NEUROIMMUNOENDOCRINOLOGY (NIE)
Introduction: Hepatic encephalopathy (HE) is one of the major complications and causes of death in acute liver failure (ALF) because of brain edema with intracranial hypertension and cerebral herniation. Several studies have used animal models to elucidate the mechanisms involved in HE pathogenesis; however, taking into account the complexity and severity of this syndrome, it is virtually impossible to accurately reproduce all human features of HE using only one model. Here, we propose an alternative murine model of HE using two consecutive doses of intraperitoneal injections of thioacetamide (TAA) in lower dosage instead of a single higher dose administration.

Methods and Results: C57BL/6 mice were submitted to intraperitoneal TAA injection in two dialy doses of 300mg/kg to induce ALF and HE. Liver injury was quantified by serum alanine aminotransferase levels (ALT) and histopathology while hepatic function was assessed through prothrombin (PT) and partial thrombin (PTT) times along with ammonia dosage. Neutrophil influx to the liver was observed by intravital microscopy and remote organ injury in the lungs and in the circulatory system was investigated using histopathology and a volume pressure recording sensor, respectively. Furthermore, neurological function was evaluated by a clinical score based on motor activity and reflexes in parallel with electroencephalography (EEG) and magnetic resonance imaging for cerebral edema. ALF induced mice developed massive liver injury measured by elevation of ALT and neutrophil accumulation in necrotic areas. Also, consequent severe coagulopathy with prolonged PT and PTT was observed in addition to neuropsychomotor abnormalities, fulfilling several ALF features observed in humans. Furthermore, TAA-treated mice presented increased serum and cerebral levels of ammonia in parallel with alterations in EEG spectrum and discrete brain edema characterizing a suggestive scenario of overt HE. In this context of liver failure and neurologic dysfunction, we also observed pulmonary inflammatory infiltration and hemodynamic changes that were indicative of multiple organ dysfunction syndrome, another major cause of death in ALF.

Conclusion: In summary, we developed a new murine model of ALF and HE that also comprise remote organ commitment, which would represent a more realistic illness faced by human patients.

Financial Support: CAPES, CNPq, FAPEMIG
ACETYLCHOLINESTERASE ACTIVITY OF THYMOCYTES AND SPLENOCYTES FROM MDX MICE WITH MUSCULAR DYSTROPHY

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Introduction: Muscular dystrophy in the dystrophin-deficient mdx mouse is characterized at early age (4-6 wks) by extensive myonecrosis, subsequent muscle regeneration (12 wks) and persistent fibrosis (24 wks). Evidence that alterations in the thymus gland correlated with muscular inflammation suggest that similar stimuli could be affecting the physiopathology of dystrophic thymus and skeletal muscles (Quirico-Santos et al., 1995). Importantly, sympathetic innervation of lymphoid organs and activation of the cholinergic anti-inflammatory pathway appear to influence immunological functions and regulate innate immune responses to inflammatory stimuli. This work aimed to determine acetylcholinesterase activity of mdx splenocytes and thymocytes during various stages of disease.

Methods and Results: This project was approved by the Animal Welfare Commission-CEPA/UFF. Mdx and C57control nondystrophic male mice were used at 4, 12, 24 weeks (wks) for isolation of splenocytes and thymocytes. After cell count adjustment, total protein and acetylcholinesterase activity were determined according to Bradford and Ellman methods. Statistical analysis was performed using unpaired Student's t test. At the height (4 wks) of myonecrosis mdx (n=7) splenocytes (424.2 ± 174.3) and thymocytes (18.8 ± 3.8) showed a 2-fold increase in the activity of acetylcholinesterase comparing to C57 nondystrophic (n=11) splenocytes (18.8 ± 3.8) and thymocytes (8.5 ± 3.3). Conversely, mdx mice at 12 wks (splenocytes: 2251 ± 328.3; thymocytes 15.0 ± 2.4) had a respective 3-fold and 19-fold decrease in the acetylcholinesterase activity comparing to C57 (splenocytes: 5999 ± 623.6; thymocytes: 285.8 ± 201.2). At 24 wks corresponding to prominent fibrosis, mdx splenocytes (1807 ± 143.2) had a 2-fold decrease in the activity of acetylcholinesterase in comparison to C57 (3373 ± 814.9), but it was not observed significant difference of acetylcholinesterase activity in mdx thymocytes (1768 ± 582) in comparison to C57 control nondystrophic (1832 ± 805.9).

