Introduction: Cyclooxygenases are rate-limiting enzymes in the metabolic pathway that converts arachidonic acid to prostaglandins. Prostaglandin E2 (PGE2) is quantitatively the major prostaglandin in the mammalian brain, and plays a central role in the modulation of synaptic signaling and excitability, both in physiological and pathological situations. The biological actions of PGE2 have been attributed to its ability to interact with plasma membrane receptors, the EP receptors (EPs). Molecular cloning has confirmed the existence of four subtypes of EPs (EP1, EP2, EP3 and EP4), which are coupled to G-proteins. It has been shown that EP1, EP3 and EP4 antagonists, as well as EP2 agonists, increase the latency for pentylenetetrazol-induced seizures, but the role of EPs in a chronic model of epilepsy has not been investigated. Therefore, the present study aimed to investigate the time-course of EPs immunoreactivity following pilocarpine-induced status epilepticus (SE) in mice. Methods: Male C57BL/6 mice (20-30 g) were injected intraperitoneally (i.p.) with methylscopolamine (1 mg/kg i.p.) 30 min before the application of pilocarpine (320 mg/kg; i.p.). All mice that developed SE received diazepam (10 mg/kg; i.p.) 60 min after SE onset. Animals were euthanized 24 h, 7 or 60 days after SE, and the hippocampi were dissected and processed for determination of EPs immunoreactivity by western blot. Data were analyzed by Student’s t test. A probability of $p<0.05$ was considered significant. Results: No changes in EP1 or EP2 immunoreactivity were found. On the other hand, an increase (251.77 %) in EP3 immunoreactivity was found 60 days after SE, whereas a decrease (34.75 %) in EP4 immunoreactivity was found 24 hours after SE. Discussion: Our present results suggest that EP3 and EP4 immunoreactivity are altered in different phases following pilocarpine-induced SE. Considering the role of EP3 and EP4 receptors in acute models of seizures, it is possible to suggest that EP3 and EP4 could be potentially valuable targets for the development of new anticonvulsants. However, additional studies are necessary to test this hypothesis, as well as its clinical implications. Animal Ethics Committee license number: 037/2012. Financial support: FAPERGS, CAPES, CNPq
Characterization of behavior and cognitive alterations after pilocarpine-induced status epilepticus in C57BL/6 mice. de Oliveira CV, Grigoletto J, Funck VR, Grauncke ACB, de Souza TL, Oliveira MS UFSM – Fisiologia e Farmacologia

Introduction: Epilepsy is a common neurological disorder that affects approximately 0.6% of the entire population. Temporal lobe epilepsy (TLE) is the most prominent of the acquired epilepsies, it is considered the most common type of partial complex seizure in adulthood. Some of the mean characteristics of TLE are the behavioral alterations, such as depression, anxiety, and psychosis, and impaired cognitive performance. Most of the characteristics of TLE can be reproduced in chronic animal models of TLE, such as the pilocarpine model. Pilocarpine model result in abnormal behaviors that are similar to behavioral abnormalities in patients with TLE and thus are useful to study the relationship between epilepsy and behavioral comorbidities. C57BL/6 mice are one of the oldest and most widely used inbred strains in biomedical research, mainly in behavior studies. Previous studies using a behavioral test battery for studying behavioral and cognitive alterations in the pilocarpine model of TLE in C57BL/6 mice showed that epileptic mice in this model exhibit behavioral and cognitive alterations, which reflecting several the disturbances that are associated with epilepsy in humans. Nevertheless the influence of the gender in the behavioral alterations in occurrence of the SE pilocarpine-induced in C57BL/6 mice was not studied yet. So, the aim of this study was to make the behavioral characterization of male and female C57BL/6 mice after SE pilocarpine-induced, thus allowing to determine the impact of gender in this model of TLE. Methods: Male and female C57BL/6 mice (20-30g) were used. To induce the status epilepticus (SE) in mouse, pilocarpine was injected intraperitoneally (i.p.). In order to avoid peripheral cholinergic effects, methylscopolamine (1 mg/kg i.p.) was administered 30 min before the application of pilocarpine. All mice that developed the SE received diazepam (10 mg/mL) after 60 min to SE, to stop the convulsions. 30 to 60 days post SE, epileptic animals were submitted a sequence of behavior test. Results: Statistical analysis revealed that epileptic female mice presented a lower number of rearings [F(1,38) = 8.726; P< 0.05] as well lower latency to cross the beam F(1,27) = 6.908; P< 0.05] and number of footslips [F(1,45) = 5.127; P< 0.05], besides these female mice [F(1,36) = 4.286; P< 0.05] spent less time in periphery of the arena in open field than epileptic male mice. Conclusion: In our study using the behavioral test battery for studying behavioral and cognitive alterations in model of TLE pilocarpine-induced in C57BL/6 mice, the results showed that pilocarpine-treated mice seem to reflect several of the behavioral and cognitive disturbances that are associated with epilepsy in humans. We noted that the changes found in epileptic male C57BL/6 resemble the changes found in epileptic females of this rodent species, indicating that the female C57BL/6 can be used for experiments in this model of TLE. Animal Ethics Committee license number: 037/2012. Financial support: FAPERGS, CAPES, CNPq
Potentiation of the effect of phenobarbital with Montelukast in pentylenetetrazol-induced seizures. Jesse AC, Marafiga JR, Fleck J, Mello CF UFSM – Physiology and Pharmacology

Introduction: Montelukast, an antagonist of cysteinyl leukotriene receptors (CysLTR1), is an anticonvulsive drug, but some patients can’t answer to the treatment, being refractory. The aim of this study was to examine whether the association between Phenobarbital and Montelukast, two anticonvulsive drugs, can potentiate the action of Phenobarbital, increasing the latency to myoclonic and generalized tonic-clonic pentylenetetrazol – induced seizures. Methods: The protocols followed the official Government Ethics Guidelines and were approved by the university ethics Committee (process: 23081.014781/2010-42). Adult male Swiss mice were stereotaxically implanted with a cannula into the right lateral ventricle. The experiments were performed 5 days after surgery. The latency to myoclonic jerks and generalized tonic-clonic seizures with Phenobarbital (6 mg/Kg – i.g.) and Montelukast (3, 10, 30 or 100 nmol/µL - i.c.v.) in PTZ (60 mg/Kg, i.p.) - induced seizures was measured. The animals were placed in acrylic boxes and habituated for 10 min before the behavioral analysis. After, the animals received the treatment, Phenobarbital and Montelukast, and 30 min later the injection of PTZ. They were monitored for 20 min for the appearance of seizures. The latency of myoclonic jerk episodes and generalized tonic-clonic seizures were measured. Results: Phenobarbital (6 mg/Kg – i.g.) alone wasineffective to increase the latency to PTZ - induced myoclonic jerks and generalized tonic-clonic seizures. Phenobarbital (6 mg/Kg – i.g.) in association with Montelukast (30 nmol/µL - i.c.v.)this potentiated and increase the latency to both PTZ - induced myoclonic jerks [ F(9.20) = 4.85; p < 0.05] and generalized tonic-clonic seizures [ F(9.20) = 9.17; p < 0.05]. The dates were analysed by two-way ANOVA and Bon Ferroni post-test. Discussion: Phenobarbital associated with Montelukast increased the latency to seizures, being a therapy option to the refractory patients. These findings suggest a facilitatory role of CysLTR1 in PTZ - induced seizures. The protocols followed the official Government Ethics Guidelines and were approved by the university ethics Committee (process: 23081.014781/2010-42). Sources of research support and acknowledgements: CAPES, CNPq, FAPERGS, PRPGP/UFSM, PIBIC/UFSM.

Decreased Na⁺,K⁺-ATPase activity after pilocarpine-induced status epilepticus in mice.

Grauncke ACB, Funck VR, de Oliveira CV, Grigoletto J, de Souza TL, Pereira LM, Guerra GP, Oliveira MS

Introduction: Na⁺,K⁺-ATPase activity is essential for the maintenance of the electrochemical gradient across the plasma membrane underlying resting and action potentials and modulation of neurotransmitter release and uptake. Consequently, changes in Na⁺K⁺-ATPase activity affect neurotransmitter signaling, neural activity, as well as animal behavior. In addition, it has been suggested that Na⁺,K⁺-ATPase plays a role in several neurological disorders, including seizure activity and epilepsy. Therefore, we decided to investigate whether the status epilepticus (SE) induced by pilocarpine alters Na⁺,K⁺-ATPase activity, immunoreactivity and phosphorylation state in the mice hippocampus.

Methods: Male C57BL/6 mice (20-30 g) were injected with methylscopolamine (1 mg/kg; i.p.) 30 min before the application of pilocarpine (320 mg/kg; i.p.). After 60 minutes of SE (defined as continuous limbic seizure activity) mice received diazepam (10 mg/kg; i.p.). Control animals received methylscopolamine and diazepam, but were injected with vehicle (0.9 % NaCl) instead of pilocarpine. Animals were euthanized 24 h, 7 or 60 days after SE, and their hippocampi were processed for determination of Na⁺,K⁺-ATPase activity, immunoreactivity and phosphorylation state. Na⁺,K⁺-ATPase activity was measured by a colorimetric method based on the differential sensitivity of alpha isoforms to the specific inhibitor ouabain. Immunodetection of Na⁺,K⁺-ATPase α subunit and phosphorylation state at Ser943 were performed by western blot. Data were analyzed by Student’s t test and a probability of P<0.05 was considered significant.

Results: Statistical analysis revealed that total Na⁺,K⁺-ATPase activity decreased by 38.63 % at 60 days post SE. In addition, α₁ Na⁺,K⁺-ATPase activity decreased by 53.73 % and 36.3 % at 24 hours and 60 days after SE, respectively. Moreover, α₂/₃ Na⁺,K⁺-ATPase activity decreased by 40.94 % at 60 days post SE. Interestingly, no changes were detected in the immunoreactivity of Na⁺,K⁺-ATPase α subunit. Conversely, a decrease (54.61 %) in the phosphorylation state of Ser943 at Na⁺,K⁺-ATPase α was found 24 hours after pilocarpine-induced SE.

Discussion: Our results indicate that hippocampal Na⁺,K⁺-ATPase activity is decreased at 24 hours and 60 days after pilocarpine-induced SE in mice. Indeed, decreased Na⁺,K⁺-ATPase activity increases brain excitability, and therefore it may contribute to the development of epilepsy following pilocarpine-induced SE. Regarding the increase in the activity of α₁ Na⁺,K⁺-ATPase 24 hours after SE, it may be a consequence of decreased phosphorylation of Ser943, which is a critical site for the regulation of Na⁺,K⁺-ATPase catalytic efficiency and membrane targeting. Additional studies are necessary to evaluate the molecular mechanisms underlying our findings as well as its clinical implications.

