimento Humano

Introduction: Orofacial pain caused by temporomandibular joint (TMJ) dysfunction is characterized by persistent pain in the TMJ and masticatory muscles. Chronic pain is modulated by different neuromodulators like brain derived neurotrophic factor (BDNF) that regulates inflammatory pain thresholds and secondary hyperalgesia. It has been cited as a pain modulator, mainly, due to its capacity to modulate the of N-metil D-aspartato (NMDA) receptors. Nerve growth factor (NGF) is involved in the neuronal plasticity linked to chronic pain. Melatonin has been used in the treatment of inflammatory and neuropathic pain and its effects could be peripheral and central. The aim of this study was to evaluate the effect of acute administration of melatonin upon BDNF and NGF central levels in a model of chronic orofacial pain.

Methods: 33 male Sprague-Dawley rats (60 days old) were divided into 6 groups: Control (not manipulated); Sham Pain + Vehicle; Sham Pain + Melatonin; Pain; Pain + Vehicle; Pain + Melatonin. The chronic orofacial pain was induced by Freund’s Adjuvant (CFA 25 µL in oil/saline total volume of 50 µL) injection in TMJ. Sham pain model received saline solution (25 µL) in TMJ. Treatment was administrated 7 days after CFA injection. Melatonin groups were treated with a single intraperitoneal injection of melatonin (50 mg/kg, final solution 50 mg/ml). Control and vehicle groups received 1% ethanol in saline. Biochemical analysis was made by ELISA method. Statistical analysis was performed by One-way ANOVA/SNK.

Results: Animals exposed to pain model showed an increase in the BDNF levels, which was reversed by melatonin at 7 days after the administration of acute dose, although this effect was also observed in the vehicle group (One-way ANOVA/SNK, F(5,30)=27.35, P<0.01). It is important to highlight that in the control/sham groups this effect it was not observed. The effects of melatonin and its vehicle in BDNF brainstem levels were state-dependent, since the effects were different between sham and orofacial pain animals that received vehicle or melatonin treatment. NGF levels increased in the orofacial pain groups, independently of received treatment, similar effect was observed in the Sham animals treated with melatonin or vehicle, suggesting vehicle effect (one-way ANOVA/SNK, F(5,26)=18.01, P<0.01).

Conclusion: Although melatonin have showed analgesic effect (data not showed) our analysis showed alterations in the neuromodulators levels induced by orofacial pain and melatonin vehicle. The effects induced by vehicle demonstrating that a single ethanol administration was able to alters neuromodulators 7 days after the injection. At the same time that this vehicle effect was a limitation for our study, it showed an interesting pharmacological data which should be considered when the researchers have to choose the vehicle that should be used in their studies, and in this way avoiding possible biases in their research. This study was approved by Ethical Committee of Animal Use of Clinics Hospital of Porto Alegre (GPPG:12-0104).

05.068 Participation of Trpa1 receptor in a trigeminal neuropathic pain model in mice.

Aim of investigation: Despite intense investigation, the mechanisms of the different forms of trigeminal neuropathic pain remain substantially unidentified. The transient receptor potential ankyrin 1 (TRPA1) channel has been reported to contribute to allodynia/hyperalgesia in some neuropathic pain models, including those produced by sciatic nerve constriction. However, the role of TRPA1 and the molecular/cellular processes that from nerve insult cause trigeminal pain-like behaviors are poorly known. We explored the role of TRPA1, monocytes/macrophages, and oxidative stress in pain-like behaviors evoked by the constriction of the infraorbital nerve (CION) in mice. Methods: Experiments were carried out according to the European Union (EU) guidelines for animal care procedures and the Italian legislation (DLgs 26/2014) application of the EU Directive 2010/63/EU. Studies were conducted under the University of Florence research permit #204/2012-B. C57BL/6 mice (male, 20-25 g, age 5 weeks), littermate wild-type (Trpa1+/+) and TRPA1-deficient mice (Trpa1−/−) (25-30 g, age 5-8 weeks, generated by heterozygotes on a C57BL/6 background (B6; 129P-Trpa1tm1Kyw/J; Jackson Laboratories, Bar Harbor, USA) were used. After CION procedure (10 days) in mice treatment with TRPA1 selective antagonists (HC-030031 or A-967079), or the antioxidant compound (α-lipoic acid), or apocynin (the inhibitor of the NADPH oxidase - NOX), or an antibody directed to the CCL2 chemokine (CCL2-ab, in order to transiently deplete the monocyte/macrophage population), or liposome-encapsulated clodronate, or vehicles. Nociceptive responses were assessed 0.5, 1, 2, and 3 hours after drug administration. Nociceptive tests were performed to detect non-evoked nociceptive behavior and mechanical/cold/chemical hypersensitivity. The levels of hydrogen peroxide and 4-hydroxynonenal were also evaluated, and intra- and peri-neural mononuclear/macrophagic invasion. Results: C57BL/6 and wild-type (Trpa1+/+) mice that underwent CION exhibited prolonged (20 days) non-evoked nociceptive behavior and mechanical/cold/chemical hypersensitivity in comparison to sham operated mice (p<0.05- p<0.001). Both TRPA1 genetic deletion (Trpa1−/−) and pharmacological blockade (HC-030031 and A-967079) abrogated pain-like behaviors (both p<0.001), which were also abated by the antioxidant, α-lipoic acid, and the NADPH oxidase inhibitor, apocynin (both p<0.001). Nociception/hypersensitivity evoked by the CION was associated with intra- and peri-neural mononuclear/macrophagic invasion and increased levels of oxidative stress byproducts (hydrogen peroxide and 4-hydroxynonenal). Attenuation of monocyte/macrophage increase by systemic treatment with an antibody against the monocyte chemoattractant chemokine (C-C motif) ligand 2 (CCL2) or the macrophage-depleting agent, clodronate (both p<0.05), was associated with reduced hydrogen peroxide and 4-hydroxynonenal perineural levels and pain-like behaviors (all p<0.01), which were also abated by perineural administration of HC-030031, α-lipoic acid or the anti-CCL2 antibody (all p<0.001). Conclusions: The present findings propose that, in the CION model of trigeminal neuropathic pain, pain-like behaviors are entirely mediated by the TRPA1 channel. Financial support: This study was supported in part by the Tuscany Region (Nutraceutica, POFCDAT_2015) and by Ente Cassa di Risparmio di Firenze (2010.1023).