Conclusion: The availability of acetylcholine in mdx splenocytes and thymocytes due changes in the pattern of acetylcholinesterase activity may be influencing the physiology of lymphoid tissues especially during specific stages (4 wks, and 12 wks) of the disease.

Financial support: FAPERJ; CAPES, FOPESQ-UFF.
ACTIVATION OF PPAR-GAMMA RESTORES MAST CELL NUMBERS AND REACTIVITY IN DIABETIC RATS THROUGH REDUCTION OF HPA AXIS ACTIVITY

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FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: Diabetes induces a reduction in mast cell function and survival (Eur J Pharmacol. 669:143-148, 2011). Since the activation of PPAR-gamma in mast cells increases its differentiation and proliferation (J Pharmacol Sci. 97:190–194, 2005), this study was undertaken to investigate the role of PPAR-gamma in the reduction of mast cell number and reactivity in diabetic rats. Methods and Results: Diabetes was induced by a single IV injection of alloxan (40 mg/Kg) and PPAR-gamma agonist rosiglitazone (0.5 mg/Kg) and/or specific antagonist GW9662 (0.5 mg/Kg) were administered after 3 days of diabetes induction, daily for 18 days (license number for this study P-66/10-4). Mast cell apoptosis and hormone levels were evaluated by analyses of DNA fragmentation and RIA, respectively. Treatment with rosiglitazone restored mast cell numbers in the thoracic cavity and in mesenteric tissue of diabetic rats (respectively from 74.0 ± 13.0 to 119.0 ± 11.0 x10³ cells/ cavity and from 5.7 ± 0.4 to 9.3 ± 0.4 cells/mm², mean ± SEM, n = 5). Rosiglitazone treatment also significantly reversed the diabetes-induced reduction in histamine release by mast cells following activation with antigen in vitro, as measured by fluorescence. Moreover, increased apoptosis in mast cells from diabetic rats were inhibited by rosiglitazone. In addition, we noted that the increase in plasma corticosterone levels in diabetic rats was inhibited by rosiglitazone administration (from 682.9 ± 147.4 to 355.7 ± 78.3 rg/mL, mean ± SEM, n = 5). PPAR-gamma blockade with GW-9662 abolished the ability of rosiglitazone to restore baseline levels of pleural and mesenteric mast cells and plasma corticosterone in diabetic rats (from 475.8 ± 16.3 to 338.3 ± 35.2 x10³ cells/cavity, from 72.0 ± 2.4 to 51.3 ± 2.9 cells/mm² and from 299.5 ± 35.9 to 476.7 ± 18.2 rg/mL, respectively, mean ± SEM, n = 5). Finally, we showed an increase in adrenal expression of ACTH receptor and in plasma ACTH levels in diabetic rats, which was reduced by rosiglitazone treatment (from 2653 ± 401.4 to 968.9 ± 189.1 rg/mL, mean ± SEM, n = 5). Conclusion: Our findings showed that the activation of PPAR-gamma restored the number and reactivity of mast cells in diabetic rats, accompanied by suppression of apoptosis, in parallel with attenuation of alloxan-induced hypercorticolism, indicating that the nuclear receptor has an important role in these phenomena. Financial support: CNPq and FIOCRUZ.
ACUTE INFLAMMATION INDUCES OBESITY IN MICE: POSSIBLE ROLE FOR SERUM AMYLOID A (SAA)