Animal Ethics Committee license number: 037/2012. Financial support: FAPERGS, CAPES, CNPq
Long-term treatment with Cilostazol reverts the retrograde amnesia caused by chronic cerebral hypoperfusion in middle-aged rats. Godinho J, Bacarin CC, Ferreira EDF, Zaghi GGD, Oliveira RMMW, Milani H UEM – Farmacologia e Terapêutica

Introduction: Chronic and progressive cerebral hypoperfusion (CCH) may be causally related to certain states of aging-related dementias, including the Alzheimer type of dementia. Cilostazol, a selective PDE3 inhibitor, has been used for prevention and/or treatment of brain hypoxia/ischemia-related conditions. Cilostazol exerts distinct effects including antiplatelet aggregation, reduction of oxidative stress, and vasodilation. These effects might be associated to an increased level of intracellular cAMP and endothelial nitric oxide synthase activity. Thus we evaluated if long-term treatment with cilostazol (Cebralat®) could attenuate both neurodegeneration and memory deficit caused by chronic cerebral hypoperfusion (CCH) in middle-aged rats. Methods: Male, Wistar rats (12–15-month-old) were trained for 15 days up to reach asymptotic learning performance in a non-food rewarded, eight-arm radial maze task, and then assigned to one of the following groups: sham-operation (n = 17), CCH + vehicle (n = 14), and CCH + cilostazol (n = 13). CCH was induced by permanent, stepwise 4-vessel occlusion/internal carotid artery (4-VO/ICA model). Memory performance of the previously acquired cognition was assessed weekly at 7, 14, 21, 28 and 35 days after 4-VO/ICA, and expressed by three parameters: (i) the latency to complete the task, (ii) the number of reference memory errors, and (iii) the number of working memory errors. Cilostazol (50 mg/kg) was administered by oral route, at once a day, starting soon after the first occlusion stage of 4-VO/ICA, and continued up to the end of behavioral testing. This protocol had the approval of internal Ethical Committee (CEEA No 137). Results: The performance of sham-operated rats did not differ from pre- to post-surgery assessment (within-group comparison, p > 0.05). Compared to sham, the vehicle-treated group showed a significant and persistent increase in latency, and number of errors (p < 0.05), clearly indicating a state of retrograde amnesia. This memory deficit was significantly reduced by Cilostazol (p <0.05 vs. vehicle), which performance returned at the level of the sham-operated group. Preliminary examination shows that cilostazol alleviate also the degree of CCH-induced neurodegeneration. Discussion: The results show that the Cilostazol was able to reverse the retrograde amnesia caused by CCH in middle-aged rats. Additional examination should confirm the neurohistological protection by cilostazol. References: Ferreira, E.D.F., Romanini, C.V.; Cypriano, P.E.; Oliveira, R.M.W.; Milani, H. Sildenafil provides sustained neuroprotection in the absence of learning recovery following the 4-vessel occlusion/internal carotid artery model of chronic cerebral hypoperfusion in middle-aged rats. Brain Res. Bull. 2013, vol. 90, pp. 58. Research support and acknowledgments: CAPES and State University of Maringá (UEM).
Effects of fish oil on ischemia-induced retrograde amnesia, oxidative stress, and neurodegeneration. Bacarin CC¹, de Sá-Nakanishi AB², Bracht A², Ferreira EDF¹, Oliveira RMMW¹, Milani H¹¹UEM – Farmacologia e Terapêutica, ²UEM – Bioquímica

Introduction: Cognitive impairment and neurodegeneration are the major outcomes of transient, global cerebral ischemia (TGCI), as it occurs after reversible cardiac arrest. Oxidative stress is an important pathway leading to tissue damage after cerebral ischemia. Fish oil (FO) constitutes a rich source of omega-3 polyunsaturated fatty acids, mainly docosahexaenoic acid (DHA). We aimed to investigate whether post-ischemic treatment with commercial, high-grade DHA-containing FO could be effective in alleviating both the cognitive and neurodegenerative outcomes of TGCI in rats. Additionally, we also evaluate whether FO could exert antioxidant effect after TGCI. Methods: Male, Wistar rats (4 months of age) were trained for 10 days for getting asymptotic learning performance in an aversive, eight-arm radial maze task, and then subjected to TGCI for 15 min (4-VO model). FO administration (300 mg/kg DHA, p.o.) was given once daily, starting at 4 hours post-ischemia and continued for 9 days consecutively. Retention of the previously acquired cognition was assessed weekly at 15, 22, 29, 36 and 43 days after TGCI, and measured by three behavioral parameters: (i) latency, (ii) number of reference memory errors, and (iii) number of working memory errors. The extent of pyramidal cell death in the hippocampus and cerebral cortex was examined at the end of the behavioral analysis. For determination of the antioxidant effect of FO, rats were treated with FO 3 days prior to and 1 day after TGCI. Twenty-four hours later, the brain was processed for (i) activities of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px); (ii) concentration of the endogenous antioxidant glutathione (GSH); and (iii) protein carbonylation. ANOVA was used to quantify the data. Results: Compared to sham, TGCI disrupted memory performance as measured by latency (145.0 ± 13.14 vs. 65.93 ± 2.75, P<0.001), reference memory errors (2.81 ± 0.44 vs. 0.93 ± 0.15, P<0.01) and working memory errors (1.60 ± 0.30 vs. 0.13 ± 0.04, P<0.001). This TGCI-induced amnesia was abolished by FO as measured by all three parameters (69.0 ± 5.20, 0.86 ± 0.15, 0.17 ± 0.04, P<0.001-0.05 FO vs. vehicle). This protective effect of FO against learning/memory dysfunction was clearly observed after both daily and cumulative data analysis. FO failed, however, to prevent ischemia-induced hippocampal and cerebral cortex damage. Compared to sham, ischemic animals treated with vehicle showed an increased oxidative stress as measured by the level of protein carbonilation (sham: 6.43 ± 0.96, vehicle: 10.17 ± 1.08, P<0.05), an effect that was reverted by FO (6.26 ± 0.76, P<0.05 vs. vehicle). FO also improved the antioxidant activity of SOD (P<0.05 vs. veh), and glutathione concentration (P<0.05 vs. veh). Discussion: The present findings suggest that FO treatment is able to prevent the cognitive impairment caused by TGCI, an effect that was not accompanied by histological protection. FO treatment also showed antioxidant properties, which might have contributed to the antiannemic effect of FO following TGCI. Research support: CAPES, CNPq and Universidade Estadual de Maringá (UEM).
CysLTR antagonists decrease pentylentetrazol-induced seizures. Marafiga JR, Lenz QF, Jesse AC, Mello CF UFSM – Physiology and Pharmacology

Introduction: Clinical and experimental evidence suggests that inflammation plays an important role in the pathophysiology of epilepsy. In line with this view, selected pro-inflammatory arachidonic acid derivatives have been reported to facilitate seizures. The aim of this study was to examine whether leukotrienes receptors (CysLTR) antagonists (Bay-u9973 – CysLT1/2R dual antagonist and pranlukast – selective CysLT1R antagonist) decrease pentylentetrazol – induced seizures. Methods: Adult male Swiss mice were stereotaxically implanted with a cannula into the right lateral ventricle, and two electrodes were placed over the parietal cortex along with a ground lead positioned over the nasal sinus for EEG recording. The experiments were performed 7 days after surgery. The effects of Bay-u9973 (0.3, 3 or 30 nmol/1µL, i.c.v.) and pranlukast (1 or 3 µmol/1µL, i.c.v.) on PTZ (1.8 µmol/2µL, i.c.v.)-induced seizures were evaluated. The animals were placed in acrylic boxes and habituated for 20 min before the EEG recording. After this period, the electrode was connected to the digital electroencephalograph. The baseline EEG activity was recorded for 10 minutes. The animals were then injected with the antagonist or vehicle 30 min before the administration of PTZ. After the injection of PTZ, the animals were monitored for additional 30 min for the appearance of seizures, by electrographic and behavioral methods. The latency to and number of myoclonic jerk episodes and generalized tonic-clonic seizures were measured, as well as EEG mean amplitude.

Results: Bay-u9973 increased the latency to generalized seizures [H(3)=20.63; p < 0.001], but did not alter the latency to myoclonic jerks. Moreover, Bay-u9773 decreased the mean amplitude (in µV) of EEG traces after PTZ administration in the two recording leads, in a 30 min observation period [F(3,20) = 4.17; p <0.01]. Pranlukast decreased seizures susceptibility as measured by a significant increase in the latency to PTZ-induced clonic [H(2)=8.704; p <0.05; Fig. 7A] and generalized seizures [H(2)=10.98; p < 0.01; Fig. 7B] as well as decreased the mean amplitude (in µV) of EEG recordings of PTZ-induced seizures [F(2,15) = 6.05; p<0.01].

Discussion: Nonselective and selective antagonists of CysLTR receptors increase the latency to seizures and decrease the mean amplitude of seizure-related encephalographic recordings. These findings suggest a facilitatory role of CysLTR1 in PTZ-induced seizures and CysLT1 receptors may be a suitable target for anticonvulsant development.

Sources of research support and acknowledgements: CAPES, CNPq, FAPERGS, PRPGP/UFSM, PIBIC/UFSM. The protocols followed the official Government Ethics Guidelines and were approved by the University Ethics Committee (Nº 69/2010).

Fish oil reduces cognitive deficits after acute cerebral ischemia, but not after chronic cerebral hypoperfusion in middle-aged rats. Ferreira EDF, Zaghi GGD, Romanini CV, Oliveira RMMW, Milani H UEM – Farmacologia e Terapêutica

Introduction: Ischemic brain disease results in serious neurodegeneration and cognitive impairments, and might be related to the development age-related dementias. Omega-3 polyunsaturated fatty acids, mainly docosahexaenoic acid (DHA), has been show to afford neuroprotection in animal models of cerebral ischemia. We reported the treatment with fish oil (FO), a rich natural source of DHA, prevented the retrograde amnesia caused by transient, global cerebral ischemia (TGCI) in young rats. Objective: Here we evaluated whether FO could be effective when given to middle-aged rats subjected TGCI or chronic cerebral hypoperfusion (CCH). Method: Naïve rats were trained for 15 days in radial maze task. On day 16 they were subjected to TGCI (15-min, 4-VO model) or CCH (4-VO/ICA model). Retention of the previously acquired cognition (i.e., memory) was assessed weekly on days 30, 37, 44, 51 and 58 after TGCI or CCH. FO treatment (DHA 300 mg/kg, p.o., once a day) started at 4 hours after TGCI and continued for 9 days consecutively. The same treatment was given after CCH, except the duration of treatment extended up to the 57th day of 4-VO/ICA. On the end of behavioral testing, neurodegeneration was examined by Nissl staining. The procedure was approved by the local Ethics Committee on Animal Experimentation (Protocol no. 044/2008). The Kruskal-Wallis ANOVA was used for between-group comparisons across the various retention testing. Results: Memory retention is reflected by the ability of rats to remember the goal box location learned during the preoperative training, and it is expressed by the parameters: latency, number of reference memory errors and number of working memory errors. Compared to sham, the rats subjected to TGCI and treated with vehicle showed increased latency (554.2 ± 59.1 vs. 234.0 ± 64.7), reference errors (13.94 ± 1.4 vs. 3.05 ± 1.9), and working errors (3.97 ± 1.4 vs. 0.46 ± 0.6). Similar latency and number of errors were observed after CCH, indicating profound and sustained memory deficit in relation to the respective sham-operated groups (p < 0.0001 to 0.05). Compared to vehicle, the FO treatment attenuated TGCI-induced amnesia (latency: 411.0 ± 96.0, p < 0.05; reference error: 7.97 ± 4.1, p < 0.01; working errors: 1.09 ± 0.8, p < 0.01). FO did not change, however, the cognitive effect of CCH (p > 0.05, vehicle vs. FO). This antiamnesic effect of FO occurred in the absence of neurohistological protection measured in both hippocampus and cerebral cortex. Conclusion: Both TGCI and CCH causes profound memory impairments (amnesia) and neuronodegeneration in middle-aged rats. FO treatment reverted amnesia after TGCI, but not after CCH. However, the positive effects of FO on memory recovery (or preservation) were not accompanied by attenuation of hippocampal and cortical neurodegeneration. Reference: Fernandes, J.S., Mori, M.A., Ekuni, R., Oliveira, R.M.W., Milani, H., 2008. Long-term treatment with fish oil prevents memory impairments but not hippocampal damage in rats subjected to transient, global cerebral ischemia. Nutrition Research 28, 798–808. Supported by CAPES, FundaçãoArâucária and UEM.