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Introduction. Although obesity is associated with a well-characterized chronic low-grade inflammatory state, more recently it has been suggested that obesity may be the consequence of an inflammatory event. Previously we showed that the acute phase protein serum amyloid A (SAA) alters proliferation, differentiation and metabolism of 3T3-L1 pre-adipocytes thus contributing to the inflammatory state of adipose tissue (Filippin-Monteiro et al., Int J Obes, 2012). Methods and Results. In this study we assessed whether acute inflammation (i.p. administration of 8 consecutive doses of 10 mg/kg LPS for 24 days) induces mouse adipose tissue modification and seek evidence for SAA participation in this process. Animals were fed a normal diet during the period of LPS administration, followed by a 3 weeks 60% high-fat diet. We also evaluated the effects of acute phase serum on the viability and proliferation of 3T3-L1 pre-adipocytes. Mice on a high-fat diet pre-treated with LPS gained more weight than mice pre-treated with saline (52.3±0.5 vs 47.8±0.3 g, **p<0.01) and the epididymal fat contributed more to body weight (7.7±0.2 vs 5.4±0.2 %, **p<0.01, LPS vs saline, respectively) with approximately 25% more adipocyte hypertrophy. However, these mice on a high-fat diet pre-treated with LPS showed reduced inflammatory markers such as SAA and F4/80 and an increased expression of the adipose tissue protective protein perilipin. Acute phase serum was capable of enhancing the survival of 3T3-L1 pre-adipocytes (92.1±2.6 vs 78.8±3.2 %, *p<0.05, LPS vs saline, respectively) and also enhance the proliferation of 3T3-L1 cells in 48 hours (10.7±1.1 vs 8.8±0.7 x 10⁴ cells/well, *p<0.05, LPS vs saline, respectively) Conclusion. Our findings indicate that repeated events of acute inflammation are capable of promoting increased weight gain in mice. Our data is supported by the fact that mice lacking SAA1.1 and SAA2.1 (SAAKO) mice gain less weight and show less macrophage infiltration in adipose tissue with time under standard conditions when compared with the wild type mice. Our results support our hypothesis that SAA is a key molecule in adipogenesis modulation.

Financial support: FAPESP, CAPES and CNPq
The effects of stress cause significant changes on the immune system. Dendritic cells and lymphocytes are key cells of the acquired immune response. The first are the main antigen-presenting cells and, the second are the effectors of acquired immune response. Therefore, we evaluated, herein, dendritic cells and lymphocytes alterations in BALB/c male mice caused by acute restraint stress model (protocol of bioethics committee 2568/2012). The experimental group was undergone three sessions (on alternate days) of restraint stress of placing animals in plexiglas tubes (14x7cm) lasting 2 hours each session. Immediately after the stress session, all the animals were euthanized for blood collection and removal of the spleen. The phenotypic analysis of dendritic cells and lymphocytes present in the spleen was performed by flow cytometry with the following markers: MHCII, CD11c, CD80, CD86, CD3, CD4, CD8 and CD28. The results show that restraint stress did not significantly alter the phenotype of spleen DCs, however it alter the phenotype of CD4 and CD8 lymphocytes populations. The next experiments will investigate the effect of this model of stress on function of these cells by lymphocyte proliferation and endocytosis assays.
ANTIDEPRESSANT EFFECTS OF THE NOCICEPTIN/ORPHANIN FQ RECEPTOR ANTAGONIST UFP-101 IN A MODEL OF LPS-INDUCED DEPRESSION-LIKE BEHAVIOR IN MICE

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Introduction: Nociceptin/orphanin FQ is a 17 aminoacid peptide, and it is the natural ligand of a Gi-protein coupled receptor named NOP. The peptide and its receptor are expressed in brain areas crucial to mood control. The supraspinal administration of the NOP antagonist UFP-101 elicits antidepressant-like effects in rodents subjected to behavioral despair assays. Accumulating evidence suggests that there is a close relationship between the neuroimmune system and emotional states. Thus, the present study aimed to investigate the effects of UFP-101 in the depression-like behavior induced by the systemic administration of lipopolysaccharide (LPS) in mice.

Methods and Results: Experiments were conducted using male Swiss mice (30 g) which were pretreated with LPS (0.8 mg/kg, intraperitoneally [ip]) or vehicle (saline, ip) 24 h before the behavioral assays. The tail suspension test (TST) was used to evaluate the experimental depression, while the distance moved was assessed in the open field test (40x40x40 cm). In the TST, mice were suspended above the floor by an adhesive tape placed 1 cm from the tip of the tail. The time that animals remained immobile during the 6-min session was recorded. Nortriptyline (NTP; 30 mg/kg, ip) was used as standard antidepressant 60 min prior the TST. UFP-101 (10 and 3 nmol, 2μl) was administrated intracerebroventricularly (icv) 5 min before the TST via a stainless-steel cannula permanently implanted. Experimental procedures were approved by Local Ethics Committee (License N° 042/2012). LPS injection increased the immobility time in TST compared to control, which was reversed by NTP. Indeed, NTP per se reduced immobility time (saline=75.2±8.4; LPS=125±8.1*; NTP=32.3±9.2; LPS+NTP=22±6.4; *p<0.05 vs saline; #p<0.05 vs LPS; n=10). No effects in the distance moved were detected in mice treated with LPS, thus suggesting a genuine depressive-like behavior. Icv administration of UFP-101 10 nmol, but not 3 nmol, reduced the immobility time in LPS-injected animals in TST (saline+saline=82.3±10.3; LPS+saline=127.7±7.7; LPS+UFP-101 3 nmol=111.9±26.0; LPS+UFP-101 10 nmol=81.3±10.6; "p<0.05 vs saline; "p<0.05 vs LPS; n=12), while UFP-101 was inactive per se.

Conclusion: The treatment with NTP and UFP-101 10 nmol reversed the LPS-induced depression-like behavior. These behavioral data suggest that UFP-101 may counteract the negative modulation induced by LPS on central neurotransmission.

Financial support: CNPq (N° 476291/2012-7) e CAPES.
ANTIOXIDANT TREATMENT RESTORES MAST CELL NUMBERS AND REACTIVITY IN ALLOXAN-DIABETIC RATS BY REDUCING PLASMATIC LEVELS OF GLUCOCORTICOIDS.

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IOC/ FIOCRUZ, RIO DE JANEIRO - RJ - BRASIL.

Introduction: In our previous studies we demonstrated that diabetic rats showed a decrease in the number of pleural and mesenteric mast cell in close relationship with hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and increase of glucocorticoid levels. Moreover, some diabetes-related morbidities are associated with an increase in reactive oxygen species (ROS). Therefore, in this study we investigated the role of ROS in the decreasing of mast cell number and reactivity observed in diabetic rats. Methods and results: All the procedures used in this study were in accordance with the guidelines of the Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License LW – 23/10. Diabetes was induced by a single IV injection of alloxan into fasted rats and antioxidant Vitamin E or NAC (40 mg/kg and 150 mg/kg p.o. respectively) were administered 3 day after diabetes induction, daily for 18 days. Some animals received a heme oxygenase-1(HO-1) inducer CoPPIX 5 days after diabetes induction, once a week for 3 weeks. Analyses were made 21 days after the diabetes induction and included plasmatic corticosterone levels evaluation by RIA, expression of HO-1 through immunohistochemistry and malondialdehyde (MDA) levels determination by TBARS test. Diabetic animals presented a decrease on both pleural and mesenteric tissue mast cells that was reversed by treatment with vitamin E (respectively from 382 ± 21.3 to 603 ± 59.3 x103 cells/ cavity and from 70 ± 8.6 to 95 ± 4.1 cells/mm2, mean ± SEM, n = 6). Treatment with vitamin E also restores peritoneal mast cells histamine release measured by fluorescence after immunological and non-immunological activation in vitro. In pituitary, diabetic rats presented a decrease on expression of the antioxidant enzyme HO-1 and on the activity of catalase in parallel with an increase on MDA. Treatment with vitamin E and NAC decreased the corticosterone levels in parallel with increased expression of HO-1 in the pituitary of diabetic animals. In addition, treatment with CoPPIX was able to restore the number of pleural mast cells (from 154 ± 2.2 to 280 ± 13.3 x103 cells/ cavity, mean ± SEM, n = 7) and corticosterone levels in diabetic animals. Conclusion: Our results show that ROS are involved in HPA axis hyperactivity and consequent increase on corticosterone which leads to a decrease in the number and reactivity of mast cell in diabetic rats. Financial support: CNPq and FIOCRUZ.
ASTROGLIAL RESPONSE AFTER FATTY ACID TREATMENT

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Introduction: High fat diet causes inflammation on hypothalamus, mainly on neurons that control the satiety and the thermogenesis. Also, apoptosis via TLR4 (toll like receptor 4) activation is observed together with inflammatory condition (Arq Bras Endocrinol Metab. 53:151, 2009/ Plos one. 4:5045, 2009). Neuron death is a process that involves glia and cytokines release. Microglia has immune function in nervous tissue but astroglia, the major component of glia cells, is closer of synaptic plasticity and chronic responses in the central nervous system. In this context, we investigated the astrogliosis reaction after fatty acid treatment on astrocyte cultures.