Introduction: Parkinson’s disease (PD) is a neurological disorder characterized by a progressive loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) associated with a-synuclein deposition and Lewy body formation in various regions in the central nervous system, besides the progressive motor impairment (Hirsch et al. 2013). In addition to motor symptoms, depression and anxiety are frequently observed in parkinsonian patients. Rotenone is a pesticide extracted from _Leguminosa_ plants that acts by inhibiting complex I of the electron transport chain. Several studies have demonstrated the capacity of this toxin in mimetizes the major symptoms of PD, specially the motor ones (Bové and Perier, 2012). In our study we evaluated the potential of oral and prolonged administration of rotenone to induce motor and non-motor symptoms of PD in mice. Methodology: Male Swiss mice (~30 g) were randomly distributed in 2 groups: vehicle (sunflower oil) and rotenone (15 mg/kg). Rotenone or vehicle was administrated by gavage (10 ml/Kg), daily, for 28 days. Open-field test was conducted on days 29, 35, 42 and 49. In addition, forced swimming test (FST) and marble burying test (MBT) were performed on day 50, while tail suspension test (TST) and elevated plus maze test (EPM) were performed on day 51. All data were analyzed by ANOVA, followed by the Tukey’s test. Statistically significant differences were set at p <0.05. Results are expressed as mean ± SEM and reported as vehicle and rotenone groups respectively (n= 10-14). Our protocol complies with the recommendations of the Federal University of Paraná and was approved by the University Ethics Committee (protocol n. 589). Results and discussion: Our results demonstrate that administration of rotenone is able to induce severe and progressive motor impairment characterized as hypolocomotion (traveled distance) on days 29 (17.55 ± 1.14; 13.43 ± 1.03) 35 (14.30 ± 1.22; 10.87 ± 0.93) and 42 (12.00 ± 1.13; 8.74 ± 1.00). In our protocol, rotenone was not able to induce depressive-like behavior in both, FST (swimming 197.57 ± 5.90; 196.92 ± 6.84/ climbing 5.07 ± 1.95; 36.92 ± 7.29/ immobility 37.35 ± 6.37; 6.14 ± 2.32) and TST (immobility 99.90 ± 11.06; 113.20 ± 7.45). In the other hand, animals treated with rotenone exhibited an anxiogenic-like behavior on EPM test (% time in open arm: 32.65 ± 5.22; 17.14 ± 2.23). However, no differences were observed between groups on MBT (% marble burying 38.00 ± 5.28; 26.29 ± 5.91). This is the first time, to our knowledge, that oral administration of rotenone is described as capable of inducing, in addition to motor impairment, anxiogenic-like behavior in mice. Thus, we believe that new studies are needed to verify if the biological mechanisms mediating these responses are the same as those underlying the human behavior. Key-words: Parkinson’s disease; Rotenone; Depression; Anxiety References: Hirsch EC, et al. Movement Disord, v. 28, 24, 2013. Bové J, et al. Neuroscience, v. 1, 211, 2012. Sponsored: CAPES, CNPq, Fundação Araucária
Anxiolytic-like effect of acute podoandin administration in mice. Bonato JM, Schiavon AP, Amoah SKS, Biavatti MW, Oliveira RMMW. Pharmacology and Therapeutics, UFSC - Pharmaceutical Sciences

Introduction: *Hedyosmum brasiliense* is an aromatic shrub, commonly known as “cidrão”, which belongs to the largest genus of the Chloranthaceae family. The genus consists of 46 species found in tropical and subtropical regions of America and is endemic to Brazil. Although widely used as a calmative, hypnotic, stomachic, aphrodisiac, to treat migraine, and for diseases of the ovaries, studies attempting to validate the pharmacological effects are still limited. Recently, it was demonstrated that the ethanol extract of the leaves of *Hedyosmum brasiliense* and podoandin, which is an isolated sesquiterpene lactone of the guaianolide type, exhibit antidepressant-like effects in the tail suspension and forced swimming tests in mice. The aim of this study was to investigate the anxiolytic-like effect of acute administration of podoandin in mice.

Methods: Male Swiss mice at 7 weeks of age, (35–40 g) received an acute intraperitoneal (i.p.) administration of vehicle (saline with 4% DMSO), podoandin (10 and 20 mg/Kg) or diazepam (1 mg/Kg), one hour before being submitted to the elevated plus maze (EPM). The number of closed arm, and open arm entries, and the time spent in the open and closed arms were recorded for 5 min for each animal. The percentage of open arm entries (%OAE=100 X open/total entries) and of the time spent in the open arms (%OT=100 X open/open+closed time) were calculated. The experimental procedures performed adhere to the ethical principles set down by the Brazilian College of Animal Experimentation (COBEA), and approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEEA 42/2012), Paraná, Brazil. Statistical analysis was performed by one-way ANOVA followed by Tukey’s test for post hoc comparisons.

Results: All data are given as the mean ± S.E.M. Acute treatment with podoandin 20 mg/Kg showed a significant increase the %OT (F3,38= 6.99, P=0.0008; vehicle=19.52 ± 2.8, podoandin 10 mg/Kg=15.39 ± 2.3, podoandin 20 mg/Kg=37.14 ± 5.9, diazepam=34.81 ± 5.8) and a statistical trend to increase the %OAE (F3,38= 2.48, P=0.078; vehicle=30.75 ± 3.2, podoandin 10 mg/Kg=30.03 ± 3.7, podoandin 20 mg/Kg=41.73 ± 3.1, diazepam=39.82 ± 4.6). Without changing the number of closed arm entries (F3,38=2.49, p>0.05; vehicle=10.67 ± 0.8, podoandin 10 mg/Kg=10.55 ± 1.1, podoandin 20 mg/Kg=11.00 ± 1.4, diazepam=14.11 ± 1.1) in the EPM when compared with the positive control.

Conclusions: Acute administration of podoandin results in anxiolytic-like effect in mice.

Keywords: *Hedyosmum brasiliense*, podoandin, elevated plus maze, mice.


Introduction: One of the most common nonmotor symptoms of Parkinson’s disease (PD) is depression, which affects approximately 35% of patients and may impair their quality of life. Curcumin, a major active compound of Curcuma longa, has been found to have several pharmacological properties. It has anti-inflammatory activity, inhibits monoamine oxidase, and has neuroprotective effects. This compound has already been extensively studied in various models of depression and, more recently, it has been studied in PD models. However, little is known about its effects on depression associated with PD. The present study investigated the antidepressant-like effect of curcumin in an animal model of 6-hydroxydopamine (6-OHDA)-induced PD. Methodology: Male Wistar rats were randomly distributed into 4 groups (n=9-14/group): sham-vehicle; sham-curcumin; 6-OHDA-curcumin and 6-OHDA-vehicle. All of the animals were anesthetized with equitesin (0.3 ml/kg, i.p.), and 6-OHDA was bilaterally infused (6 μg/μl of cerebrospinal fluid + 0.2% ascorbic acid) into the substantia nigra pars compacta (SNpc) during a stereotaxic surgery, using a 27-gauge needle attached to a 10 μl syringe (Hamilton, USA). The sham groups also underwent the same procedure but received artificial cerebrospinal fluid instead of 6-OHDA. All of the rats were treated for 21 days with curcumin (30 mg/kg, p.o.) or vehicle (sunflower oil, p.o.) beginning 1 h after surgery. Twenty four hours and 21 days after surgery the animals were subjected to the open field test. Immediately after, the rats were evaluated in the forced swimming test (FST). Another group of rats were subjected to the sucrose preference test that was conducted before surgery (basal) and subsequently performed weekly until day 21. After the last sucrose preference test, the striatum and hippocampus of some rats were dissected to quantify monoamines. The remaining animals were submitted to a perfusion process for immunohistochemical analysis. The statistical analyses used were one-way ANOVA followed by the Newman-Keuls post hoc test and two-way ANOVA followed by the Bonferroni post hoc test (sucrose preference test). Our protocol was approved by the Federal University of Paraná Ethics Committee (protocol #590). Results and discussion: 6-OHDA groups presented a reduction in motor parameters 1 day after surgery (sham-vehicle 99.3 ± 7.58, 6-OHDA-vehicle 49.4 ± 14.5, 6-OHDA-curcumin 28.9 ± 9.4, p<0.01) which was reversed on 21th day (sham-vehicle 79.8 ± 9.0, 6-OHDA-vehicle 69.2 ± 10.2, 6-OHDA-curcumin 71.6 ± 12.7). The animals 6-OHDA treated with curcumin exerted an antidepressant-like effect in both FST (immobility: 6-OHDA-curcumin 10.2 ± 3.2, 6-OHDA-vehicle 68.7 ± 14.5, p<0.01) and sucrose preference test. Neurochemical analyses showed that curcumin treatment increased dopamine levels on striatum (6-OHDA-curcumin 4.9 ± 0.7, 6-OHDA-vehicle 2.7 ± 0.1, p<0.01). Moreover, curcumin was able to prevent death of dopaminergic neurons in the SNpc, as observed on immunohistochemical analyses (6-OHDA-curcumin 98.1 ± 4.1, 6-OHDA-vehicle 83.1 ± 5.1, p<0.01). These findings suggest antidepressant-like and neuroprotective effects of curcumin in 6-OHDA model of PD.

Introduction: Parkinson’s disease (PD) is a progressive neurodegenerative disease characterized by loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc) and consequent decreased levels of dopamine (DA) in the striatum [1]. PPAR-α receptor agonists, such as fenofibrate, has been shown a major role in the regulation of inflammatory processes. PPARα is expressed in various tissues, including CNS in the SNpc and striatum [2]. In this study we evaluated the effects of treatment with fenofibrate 100 mg/kg (po) starting 5 days before administration of rotenone ip 2.5 mg/kg, and continuing with rotenone for 10 days.

Methods: The rats were divided into 4 groups: CM C (fen vehicle)/girasol oil (rot vehicle), CMC/Rot, fen/oil and fen/Rot. After undergoing the treatments was performed modified open field (OF) test 1, 7, 14 and 21 days after the last day treatment (day 15). On day 22 was performed forced swimming (FS) test. On day 23 the striatum were removed for determination of DA. The protocol complies with the recommendations of UFPR and was approved by the Institutional Ethics Committee n° 590. Results: FS and dosage DA: one-way ANOVA followed by Tukey test. OF and AT: two-way ANOVA followed by Bonferroni test. Values are expressed as mean ± standard error of mean (SEM). Significance level: p≤0.05. Data show a significant difference (SD) between CMC+oil and CMC+rot groups and a SD between CMC+rot and CMC+fen groups on day 15 in the distance and speed depicted in the OF. On days 7, 14 and 21 there wasn’t SD between the groups in to treatment [F(3.116)=3.562; p=0.0164], weeks time [F(3.116)=13.34; p=0.0001] and interaction [F(9.116)=5.003; p<0.0001] in the distance and to treatment [F(3.116)=4.241; p=0.1553], weeks time [F(3.116)=1.778; p=0.1553] and interaction [F(9.116)=2.183; p=0.0280] in the speed depicted. In the FS, the immobility time showed a SD between CMC+rot and CMC+oil groups and was observed a SD between CMC+rot and fen+rot groups [F(3.30)=10.09; p=0.0001]. In addition was showed a SD between fen+rot and CMC+rot groups in the swimming time [F (3.30)=6.142; p=0.0025]. The striatal DA showed SD between CMC+rot and CMC+oil groups. In addition, been found SD between fen+rot and CMC+rot groups and showed no significant difference with the CMC+oil group [F(3.23)=4.938; p=0.0100].