Methods and Results: Dissociation of cortices from Swiss newborn mice (1-2 days, both sexes) was used for establishment of purified astrocyte cultures (protocol CEUA/UFU040/10). The treatments with different doses (J Neurosci. 29:359, 2009) of stearate and palmitate (50, 100, 200 and 500 µM) were performed during different periods (12, 24, 72 hours or 5 days). Immunocitochemistry was realized with GFAP antibody, an astroglial marker, for reaction level evaluation. Curves for all doses and periods to both fatty acids were built. We could see a progressive response of astrocytes cell to fatty acid treatment. After 5 days of treatment, more intense reaction were observed on doses of 100 µM and 200 µM of stearate and 200 µM and 500 µM of palmitate.

Conclusion: Both fatty acids, stearate and palmitate, induced astroglial reaction after five days of treatment in vitro. This result can indicate a possible contribution of the astrocyte to hypothalamic inflammation observed during high fat diet ingestion.

Financial support: CNPq and PROPP/UFU.
Introduction

Obesity is defined as an excessive accumulation of adipose tissue and occurs when energy intake exceeds energy expenditure (JAMA 303, 235-41, 2010). It is known that type II diabetes, cardiovascular diseases and many types of cancer are associated with obesity (Lancet 371, 569-578, 2008). These associations are given the alarming rise in childhood obesity, which portends an increased incidence of these diseases in obese children reaching adulthood (Int J Cancer 112, 348-351, 2004). Animal models are widely studies to elucidate the development of several diseases. There are reports that BALB/c mice are resistant to obesity and the consumption of diets with a high fat content (HFD) had little effect on the body weight of these animals (Int J Obesity 34:1415-1426, 2010). In this work, we investigate the weight gain and biochemical profile of female BALB/c mice subjected to high-fat diet.

Methods and Results:

The 6-week-old female BALB/c mice were fed HFD (60 kcal% fat) or control diet (CD 10 kcal% fat) for 16 weeks. At the end of this period, cholesterol, triglycerides, leptin and glucose levels of these animals were measured. The weight of the body and perigonadal fat were also evaluated. Since 12 weeks of diet, the HFD animals showed difference in body weight compared to controls (CD 24.34 ± 1.68 and HFD 27.25 ± 3.28) (n = 25). This difference was also observed at 16-weeks (CD 24.66 ± 1.69 and HFD 29.05 ± 4.18) (n = 25). At 16 weeks of diet, weight of perigonadal fat was higher in HFD mice (CD 0.371 ± 0.043 and HFD 1.613 ± 0.358 HFD) (n = 4). The levels of total cholesterol (CD 65.8 ± 7.19 and HFD 119.4 ± 24.8), HDL (CD 66.2 ± 18.95 and HFD 132.4 ± 44.35), leptin (CD 870 ± 231.7 and HFD 5879 ± 5337) and fasting glucose (CD 90.2 ± 8.52 and HFD 107.7 ± 13.15) were also higher in HFD animals (n=5).

Conclusion: Long-term consumption of a HFD, without any reduction in the intake of protein, minerals, vitamins and fiber has an effect on body weight gain, increased perigonadal fat and biochemical parameters related to metabolic disorders and obesity in BALB/c mice. These alterations can compromise the immune system response since the obesity induces a low grade chronic inflammation.

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BENEFITS OF PHYSICAL ACTIVITY IN PERIPHERAL AND CENTRAL INFLAMMATORY RESPONSES INDUCED BY A TOTAL SLEEP DEPRIVATION

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Introduction: Controlled, experimental studies on the effects of acute sleep loss have shown that mediators of inflammation are altered by sleep loss. Also there is increasing evidence that physical activity has beneficial effects on brain function. We evaluated the effect of regular exercise on inflammation induced by sleep loss. Methods and Results: Animals were randomly allocated into four groups: a sedentary group (n = 10) performing a normal sleep; a sedentary group (n = 10) performing a sleep deprivation, an exercise-trained group (n = 10) performing a normal sleep and an exercise-trained group (n = 10) performing a sleep deprivation. Experimental training protocol: The trained animals were exercised by running on a motorized treadmill during 7 weeks. Sleep deprivation protocol: Rats remained singly-housed throughout the sleep deprivation experiments in activity wheels validated previously (J. Sleep Res.17:376–384, 2008). The TNF-α and IL-1β concentrations were higher in the hippocampus of sleep-deprived sedentary rats compared to sedentary rats performing a normal sleep (2.03±0.33 pg/mg protein vs 1.08±0.30, p<0.05; 3.07±0.26 vs 1.16±0.19, p<0.05; respectively). No statistically significant effect of sleep deprivation on TNF-α and IL-1β contents was observed in the hippocampus of trained rats. The IL-6 concentration was higher in the frontal cortex of sleep-deprived sedentary rats compared to sedentary rats performing a normal sleep (11.13±0.55 vs 8.67±0.67, p<0.05). No statistically significant effect of sleep deprivation on IL-6 content was observed in the hippocampus of trained rats. Serum levels of cytokines: TNF- and IL-6 levels were higher for sleep-deprived sedentary rats compared to sedentary rats performing a normal sleep (3.85±0.50 pg/ml vs 1.67±0.29, p<0.01; 41.93±4.47 vs 18.65±2.69, p<0.001; respectively). Conclusion: In the present study, we observed a pro-inflammatory response in serum and brain areas after a sleep deprivation. We demonstrated for the first time that a physical training reduces the increase of TNF-alpha and IL-1β in hippocampus, IL-6 in frontal cortex and TNF-α and IL-6 levels induced by sleep loss. Theses results support previous reports which infer that physical training may possess anti-inflammatory properties. Also we hypothesis that TLR4 may play a role regulating the link between inflammatory cytokine production induced by sleep deprivation and a physical training.

Financial support: Grants from DGA (08ca704).
Introduction: We now know that the sickness syndrome can be divided into at least two response patterns, one that is best known and includes fever, and another that is poorly understood and includes hypothermia. A switch from the fever to the hypothermia pattern is known to occur as systemic inflammation becomes more severe, but the neuroimmune mechanisms responsible for this switch are elusive. We evaluated whether bacterial lipopolysaccharide (LPS) acting directly on the brain could promote a fever-hypothermia switch as well as the hypotension that is often associated with hypothermia in models of systemic inflammation.

Methods and Results: At an ambient temperature of 22°C, freely moving rats received intracerebroventricular (i.c.v.) injections of LPS at doses ranging from 0.5 to 25 µg. Despite the use of such high doses, the prevailing thermal response was fever. Nevertheless, an analysis of individual responses revealed that two out of eighteen rats displayed some decrease in body temperature. To investigate if a hypothermic response could be hidden within the prevailing febrile response, rats were pretreated with a cyclooxygenase-2 inhibitor (SC-236, 3.5 mg/kg i.v.) known to block fever. This strategy also failed to reveal any consistent hypothermic response following i.c.v. LPS. At the doses tested, i.c.v. LPS was similarly ineffective at inducing hypotension. Additional doses of LPS did not need to be tested because the 25-µg dose was already sufficient to induce both hypothermia and hypotension when administered peripherally (intra-arterially).

Conclusion: Unlike fever and other sickness symptoms, hypothermia and hypotension are triggered exclusively by LPS recognized outside the brain.

Support: AHA, FAPESP
COMPLEX REGULATORY ROLE OF CAPSAICIN-SENSITIVE PEPTIDERIC SENSORY NERVES IN THE SERUM-TRANSFER ARTHRITIS MODEL OF THE MOUSE