Effect of piroxicam in depressive-like behavior in animal model of Parkinson's disease.

Santiago RM1, Barbiero J1, Tonin FS1, Zaminelli T1, Boschen SL1, Andreatini R1, da Cunha C1, Lima MMS2, Vital MABF1

1UFPR – Farmacologia, 2UFPR – Fisiologia

Depression is one of the most common psychiatric symptoms in patients with Parkinson's disease (PD) (Cimino et al., 2011). This disease has also been observed in animal models of PD the animal demonstrated depressive-like behavior (Santiago et al., 2010). Some authors reported that depression is characterized by activation of the inflammatory response (Hirsch et al., 2012). This study investigated the effects of the treatment with piroxicam in depressive-like behavior of rats injured with 6-OHDA in the substantia nigra.

Methods: The rats were randomly distributed into four groups: sham+saline, sham+10 mg/kg piroxicam, 6-OHDA+saline, 6-OHDA+10 mg/kg piroxicam. On day 0 the animals underwent stereotactic surgery, when the animals in the experimental group received bilateral infusion in the substantia nigra of 6-OHDA and sham group animals received bilateral infusion of cerebrospinal fluid. One hour after the stereotaxic surgery the animals were treated with 10mg/ kg piroxicam or saline 0.9% (p.o) once a day for 22 days. Open field test was conducted in the subsequent 1st and 21st days. In addition, the same animals were tested in the modified forced swim on day 22. Another set of animal underwent analogous randomization were submitted to the sucrose preference consumption test 7th and 22st days. The protocol complies with the recommendations of Federal University of Paraná and was approved by the University Ethics Committee (protocol number 470).

Results: In the swimming 6-OHDA+saline group presented significant reductions in comparison to the sham+saline group and increased immobility time compared to the control group. The sucrose preference test the animals in 6-OHDA+piroxicam group showed no reduction in preference for sucrose compared to control group. The analyses of the 5HT within the hippocampus was found significantly decreased in the 6-OHDA+saline group (P<0.001) compared to control group on day 21.

Discussion: The treatment for 21 days with the piroxicam was capable to reverse the onset of depressive-like behavior in treated animals and prevent the reduction of hippocampal 5HT.

Acknowledgments: This work was supported by grants from CNPq, and CAPES that had no further role in the study design, in the collection, analysis and interpretation of data, in writing of the report, and in decision to submit the paper for publication. RA, CC and MABFV are recipient of CNPq fellowships.

GABAB receptor agonist only reduces ethanol drinking in light-drinking mice. Lima MR1, Villas Boas GR1, Silva AP1, Trufini RF1, Zamboni CG2, Lacerda RB2 – 1Uniamerica – Farmacologia, 2UFPR – Departamento de Farmacologia

Introduction: Baclofen, a GABAB agonist, reduces ethanol intake in animals and humans, but the contrary or no effect has also been reported. Our previous study demonstrated that mice characterized as “loss of control over ethanol intake” had different Gabbr1 and Gabbr2 transcription levels, which express, respectively, the GABAB1 and GABAB2 subunits in brain areas related to addictive behavior. In the present study, we tested baclofen on ethanol intake in mice exposed to the free-choice paradigm.

Methods: Sixty adult male Swiss mice, individually housed, had free access to three bottles: ethanol (5% and 10%) and water. The protocol had four phases: acquisition (AC, 10 weeks), withdrawal (W, 4 cycles during 2 weeks of 2 day-free-choice and 2 day-only-water), reexposure (RE, 2 weeks), and adulteration of ethanol solutions with quinine (AD, 2 weeks). Then, mice characterized as “loss of control” (A, n = 11, preference for ethanol in AC and maintenance of ethanol intake levels in AD), heavy (H, n = 11, preference for ethanol in AC and reduction of ethanol intake levels in AD), and light (L, n = 16, preference for water in all phases) drinkers were randomly distributed into two subgroups receiving either intraperitoneal injections of all doses of baclofen (1.25, 2.5, and 5.0 mg/kg, given each dose twice in consecutive days) or saline, being exposed to free-choice. Fluid consumption was measured 24 h later. All animal maintenance, care, and treatment procedures were controlled and approved by the Ethics Committee for Animal Experimentation of the Biological Science Department, Universidade Federal do Paraná (process nº. 23075.105451/2009-19; approved in November 10, 2009).

Results: Baclofen reduced ethanol intake in group L. In group H a reduction compared to AC was observed. Group A maintained their high ethanol intake even after baclofen treatment.

Discussion: Activation of the GABAB receptor depends on the precise balance between the GABAB1 and GABAB2 subunits, so the disproportionate transcription levels, we reported in group A, could explain this lack of response to baclofen.

Conclusion: This work highlights the importance of testing baclofen in individuals with different ethanol drinking profiles, including humans. And it points to the fact that the GABAB receptor agonist only reduces ethanol drinking in light-drinking mice. Our results show the relevance of studies with baclofen effects on different ethanol intake profiles in animals and maybe also in humans with different degrees of alcoholism.

Acknowledgments: We thank Silvia N.C. Genaro for technical assistance. No specific funding for this study was provided. The only support provided was from the Universidade Federal do Parana to G.R.V.B. and D.C., recipients of fellowships from CAPES, and A.V.R.L. and M.P., recipients of fellowships from FAPESP and CNPq respectively.

Presynaptic M₁ and A₂A receptors play roles in facilitatory effect caused by methylprednisolone in neuromuscular transmission. Oliveira L¹, Costa AC², Noronha-Matos JB¹, Silva I¹, Ambiel CR², Corrado AP³, Alves-do-Prado W⁴, Correia-de-Sá P¹ ¹Imuno-Fisiologia e Farmacologia, ²UEM – Ciências Fisiológicas, ³FMRP-USP – Farmacologia, ⁴UEM – Farmacologia

It has been well determined that methylprednisolone (MP, 0.3 mM) acting on motor nerve terminal is able to increase the acetylcholine release, but the mechanisms involved with such effect caused by the corticoid were not completely elucidated. Since muscarinic (M) cholinergic (facilitatory-M₁, inhibitory-M₂) and adenosine (facilitatory-A₂A, inhibitory-A₁) receptors on motor nerve terminal play key roles in neuromuscular transmission, we investigated the effects caused by selective blockers of M₁ (pirenzepine; PZP, 10.0 nM), M₂ (AF-DX 116, 10 nM), A₂A (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol; ZM241385; 10.0 nM) and A₁ (DPCPX, 2.5 nM) receptors on the facilitatory effect caused by MP in the phrenic nerve diaphragm muscle of rats indirectly stimulated at 50 Hz. Rats (Wistar, 150–250 g) of either sex were killed after stunning followed by exsanguination. Animal handling and experiments followed the guidelines defined by the European Communities Council Directive (86/609/EEC).

The procedures used for montage of neuromuscular preparations, labeling the neurotransmitter, and measuring evoked [³H]acetylcholine ([³H]ACh) release according Correia-de-Sá et al. 1996 (Correia-De-Sá, J. Neurophysiol. 76, 3910, 1996). [³H]ACh release was evoked by two periods of electrical stimulation (50 Hz-bursts, 5 trains of 5 sec duration, with 20 sec intertrain interval) of the phrenic nerve, starting at the 12th (S1) and 39th (S2) minutes after the end of washout (zero time). Data obtained in presence of MP separately and combined with PZP, ZM241385 or atropine were evaluated at S2 instant. Drugs were added in the bath 15 min before S2 and statistical analysis of data were carried out using ANOVA followed by Dunnett’s (p< 0.05). The facilitatory effect (40 ± 11%, n=5) caused by MP (0.3 mM) was reduced (p < 0.05) by 10.0 nM PZP (from 40 ± 11%, n=5 to 13 ± 8%, n=6) and 10 nM ZM241385 (from 40 ± 11%, n=5 to 21 ± 9%, n= 4). The facilitatory effect caused by MP was not (p > 0.05) changed by 10 nM AF-DX 116 (n=4) and 2.5 nM DPCPX (n=3), selective blockers of presynaptic inhibitory-M₂ and inhibitory-A₁ receptors, respectively. In contrast, it was verified that the effect of MP was incremented by 0.2 µM Atropine (from 40 ± 11%, n=5 to 77 ± 15%, n=4). Exocytose caused by MP was confirmed by real-time video microscopy using the FM4-64 fluorescence dye. The effect caused by atropine in the facilitatory effect caused by MP was also confirmed in myographic records. In such conditions, atropine produced a dramatic (192%) increase in facilitatory effect induced by MP. Data indicate that part of effects obtained with the combination of atropine with MP were not solely determined by blockage of presynaptic muscarinic receptors, but the facilitatory-M₁ and facilitatory-A₂A receptors on motor nerve terminal play key roles in facilitatory effect caused by MP in neuromuscular transmission.

Financial supports: CNPQ/Fundação Araucária
Cannabidiol treatment reduces long term memory impairment promoted by aging process.

Introduction: The set of biological changes triggered by aging process create several modifications at molecular and cellular levels that culminate in mnemonic and cognitive deficits. Cannabidiol (CBD) is the major non-psychotomimetic constituent of Cannabis sativa with a large number of pharmacological effects such as anti-inflammatory and neuroprotective. The aim of this study was to evaluate if CBD chronic treatment affects the mnemonic profile of aged mice.

Methods: C57BL6 male mice (3, 12 and 18 months old) were treated daily with CBD (30mg/Kg/day) or vehicle injections (2% tween + 98% saline) for 14 days. On day 15th, twenty four hours after the last CBD or vehicle injections, all groups were submitted to the Object Recognition Task (OR). Results were expressed by mean +/− SEM; p<0.05. All experiment protocols were approved by the local ethics committee (protocol 104/209). Results: Aged mice (12 and 18 months old) displayed long term memory impairment in the OR when compared to young controls (3 months old). CBD treatment reverted the memory impairment induced by aging process at 12 months but not at 18 months old treated mice (vehicle: young: 65.5+/- 3.9; 12 months old: 53.5+/- 4.9; 18 months old: 53.5+/- 3.7 CBD: young: 66.3+/- 5.6 ; 12 months old: 72.8+/- 5.5 * ; 18 months old: 53.9 +/-4.6). No effects on distance travelled were found.

Discussion: The results presented here suggest that CBD chronic treatment decreases memory impairment induced by aging process. Financial support: CNPq
RNA interference with nNOS protects SH-SY5Y cells from interferon gamma injury. Silva SS, Lustosa CF, Ferreira NR, Del Bel EA, Titze-de-Almeida R. FAV-UnB – Terapia Gênica, FORP-USP – Neurofisiologia e Biologia Molecular

Introduction: The vulnerability of dopaminergic neurons to chemical insults remains a relevant issue in neuropathology. Previous studies found increased levels of interferon gamma (IFN-γ) and the neuronal nitric oxide synthase enzyme (nNOS) during neuronal injury. No previous work, however, has evaluated whether nNOS affects the viability of dopaminergic neurons exposed to IFN-γ. We used siRNAs and short-hairpin RNA expression vectors to silence nNOS, and evaluated their effects on IFN-γ-injured SH-SY5Y cells.

Methods: Firstly, neuroblastoma cells (ATCC® CRL-2266™ - SH-SY5Y) were plated in 96-well plates (5X10⁴ cells/well) and incubated for 24 hours with the injuring agent IFN-γ (37.5 ng/mL, Interferon Gamma®, Invitrogen). After that, cells received siRNA nNOS (4400) or the negative control scramble (18.75 nM), both mixed with Lipofectamine. They were incubated for 8h or 24h. The cell viability was determined by using the colorimetric MTT (3-(4,5)-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide) assay. For that, 15 μL of the MTT-labeling reagent (0.5 mg/mL, Invitrogen) was added to each well and the plate was maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for an additional 3h-period. Insoluble formazan was dissolved with dimethylsulfoxide, and the MTT reduction was measured at 595 nm. Control cells without treatment were taken as 100% viability. Regarding to expression vector’s neuroprotective effects, we examined whether pnNOS (4400) would ameliorate the viability of SH-SY5Y cells injured by IFN-γ. For that, the cells (5X10⁴) were first incubated for 24 hours with the injuring agent IFN-γ (37.5 ng/mL), in 96-well plates. Effects on cell viability were determined by MTT at two vector doses (0.2 μg or 0.4 μg) and three time points (8h, 24h or 48h).

Results: Treatment for 24h with siRNA nNOS (4400) caused a significant increase in cell viability, in comparison with the mock-treated group (62% versus 57%, respectively; P < 0.05). The ability of pnNOS (4400) to improve the cell viability after injury was also examined. At 8h post-transfection, the vector (0.2 μg) increased 9.7% the cell viability of injured cells (90.2% versus 99.9%; P < 0.05). The effects were dose-dependent. The higher plasmid dosage (0.4μg) caused the highest neuroprotective effect, at 24h post-transfection. The viability of injured neuroblastoma cells improved 15.8% in the group treated with pnNOS (4400), compared to the mock-treated group (99.0% versus 83.2%, respectively; P < 0.05). At 48h post-transfection, no positive effects occurred in any plasmid doses.

Discussion: To our knowledge, no previous work has addressed the role of nNOS in neuronal damage caused by IFN-γ. The present study reveals that both siRNA nNOS (4400) and the vector pnNOS (4400) have ability to protect SH-SY5Y cells from the injury caused by IFN-γ. In conclusion, our data suggest the nNOS enzyme plays at least a partial role in cell degeneration caused by IFN-γ.


Financial support: CAPES, CNPq, FAP-DF.
Alpha-melanocyte stimulating hormone (α-MSH) does not alter pilocarpine-induced seizures. Temp FR, Santos AC, Marafiga JR, Jesse AC, Guerra GP, Scimonelli TN, Mello CF. 1UFSM – Fisiologia e Farmacologia, 2UFSM – Fisiologia e Farmacologia, 3UTFPR, 4Universidade de Córdoba

Introduction: Epilepsy is a brain disorder characterized by an enduring predisposition to generate epileptic seizures. Several lines of experimental and clinical evidence support a relationship between inflammation and epilepsy. Alpha-melanocyte stimulating hormone (α-MSH) is involved in different neurological functions, which include anti-inflammatory effects in central nervous system (CNS). In this study we investigated whether α-MSH decreases pilocarpine-induced seizures.

Methods: Adult male Swiss mice were used. α-MSH (0.1, 0.3 or 1 mg/kg, i.p. or 1.66, 5 or 15 μg/3 μL, i.c.v.) or vehicle (sterile 0.9% NaCl, 10 mL/kg, i.p. or phosphate buffered saline –PBS–, pH 7.4, 3 μL, i.c.v.) was administered thirty minutes before pilocarpine (370 mg/kg, i.p.). In the following forty minutes the animals were observed for the appearance of seizures. Latency to generalized tonic-clonic seizures, number of seizure episodes, total time spent seizing and Meurs scale were recorded.

Results: Regardless of the administration route, α-MSH did not alter the latency to pilocarpine-induced tonic-clonic seizures, number of seizure episodes, total time spent seizing and Meurs score.

Repeated cannabidiol administration results in antidepressant-like effect in mice. Schiavon AP, Bonato JM, Guimarães FS, Milani H, de Oliveira RMMW. UEM - Farmacologia e Terapêutica, FMRP-USP - Farmacologia.

Introduction: Cannabidiol (CBD), the main non-psychotomimetic cannabinoid derived from the plant *Cannabis sativa*, possesses a wide therapeutic potential. CBD administration has been shown to exert antipsychotic and anxiolytic effects in humans as well as in several experimental models involving rodents. However, the study of CBD actions has been mostly restricted to its acute effect, whereas its efficacy after chronic administration was largely unclear. Recently, a study has shown that repeated CBD administration prevented the anxiogenic effect of chronic unpredictable stress in mice as evidenced by decreasing the latency to eat in the novel environment in the novelty suppressed feeding test and by increasing the open arm exploration in the elevated plus maze (EPM). The aim of this study was to investigate the effect of repeated CBD administration in mice subjected to the EPM and tail suspension (TST) tests. Methods: Male Swiss mice at 7 weeks of age, weighing 35–40 g received intraperitoneal (i.p.) administration of CBD (30 mg/Kg) or appropriate vehicle (saline with 1% Tween 80) during 15 consecutive days. The last doses of the treatments were administered one hour before the behavioral testing. The mice were firstly submitted to the EPM (5 min) and subsequently to the TST (6 min). In the EPM, the percentage of open arm entries (%OAE=100 X open/ total entries), the percentage of the time spent in the open arms (%OT=100 X open/ open + closed time) and the number of closed arm entries were calculated. In the TST, the latency to present the first episode of immobility and the immobility time during the last 4 min of the test were registered. All experimental procedures adhered to the ethical principles set down by the Brazilian College of Animal Experimentation (COBEA), and were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEEA 42/2012), Paraná, Brazil. Statistical analysis was performed by one-way ANOVA followed by Newman-Keuls’ test for post hoc comparisons. Results: Data are given as the mean ± S.E.M. ANOVA showed no significant differences for %OAE (F(2,28)= 0.57, P=0.57), %OT (F(2,28)= 1.20, P=0.32) or the number of closed arm entries (F(2,28)= 1.57, P=0.22) among the groups. Repeated treatment with CBD demonstrated no significant differences concerning the latency for the first episode of immobility (F(2,28)=2.36; P=0.11) but resulted in significant decrease in immobility time (F(2,28)=11.11; P=0.003; vehicle=126.6 ± 6.21; CBD=95.9 ± 12.38; imipramine=59.27 ± 9.12) when compared to the vehicle group. Discussion: Repeated CBD administration resulted in decreased of immobility time in mice subjected to the TST, which is indicative of antidepressant-like effect. Keywords: Cannabidiol, elevated plus maze, tail suspension test, mice. Financial support: CAPES, CNPq and UEM.
Alpha-melanocyte stimulating hormone (α-MSH) does not prevent pentylenetetrazol-induced seizures. Santos AC1, Temp FR1, Marafiga JR1, Jesse AC1, Guerra GP2, Scimonelli TN3, Mello CF1 UFSM – Fisiologia e Farmacologia, 2UTFPR, 3Universidade de Córdoba

**Introduction:** Current evidence suggests that inflammation plays a role in the pathophysiology of seizures [1]. α-Melanocyte stimulating hormone (α-MSH) is involved in different neurological functions and also exerts anti-inflammatory effects, including in the central nervous system (CNS) [2,3]. Considering that several studies have suggested a role for brain inflammation in PTZ-induced seizures [4, 5] and that α-MSH has anti-inflammatory effects in the CNS, we investigated whether the systemic administration of α-MSH decreases the PTZ-induced seizures.

**Material and Methods:** Adult male Swiss mice were used. α-MSH was injected at the doses 0.1, 0.3 or 1 mg/Kg (i.p.), or sterile 0.9% NaCl (10 ml/kg, i.p.), 15 or 30 minutes before the injection of PTZ (60 mg/Kg, i.p.). Immediately after PTZ injection, the animals were monitored for thirty minutes for the appearance of behavioral seizures. The analyzed parameters were: Latency to myoclonic jerks; latency to tonic-clonic seizures; number of seizure episodes; total time spent seizing and Racine scale. α-MSH doses and injection intervals were chosen based on a previous study [6].

**Results:** α-MSH (0.1, 0.3 or 1 mg/Kg, i.p.) administered 15 or 30 minutes before PTZ, did not alter the latency to PTZ-induced myoclonic jerks, tonic-clonic seizures, number of seizure episodes, total time spent seizing and Racine scale. α-MSH also did not alter seizure severity, as assessed by the modified Racine score [7].

**Discussion:** The current study shows that systemic administration of α-MSH does not attenuate seizures, regardless of α-MSH dose or time between peptide and convulsant injection. These findings may have been due to poor diffusion of the peptide across the blood-brain barrier (BBB) [8, 9]. The current results do not support a protective role for α-MSH against PTZ-induced seizures.

**Acknowledgements:** CAPES, CNPq, FAPERGS, PIBIC/UFSM, PRPGP/UFSM. The protocols followed the official Government Ethics Guidelines and were approved by the University Ethics Committee (Nº 008/2013).

Spinal cord trpV1 receptor activation by nitric oxide mediates nociception. Rossato MF¹, Beck VR², Hoffmeister C², Ineu RP², Funck VR¹, Oliveira M¹,², Ferreira J¹,²,³. ¹UFSM – Biochemical Toxicology, ²UFSM – Pharmacology, ³UFSC – Pharmacology

Introduction: Nitric oxide (NO) is produced in spinal cord during nociceptive events. It may react with molecular oxygen or superoxide anion to produce peroxinitrite, both been neutralized by antioxidants, as glutathione. Accordingly, intrathecal (i.t.) administration antioxidants may induce antinociception in vivo. Moreover, it has been demonstrated that NO may activate TRPV1 in vivo, a receptor involved in detection and transmission of nociceptive stimuli, in vitro. Thus, we decided to investigate whether spinal cord NO/peroxinitrite production may elicit TRPV1 activation and produce nociception in vivo. Methods: Male Swiss mice (25-35 g, n=6 per group) received an i.t. injection of vehicle, N-acetylcysteine (antioxidant), L-NAME (NO synthase inhibitor) or SB366791 (TRPV1 antagonist). Fifteen minutes after, they received an intraplantar injection of capsaicin. Overt nociception and heat hyperalgesia were measured from 0 to 5 minutes and 15 to 90 minutes after capsaicin, respectively. Different groups of animals were euthanized 30 minutes after capsaicin injection and their lumbar spinal cord was collected to quantify the amount of thiols (SH), stable NO metabolites (nitrite/nitrate, NOx) and product 3-nitrotyrosine (3-NT), formed by peroxinitrite. Also, we evaluated the effect of SB366791 over the hyperalgesia induced by i.t. injection of butionine-sulfoxamine (BSO – inhibitor of glutathione synthesis, a NO/peroxinitrite scavenger), arginine (ARG – substrate for NO synthase), nitroglycerine (NTG – nitric oxide donor) and SIN1 (peroxinitrite donor). This project was approved by CEUA/UFSM process number 124/2011. Results: The intrathecal pretreatment with NAC, L-NAME and SB366791 prevented both overt nociception (100.0 ± 9.5; 95.1 ± 16.1 and 76.9 ± 16.5%, respectively) and heat hyperalgesia induced by capsaicin (73.3 ± 6.9; 42.8 ± 4.9 and 58.8 ± 10.4% inhibition, respectively). Furthermore, the pre-treatment with N-acetylcysteine and L-NAME also prevented the decrease in SH levels (122.9 ± 42.1 and 76.9 ± 16.5%, respectively) and the increase in NOx (104.4 ± 29.1 and 73.97.1%) and 3-NT production (68.3 ± 11.0 and 116.2 ± 30.1%) caused by capsaicin. Also, the pre-treatment with SB366791 fully prevented the hyperalgesia induced by BSO, ARG, NTG and SIN1 (100.2 ± 17.1, 90.5 ± 7.5, 94.7 ± 21.4 and 100.0 ± 2.2% inhibition, respectively). Discussion: The nociception caused by peripheral injection of capsaicin seems induces the production of NO/peroxinitrite, responsible for the spinal activation of the TRPV1 receptor. Acknowledgements: Financial aid supported by CAPES, CNPq and UFSM.
µ and κ opioid-receptors in the prelimbic cortex have a facilitatory influence on the cardiovascular responses to acute restraint stress in rats. Fassini A, Scopinho AA, Resstel LBM, Corrêa FMA FMRP-USP – Farmacologia

Introduction: The ventral medial prefrontal cortex (vMPFC) is a limbic structure divided in prelimbic (PL) and infralimbic portion (IL). Electrical or chemical stimulation of PL cause cardiovascular responses. Restraint stress (RS) causes cardiovascular responses such as increased of blood pressure and heart rate. Microinjection of the unspecific synaptic blocker in the PL increased HR response to RS, without effect on the blood pressure response, suggesting that PL synaptic mechanisms have an inhibitory influence on restraint-evoked HR changes. However, the possible neurotransmitters present in vMPFC that are involved in this modulation have not yet been identified. Opioid peptides and their receptors are present in the PL. Furthermore, the central opioid system is known to modulate the cardiovascular system, even during aversive situations. Hence, the objective of this study was to investigate the role of opioid-receptors in PL on the cardiovascular responses caused by RS. Methods: Experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto (n°.173/2011). Male Wistar rats (240-280g) were used. Guide cannulae were implanted bilaterally in the PL for drug injection of naloxone (nonselective opioid antagonist in the doses of 0.003, 0.03, 0.3, 3 and 30nmol), CTAP – D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂ (µ-selective antagonist in the dose of 0.3nmol/100nL), nor-binaltorphimine dihydrochloride – nor-BNI (κ-selective antagonist in the dose of 0.003nmol/100nL), naltrindole hydrochloride (δ-selective antagonist in the dose of 0.3nmol/100nL) or vehicle (artificial cerebrospinal fluid, aCSF, 100nL) and a polyethylene catheter was implanted in the femoral artery for MAP and HR record, using a computerized acquisition system. 10 minutes before microinjection of drugs or vehicle into the PL, rats were subjected to RS. Results: Non-linear regression analysis of bilateral microinjection of naloxone in the PL generated inverse U-shaped dose-inhibition curves for both MAP (r²=0.6514, df=29, P<0.05) and HR (r²=0.4679, df=29, P<0.05) values. Bilateral microinjection of CTAP at dose of 0.3nmol/100nL RS (n=7; MAP: F₁,72=15.81, P=0.0002 and HR: F₁,72=18.85, P<0.0001) or nor-BNI at dose of 0.003nmol/100nL in PL (n=6; MAP: : F₁,66=17.67, P<0.0001 and HR: F₁,66=6.4, P=0.01) reduced the increase of MAP and tachycardia caused by RS. On the other hand, naltrindole at dose of 0.3nmol/100nL (n=6) had no effect on the restraint-evoked MAP (F₁,66=0.37, P=0.54) and HR (F₁,66=0.005, P=0.94) increases. Conclusion: The current results demonstrate that the µ and κ opioid-receptors in PL modulate the cardiovascular responses caused by RS, suggesting a facilitatory role of this structure in this aversive situations. Bibliographic references: TAVARES, J Neurosci Res. 87(11):2601-7, 2009. KIRITSY-ROY, J Pharmacol Exp Ther. 239(3):814-22, 1986. Financial Support: CAPES, FAPEA and FAPESP
Introduction: It is widely recognized that music exerts physiological effects in the whole body leading to various behavioral and cognitive changes. Furthermore, music has been reported to foster the activation of neural circuits and parts of the brain linked to emotional behavior. However, many gaps still exist regarding the therapeutic properties of music that deserve to be investigated. Previous studies have demonstrated that Mozart’s music has a modulatory effect on brain activity, mainly due to the increase of dopamine levels of the central nervous system, suggesting that dopamine could be one the main neurotransmitter responsible for different types of behaviors. Thus, in this work we aimed to study the interference of music in different stages of development of the central nervous system of rats.

Methods: All animal procedures were in accordance with the ethical committee for animal care (UnBdoc No. 59772/2013). 21-days-old female Wistar rats (n= 30) were divided into two groups, (1) untreated (controls), (2) exposed to music (music), and placed in a closed room equipped with a speaker, where taped music (average sound level: 65 dB) was played. Mozart’s sonata for two pianos (K. 488) was played repeatedly for 4h (two sessions of 2h each) per day over 4 days. Animals were submitted to open field test, elevated plus-maze test (EPM), forced swimming test, and step-down inhibitory avoidance test, to evaluate locomotor activity, anxiety- and depression-like behaviors, and short and long-term memories, respectively. Animals were retested at two and three months of age.

Results: Mozart’s sonata modified the behavioral response in a significant way only at 2 months old age, as observed by the increase in the percentage of open arm time (control= 4.91 ± 1.69; music= 15.12 ± 2.40; p<0.05) at EPM. This effect seems independent of locomotor activity, as no significant change was observed when the animals were evaluated in the open field test. At the forced swimming test, 21-days-old rats increased the immobility time (control= 22.13 ± 5.45; music= 75.73 ± 8.01; p<0.05), and no relevant effect in short- and long-term memories was observed at the investigated ages.

Conclusion: Based on these results we observed that the behavioral changes resulting from music therapy seems to be age- and/or experimental model-dependent, whereas the anti anxiolytic effect with music was only observed at two months of age. The behavior of animals in the forced swim test, culminating in a time of immobility greater than the control, seems to reflect only the low weight of the animals, and not necessarily reflects a depressive disorder. The mechanisms of action and the neurotransmitters involved in these responses are still being investigated.

Acknowledgements: PIBIC/CNPq/UnB.
Introduction: Ubiquitous nature of oleanic acid (OA) has made it a common ingredient present in many fruits and herbs. OA has been widely consumed for many centuries without health hazard. Previous results had associated anxiolytic and antidepressive like properties of Pimenta pseudocaryophyllus to the presence of oleanic acid (Fajemiroye et al., 2012 & 2013). Multifunctional nature of OA can be partly justified by its susceptibility to chemical modification on its C-3 OH, the C-12–C-13 double bond and the C-28 COOH that produces series of new synthetic oleanane triterpenoids. According to Jacob and Alain (2012) OA as a natural substrate could release bioactive metabolites. Hence, present work probes antianxiety – antidepressive like property of OA, investigate underlining mechanism and study biological activity of its analogs towards new drug development.

Methods: Male Swiss mice (25 ± 6 g) were subjected to barbiturate sleep, light dark box (LDB), elevated plus maze (EPM), forced swimming (FS), open field (OF), quantification of brain derived neurotrophic factor (BDNF)] as approved by ethics committee (UFG -104/08). Synthesis of OA derivatives and MAO bioassays were also conducted.

Results: Oleanolic acid (20 or 40 mg/kg, p. o.) increased duration of sodium pentobarbital (40 mg/kg, i.p.) induced sleep. The LDB and EPM data showed increase in time spent in the light compartment and in the open arms respectively. Meanwhile, anxiolytic like property of OA remained unaltered with non anxiogenic dose of pentylenetetrazole (PTZ 20 mg/kg, i.p.). Unlike OA at 40 mg/kg, OA 5, 10 or 20 mg/kg reduced immobility time in the FS. In the open field OA 40 mg/kg reduced the number of sector traversed and number of rearings. Pretreatment with p-chlorophenylalanine methyl ester (PCPA 100 mg/kg, i.p), α-methyltyrosine (AMT 100 mg/kg, i.p and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido) butyl]piperazine hydrobromide (NAN 0.5 mg/kg, i.p) blocked antidepressive-like property of OA in the FS. Chronic administration of OA increased hippocampal BDNF level. Monoamine bioassays shows IC 50 > 100 μM of this triterpene and most of its analogs (methyl oleanolate, oleanolic acid 3-acetate and Michael acceptor -type of compounds). Discussion: An increase in time spent at the light area and on the open arm of LDB and EPM respectively demonstrated anxiolytic like property of OA. Unaltered effect of OA despite PTZ pretreatment suggests non-involvement of GABA A receptor. Reduction in immobility time in the forced swimming test indicates antidepressive like property. Effects of OA 40 mg/kg in the FS and OF suggest possible myorelaxant and/or sedative action. Reversal of OA activity by AMPT (catecholamine synthesis inhibitor), PCPA (serotonin depletor) or NAN (SHT 1A) pretreatments implies monoamine mechanism while an increase in BDNF levels suggests involvement of neurotrophic factors. An IC 50 values demonstrated non-inhibition of OA and most of its derivatives.

Financial supports: FUNAPE/UFG, FAPEG, CAPES, CNPq

Extracellular ATP directly triggers ligand-gated ion channels receptors (P2XR), while, the G protein coupled receptors P2YR and P1R have higher affinity to ADP and adenosine, respectively. The hydrolysis of ATP by ectonucleotidases regulates the amount of purinergic species available in the synaptic cleft, therefore, regulates which purinergic receptor subtype is activated by nerve released ATP. Ecto-nucleoside-triphosphate-diphosphohydrolases (NTPDase) and ecto-nucleotide pyrophosphatase/ phosphodiesterase (NPP) hydrolyzes ATP and ADP to AMP, while ecto-5'-nucleotidase (5'N) hydrolyses only AMP to adenosine (ADE) which is subsequently deaminated to inosine by adenosine deaminase (ADA). Understanding the dynamic of this pre and post synaptic interaction requires a model in which the activation of each receptor leads to a different functional output.

**Aim:** Taking into account that rat pineal gland present a daily variation of sympathetic tonus, and that ATP is co-released with noradrenaline, here we evaluated the relevance of the enzymes that controls ATP metabolism on the output of the purinergic stimulation. We characterized the influence of the light/dark cycle on ectonucleotidases activity in rat pineal glands and the effect of P1 and P2 agonists on melatonin synthesis.

**Methods:** Female Wistar rats (45 days-old) kept under 12/12h light/dark cycle were decapitated at ZT0, 4, 8, 12, 16, 20. Ethical protocol (CEUA/IB 175/2013). The RNA was isolated with TRIzol and the cDNA synthetized with SuperScript-III. Specific primers of Ntpdases (1-3,5,8), Npp (1-4), 5'N and the internal control Gapdh were used. For NTPDase activity the glands were homogenized in the reaction medium and 0.7 mg/mL of protein was incubated for 10min with sodium azide (10mM), prior to 20 min incubation (37°C) with ATP, ADP or AMP. Inorganic phosphate was measured by the colorimetric method. Enzyme activity was reported as nmol Pi released/min/mg protein (Int. J. Devl. Neuroscience 21(2003):75). Cultured rat pineal glands were stimulated with ISO (0.1µM, 5h) in the presence or absence of ATP (0.01–3mM), ADP or adenosine (0.01–1mM). MEL content in the medium was measured by HPLC and expressed as ng/well.

**Results:** The ectonucleotidase activity and gene transcription showed a daily variation. ATP (52.1 ± 4.6 vs 32.6 ± 1.7, n=4, p<0.05) and AMP (83.9 ± 1.2 vs 47.7 ± 5.3, n=4, p<0.05) hydrolysis increased at the dark phase. ADP and ADE hydrolysis did not change between the daytime and nighttime. In the light phase there was a peak of NTPDase3 (ZT8) and NTPDase5 (ZT12) gene transcription. Transcripts of 5'N peaked in ZT20 and others NTPDases and NPPs did not change along the day. ISO-induced MEL production (61.86 ± 6.09, n=7) was inhibited by ATP or ADP and increased by ADE. The co-stimulation of ISO (0.1µM) and ATP (3mM) reduced the content of MEL to 30.51 ± 6.8 (n=5, p<0.05), with ADP (1mM) to 30.98 ± 6.1 (n=7, p<0.05), and with ADE the MEL content was increased to 124.2 ± 22.4 (n=4, p<0.05). **Conclusion:** The variation in the activity of the enzymes suggests that purinergic neurotransmission relies on higher adenosine presence at nighttime, while at daytime ATP or ADP should be responsible for purinergic neurotransmission. Therefore, a putative contribution of ATP on melatonin production is probably related to the available purine species in the synaptic cleft, as the metabolization of ATP varies along the day.

**Support:** CAPES, FAPESP, CNPq.
Exposure of adolescent rats to methylphenidate and cross-sensitization to ethanol: Preliminary findings. Gelain MAS1, Gelain MS1, Freese L1, Pereira NSC2, Costa PA1, Caletti G1, Bisognin KM1, Souza MF1, Nin MS1, Gomez R3, Barros HMT1 1UFCSPA − Ciências Básicas da Saúde, 2UFRGS

Introduction: The methylphenidate (MPH) is the most widely used psychoactive drug in the treatment of attention deficit hyperactivity disorder (ADHD). Individuals are treated for long periods with this drug, extending up to adulthood. Drug abuse begins in adolescence, and ethanol (EtOH) is the most used drug among teenagers, and is commonly used in combination with psychostimulants. Studies show that individuals with ADHD have an increased risk of developing substance abuse. Teens of many species differ from adults in sensitivity to drugs of abuse. The chemical changes during adolescence contribute to the behavioral differences observed in this phase of life and may explain the greater vulnerability of adolescents to addiction. The sexual gender has been appointed as an influencing factor on the use and abuse of psychoactive drugs. It is known that repeated treatment with a drug of abuse produces sensitization to itself and cross-sensitization with other substances - which strongly suggests common neurobiological mechanisms. Despite the cross-sensitization between methylphenidate and other drugs have already been thoroughly addressed, not observed in the literature reporting on the phenomenon of cross-sensitization between methylphenidate and EtOH. The objective of this study is to investigate whether treatment with methylphenidate during adolescence produces cross-sensitization to EtOH in adulthood and whether there are differences between the sexes in this phenomenon.

Methods: This study was approved by Animal Ethics Committees / UFCSPA, license 1034/10. We used 14 male and 15 female Wistar rats periadolescent - postnatal day (PND) 40 and weighed 140g at the beginning of the experiment. Animals were randomly divided into two groups, male (control, MPH) and female (CTR, MPH). The MPH group received MPH 2.5 mg/kg intraperitoneal (IP), the control group (CTR) received saline (SF) IP, PND 43-48. When adults (PND 133), the male and female rats were divided per sex into three groups - MPH (received EtOH), CTR (received EtOH) and CTR (received SF). The animals were given 2g/kg of 20% EtOH IP immediately before being exposed to the open field test. All the animals were evaluated in one 10 min session. The tests were videotaped and analyzed by two blinded observers. We evaluated the locomotor activity of animals. Ethanol was diluted from 100% to a final concentration of 20% (v/v). The data were analyzed using Sigma Stat 3.0 and ANOVA. Considered statistically significant if P <0.05.

Results: No statistical differences in locomotor activity among adolescent females treated with MPH or SF and, in adulthood, exposed to EtOH. Males MPH-EtOH locomoted less than males CTR-SF (P <0.01), and CTR-EtOH (P <0.05)

Discussion: Our preliminary data indicate that there is not cross-sensitization between MPH and EtOH. Perhaps repeated exposure to MPH during adolescence would cause downregulation of dopamine receptors and protects against abuse of EtOH. References: Garland EJ. Psychopharmacol, 12, 385, 1998. Kuczenski R. J Neurochem, 68, 2032, 1997. Financial agencies: UFCSPA, CAPES, CNPq
Introduction: Besides being a relay station for auditory pathways and tonically modulated by GABAergic neurotransmission, the inferior colliculus (IC) has been involved - as other structures of the Brain Aversive System - in mediating defensive responses elicited by aversive stimuli. However, the contribution of this structure in the behavior of rats submitted to the elevated plus maze (EPM) with the use of a pharmacological agent such as the benzodiazepine midazolam, have not been studied yet. Our objective was to analyze the effects of midazolam in three different doses, injected into the dorsal (ICd) or ventral portions (ICv) of the inferior colliculus of rats submitted to the EPM. Methods: 62 adult male Wistar rats from the animal colony of USP-RP were implanted with guide cannulas into the IC via stereotactic surgery. Five days after surgery, midazolam was microinjected into IC at doses of 5, 10 or 20 nmol/0.4ml. Control animals received saline (0.9% NaCl) into the same volume and route of administration. Twenty minutes after microinjection, rats were submitted to the EPM. Each rat was placed in the center of the EPM facing one of the closed arms, and its activity was recorded for 5 minutes. We analyzed the number of entries and time spent in open and closed arms, as well as complementary ethological measures. Histological analysis later determined the sites of microinjections. Data were analyzed using one-way ANOVA followed by Newman-Keuls post-hoc test (Ethic Commission for the Use of Animals Protocol No. 11.1.308.53.9). Results: The dose-response curve of midazolam at doses of 5, 10 and 20 nmol microinjected into the IC of rats submitted to the EPM indicated the dose of 10 nmol as the lowest effective dose to achieve an anxiolytic-like effect in this model, as long as injected into the ventral portion of the IC. The anxiolytic effect detected in the animals receiving treatment via ICv was not observed in animals whose cannula was positioned in the ICd, confirming the neural segregation between these two areas. The anxiolytic effect of 10 nmol of midazolam injected into the ICv included increases in the percentage of time spent [F(3,28)=3.81; p<0.05] and number of entries into the open arms [F(3,28)=4.686; p<0.05], frequency of end arm exploration [F(3,28)=5.207; p<0.01] and head dipping [F(3,28)=5.486; p<0.01]. Midazolam in ICv also reduced the frequency of risk assessment behaviors [F(3,28)=5.718; p<0.01], which is an expected effect of anxiolytic drugs. Discussion: This study shows for the first time that midazolam acts selectively on the ICv to produce anxiolytic effects. It is remarkable the pharmacological dissociation of the reactivity of the ventral and dorsal divisions of the IC to the local injection of midazolam. Financial Support: FAPESP
Age-related changes induced by lipopolysaccharide on α2,3-Na,K-ATPase activity, cyclic GMP levels and oxidative status in rat hippocampus. Vasconcelos AR1, Yshii LM1, Böhmer AE1, Lima LS1, Alves R1, Andreotti DZ1, Marcourakis T2, Scavone C1, Kawamoto EM1 1ICB-USP, 2USP – Análises Clínicas e Toxicológicas

Introduction: Chronic neuroinflammation is a common characteristic of neurodegenerative diseases, such as Alzheimer’s and Parkinson’s diseases, that may contribute to loss of function and cell death. Sodium, potassium pump (Na,K-ATPase) plays an important role to maintain cell ionic equilibrium. Disruption of Na,K-ATPase activity and cyclic GMP (cGMP) signaling could lead to oxidative stress process which could be detrimental to the cells. In this project we compared the effects of an inflammatory stimulus induced by Lipopolysaccharide (LPS) administration on the Na,K-ATPase activity, cGMP and TBARS levels in hippocampus of young, adult and aged rats.

Methods: 4, 12 and 24-month-old male Wistar rats were euthanized 2 h after LPS i.v. (1 mg/kg). Hippocampus samples were used to measure Na,K-ATPase activity by a colorimetric assay that determines the isoforms α1 and α2,3-Na,K-ATPase activities separately, cGMP levels by enzyme-linked immunosorbent assay (ELISA), and oxidative status by thiobarbituric acid reactive substances (TBARS) assay. This research was approved by the Biomedical College of Animal Experimentation (COBEA). All procedures were also approved by the Ethical Committee for Animal Research (CEEA) of the Biomedical Sciences Institute of the University of São Paulo (protocol 53, page 17, book 2).

Results: The results showed that Aging induces a progressive decrease in hippocampal total-Na,K-ATPase activity at 12 and 24 months when compared with the values detected at 4 months, which is due to a reduction in α2,3-Na,K-ATPase activity, since α1-Na,K-ATPase and Mg-ATPase activities are not changed. Also, LPS caused a decrease of the total-Na,K-ATPase activity 2 h after intravenous injection at 4 and 12 months. This effect was also specific to α2,3-Na,K-ATPase. We also showed that cGMP levels decrease at 12 and 24 months when compared to 4-month-old animals. TBARS determinations showed that aging is linked to progressive increase in products of lipid peroxidation at 12 and 24 months when compared to 4-month-old rats. Moreover, LPS treatment decreased cGMP levels at 4 and 12 month-old animals but not at 24 months when compared with respectively aged control groups. On the other hand, TBARS levels were increased after LPS at 4, 12 and 24 month-old animals when compared to respectively aged control groups.

Discussion: Taken together, these findings suggest that aging is associated with a progressive decrease of protective signaling related to an increased risk for deficits on brain function and neurodegenerative disorders which are aggravated by inflammation. Financial support: CNPq, CAPES and FAPESP
The influence of Na,K-ATPase isoforms in ouabain signaling cascade against LPS induced NF-kB activation in glial cells. Kinoshita PF, Yshii LM, Orellana AMM, de Sá Lima L, Kawamoto EM, Scavone CI

Introduction: Ouabain is known as an endogenous hormone produced in hypothalamus and adrenal gland. This cardiac glycoside binds to Na,K-ATPase and it can activate signaling pathways in concentrations that is not linked to the common effect of the enzyme inhibition. In fact, some data have reported that ouabain can protect against some types of injury. Glial cells have an important role in the response against injury in the brain and they can also control inflammation which is crucial to trigger neurodegenerative diseases. NF-kB is a transcription factor involved in inflammation. LPS was used as a model of inflammation and the aim of the study is to evaluate if the treatment with ouabain before LPS was capable of reduce the activation of the transcription factor NF-kB caused by LPS.

Methods: the primary mouse cortical glia culture was treated with ouabain in a range of concentrations in LPS (1μg/ml) effects in cell viability (LDH) and cell proliferation (MTT). RelA (NF-kB subunit) nuclear translocation was evaluated by immunofluorescence assay. Treatments were performed in culture medium (DMEM) without serum with different ouabain concentrations (1μM, 10μM, 100μM), time of incubation (15 minutes, 1 and 6 hours) and LPS in different time points (1, 2 and 6 hours). Then, we selected the concentrations that decreased the RelA nuclear translocation in presence of LPS and we evaluate the activity of NF-kB by the electrophoretic mobility shift assay. The procedure was approved by the Ethical Committee for Animal Research (CEEA) of the Biomedical Sciences Institute of the University of São Paulo (registered under number 37 in the pages 101 of the book 2, 2011).

Results: None of the different ouabain concentrations altered the cell viability and some ouabain doses increased the cell proliferation. The pre-treatment with ouabain (10μM) for 1 hour decreased the NF-kB activation induced by LPS (1μg/ml, 1h).

Discussion: Taken together our data showed that ouabain pretreatment reversed the NF-kB activation induced by LPS in primary mouse cortical glia culture from mice which shows the anti-inflammatory effect of ouabain against LPS.

Sponsors: FAPESP, CNPq
Short-term sustained hypoxia increases the glutamatergic transmission in Nucleus Tractus Solitarius (NTS) neurons of juvenile rats. Accorsi-Mendonca D, Almado CEL, Machado BH

Introduction: The synaptic transmission in central nervous system is affected by hypoxic conditions. Chronic intermittent hypoxia (CIH), during 10 days, depresses the glutamatergic afferent neurotransmission in nucleus tractus solitarius (NTS), area related to the integration of peripheral chemoreceptor inputs. Aim: To analyze the effect of short-term SH (24 hours - 10% O₂) on the intrinsic properties and synaptic transmission of NTS neurons from juvenile rats (21 days old - Protocol of the Animal Ethic Committee # 070/2007). Methods: The electrophysiological properties of NTS neurons were recorded in brainstem slices using whole cell patch clamp technique. Results: SH produced no change in resting membrane potential (-78.7 ± 2.4 vs -72.3 ± 3.7 mV), input resistance (1.4 ± 0.4 vs 1.4 ± 0.4 GOhm) or capacitance of NTS neurons (14.2 ± 0.9 vs 12.4 ± 0.8 pF). However, SH increased the frequency of spontaneous excitatory post-synaptic currents (3.6 ± 0.4 vs 7 ± 1.5 pF) and the amplitude of evoked excitatory post-synaptic currents(-289 ± 51 vs -472 ± 64 pA). Moreover, SH also increased the depression of evoked excitatory post-synaptic currents after five stimuli on afferent fibers (amplitude of 5 evoked currents of control group: 100%, 44%, 34%, 32% and 28%, n=15; amplitude of 5 evoked currents of SH group: 100%, 40%, 28%, 22% and 17%, n=15; *p<0.001 - Two way ANOVA). Conclusion: These findings suggest that SH alters the short-term plasticity of NTS neurons due to an increase in release probability, but does not change their intrinsic properties and may play a critical in the cardiovascular and respiratory problems faced by those humans exploring high altitudes. Financial support: CAPES, FAPESP and CNPq.
Intrahippocampal injection of ouabain activates NF-kB and Wnt-beta-catenin signaling pathway in rats.

Orellana AMM, Yshii LM, Böhmer AE, Kinoshita PF, de Sá Lima L, Andreotti DZ, Kawamoto EM1, Scavone C USP – Farmacologia

Introduction: The enzyme Na⁺,K⁺-ATPase (Sodium Potassium Adenosine Trisfosfatase) is an integral membrane protein, highly conserved in eukaryotes, which through hydrolysis of one ATP molecule establishes the electrochemical gradient across the plasma membrane, which is essential to maintain the osmotic balance of cells, the resting membrane potential and the excitatory property of nerve and muscle cells. Besides its role in ion homeostasis, several recent studies suggest that this pump may also act as a signal transducer and transcription activator involved in cell growth, differentiation and programmed cell death. Ouabain (OUA), the ligand of Na⁺,K⁺-ATPase, is a steroid derivative that is produced by the adrenal cortex and hypothalamus. After OUA binding, the Na⁺,K⁺-ATPase signaling seems to activate pathways such as Src, MAPK, NF-kB and Ca²⁺. Some evidence indicate a possible crosstalk between the NF-kB signaling pathway and WNT canonical pathway, however the molecular mechanisms are still unknown. WNT canonical play important role during embryogenesis and in adult tissue homeostasis, as it is related to neurogenesis process. The aim of this study was to verify whether the intrahippocampal administration of OUA can modulate NF-kB activity and WNT signaling cascade.

Methods: Adult male Wistar rats were used at the age of four months. All procedures were approved by the Ethics Committee on Animal Experimentation of the Institute of Biomedical Sciences in accordance with the requirements described in the Brazilian College of Animal Experimentation (COBEA) protocol, registered under n° 124 on pages. 93 of Book 2. Western blot, RT-PCR and Electrophoretic mobility shift assays were used to determine changes in both canonical pathways at several intervals (1, 2, 10, 24 and 48 hours) after OUA.

Results: Results indicate that OUA (10 nM) was able to activate the NF-kB signaling pathway after 1, 10, 24 and 48 hours following an oscillatory profile. OUA also seemed to modulate WNT canonical pathway, since after 10 hours of injection there was an increased phosphorylation of GSK-3b, whereas in 24 hours, we observed increased nuclear translocation of β-CATENIN. Moreover, we found increased levels of BDNF throughout the time course studied.

Discussion: In summary, OUA 10 nM is able to first activate NF-kB signaling pathway and later modulate some proteins of WNT canonical pathway. Financial support: CNPq /FAPESP (Brazil) KEY TERMS: Ouabain, Wnt, β-CATENIN, BDNF, Na,K-ATPase
Effects of reversible inactivation of the dorsal hippocampus on the cardiovascular responses activated by the chemoreflex and the possible involvement of NMDA receptors. Kuntze LB, Ferreira-Júnior NC, Lagatta DC, Resstel LBM FMRP-USP – Pharmacology

Introduction: The chemoreflex is a regulatory mechanism of cardiovascular and ventilatory responses that ensures organism’s homeostasis even before hypoxia or hypercapnia conditions meanly regulated by the nucleus of the solitary tract and the rostral ventrolateral bulb area. There are studies that suggest the involvement of limbic structures in neural pathways that modulate the peripheral chemoreflex. So we hypothesized that the dorsal hippocampus (DH) can participate in modulation of cardiovascular responses evoked by chemoreflex. The aims are investigate the participation of DH in the modulation of the chemoreflex and assess the involvement of glutamatergic system present in this area. Methods: Male Wistar rats, weighing 200-220g, had stainless steel guide cannulae bilaterally implanted into the DH. Afterwards, a catheter was inserted into the femoral artery for mean arterial pressure (MAP) and heart rate (HR) recording. Chemoreflex activation was induced by infusion of potassium cyanide (KCN, 40 μg/0.05 ml/animal) through a second catheter implanted into the femoral vein before, 10 and 60 minutes after the bilateral microinjection of chloride cobalt (CoCl₂), DL-AP7 or vehicle into the DH. The magnitude of changes in MAP and HR was quantified at the peak and the duration and the data are expressed as means ± SEM. The experimental procedures are carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto (protocol number: 98/2013). Results: Vehicle group (500nl; n=12) showed a typical systemic cardiovascular pressor (before=61.75 mmHg ± 3.8, 10 min= 58 mmHg ± 4.1; F(2,35)= 0.27; p>0.05) and bradycardia (before= -318.3 bpm ± 15.50, after= -346.8 ± 16.16; F(2,32)=0.95; p>0.05) responses to chemoreflex. Reversible DH inactivation by CoCl₂ (1 mmol/ 500 nl; n=15) evoked a significant increase of the bradycardiac response (before= -278.3 bpm ± 18.65, 10 min= -337.1 bpm ± 13.77; F(2,44)=3.0; p<0.05), although no significant changes in the magnitude of pressor response (before= 61.20 mmHg ± 3.9, 10 min= 60.8 mmHg ± 3.9; F(2,44)=0.6; p>0.05). On the other hand DL-AP7 treatment (1 nmol/ 500 nl; n=10) was not able to affect significantly both pressor (before= 44.8 mmHg ± 5.7, 10 min= 47.6 mmHg ± 5.2; F(2,28)=0.6; p>0.05) and bradycardic responses (before= -304 bpm ± 15.63, 10 min= 334.5 bpm ± 16.26; F(2,29)=1.08; p>0.05), but a trend of increased bradycardiac was noted. The duration of all responses were not altered by any treatment. Sixty minutes after treatment the chemoreflex responses returned basal levels. Discussion: Our preliminary findings suggest that the DH neurotransmission have some modulatory effect on parasympathoexcitatory component of the peripheral chemoreflex activation and NMDA glutamate receptors are possibly involved. References: G. V. Guimaraes, Arq Bras Cardiol, 96, 161, 2011. J. Duffin, Respir Physiol Neurobiol, 173, 230, 2010. A. S. Haibara, Am J Physiol 276, R69, 1999. E. M. Granjeiro, Exp Physiol, 96, 518, 2011. L. B. Resstel, Cereb Cortex, 18, 2027, 2008. M. Kuniecki, Acta Neurobiol Exp (Wars), 62, 85, 2002. R. F. Tavares, Life Sci, 81, 855, 2007. Financial support: CNPq.
Medial prefrontal cortex cannabinoid CB1 receptors modulate autonomic responses in rats submitted to restraint stress. Moraes-Neto TB, Fassini A, Correa FMA, Resstel LBM FMRP-USP – Farmacologia

Goals: Acute restraint is an unavoidable stress situation that evokes autonimic changes, characterized by elevated mean arterial pressure (MAP), intense heart rate (HR) increases and decrease in the tail skin temperature. The prelimbic cortex (PL) and the infralimbic cortex (IL) are areas which compounds the ventral portion of medial prefrontal cortex (vMPFC). The PL and the IL glutamatergic system are involved with modulation of tachycardic responses evoked by restraint stress. Moreover, the activation of endocannabinoid CB1 receptors reduces the local PL glutamate releases. Therefore, the objective of the present work was to investigate the involvement of local PL and IL CB1 receptors in the modulation of autonomic responses evoked by restraint stress (RS) in rats. Methods and results: Male Wistar rats (250-270g) had guide cannulae bilaterally implanted in the PL or IL for drug injection and a polyethylene catheter was implanted in the femoral artery for MAP and HR recording. Tail temperature was measured using a thermal camera. The animals were submitted to restraint, which was initiated by introducing animals into a small plastic cylindrical restraining tube (diameter = 6.5 cm and length = 15 cm) and lasted for 60 minutes. The CB1 receptor antagonist AM251 (10, 100 or 300 pmol/200 nL) was administrate 10 minutes before the restraint stress. Results: The RS increased MAP (PL: F3, 864=23.97, P<0.05 e IL: F3, 864=45.73, P<0.05), HR (PL: F3, 864=21.99, P<0.05 e IL: F3, 864=21.16, P<0.05) and decreased the tail skin temperature (PL: F16, 408=65.79, P<0.05 e IL: F16, 408=51.32, P<0.05). In the IL, the administration of 10 and 300 pmol of AM251 reduced the pressor response (F3, 24=13.51, P<0.05). Moreover, the dose of 100 pmol increased the tachycardiac response (F3, 24=18.69, P<0.05) and the dose of 300 pmol reduced the decreases of tail skin temperature (F3, 24=18.69, P<0.05). In the PL the administration of 10 and 300 pmol of AM251 reduced the pressor response (F3, 24=7.9, P<0.05). Moreover, the dose of 100 pmol increased the tachycardiac response (F3, 24=10.86, P<0.001) and the dose of 300 pmol reduced the decreases of tail skin temperature (F3, 24=32.4, P<0.001). Conclusion: The present results shown that vMPFC endocannabinoid system through CB1 receptors modulates the autonomic responses evoked by restraint stress. Moreover, there is no difference between the IL and PL CB1 receptors modulating autonomic restraint stress. Financial support: FAPESP, CAPES and FAEPA. Protocol of COBEA Animal Use Ethic Committee: 063/2010