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Introduction: Capsaicin-sensitive sensory nerves regulate inflammatory processes through the released pro-inflammatory (Substance P, calcitonin gene-related peptide) and anti-inflammatory (somatostatin, opioid peptides) neuropeptides. The K/BxN serum-transfer arthritis is an appropriate model of rheumatoid arthritis to investigate neuro-immune interactions. We examined the role of capsaicin-sensitive sensory nerves in autoantibody-induced arthritis and consequent hyperalgesia. Methods: Capsaicin-sensitive sensory nerves were destroyed by resiniferatoxin (RTX) pretreatment (desensitization) in male C57Bl/6 mice (n=6-8/group). Arthritogenic (K/BxN) or control (BxN) serum (150-150 ml) was administered i.p. on days 0 and 3. Paw volume was measured by plethysmometry, touch sensitivity with dynamic plantar aesthesiometry and noxious heat threshold with increasing temperature hot plate. Cold tolerance was determined by paw withdrawal latency from 0°C icy water, arthritis severity by the grid test, semiquantitative scoring was performed and weight loss was measured during a 2-week-period. Bone morphology was monitored by micro-CT, joint matrix-metalloproteinase (MMP)-activity by fluorescence molecular tomography. Histopathological scoring was performed from the tibiotarsal joints. Results: The arthritogenic serum induced a 42.7±3.2% paw oedema on day 4, which decreased from day 11. In the early phase, a minimal thermal-, while by day 11 a 48.4±4.7% mechanical hyperalgesia was detected. RTX-desensitized mice developed significantly greater paw swelling, arthritis score, and they had worse performance in the grid test. In contrast, in the RTX-pretreated group the noxious heat threshold was significantly higher in the first 5 days, the late mechanical hyperalgesia and bone destruction were remarkably reduced. Cold allodynia and weight loss were not different in the two arthritic groups. MMP-activity significantly increased in the inflamed forepaws and tibiotarsal joints, but RTX pretreatment did not alter this inflammatory marker on day 8. Conclusion: We provide the first evidence for the involvement of sensory-immune interactions in autoantibody-induced arthritis and consequent pain. Capsaicin-sensitive afferents exert complex regulatory roles by decreasing the inflammation, but inducing hyperalgesia and bone loss. Support: SROP-4.2.2.A-11/1/KONV-2012-0024.

[1]

[1]összesen 2500 szó lehet szóközökkel együtt, szóval kicsit rövidíteni kell rajta
CROSS-TALK BETWEEN ERYTHROPOIETIN RECEPTOR AND TLR4 SIGNALING PATHWAYS MODULATES MACROPHAGES INFLAMMATORY MEDIATORS PRODUCTION.

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Introduction. Macrophages participate in various biological processes and are essential for inflammation. Erythropoietin (EPO) is a glycoprotein hormone that classically regulates red blood cells production by Erythropoietin receptor (rEPO). Although, EPO is a hormone specific for erythrocytes hematopoietic, some studies had shown that their biological effects are not limited to these targets. Our aim is this study was investigate the effects of EPO on cell signaling pathway regulation on Bone Marrow derived-macrophages (BMDM) and the release of inflammatory mediators after stimulation in vitro with LPS.

Methods and Results. For this, we used BMDM from C57BL/6 mice and proposed two protocols of treatment/stimulation in vitro: (1) EPO for 1h + LPS (100 or 500 ng/mL) for 24h or (2) LPS for 1h + EPO (0.3 Ui/mL, 3.0 Ui/mL or 30 Ui/mL) for 24h. In the experimental condition of group (1) we observed increased production of pro-inflammatory mediators (cytokines, Nitric Oxide (NO) and lipid mediators) after BMDM stimulation with LPS compared to non-stimulated macrophages. On group (2) we observed a decreased of pro-inflammatory mediators, but increased on regulatory cytokines production. In cell signaling pathway, we demonstrated different activation of p-MAPKp38, p-ERK1/2, p-PKC and p-PKA. However, different expression of p-STATs was emphasized. In order to this, we observed different regulation of some inflammatory genes expression by qRT-PCR.

Conclusion. Taken together, our results demonstrated that macrophages are direct targets for the modulatory effects of EPO on immune response, a cross-talking between rEPO and innate receptors (TLR4) signaling modulates p-STATs pathway and consequently inflammatory mediators production.

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Introduction: Chronic neuroinflammation is consistently associated with the pathophysiology of Parkinson’s disease (PD). Oxidative stress is also a key stimulator of microglial activation, which subsequently leads to the generation of reactive oxygen species (ROS) from microglia. 6-hydroxydopamine (6-OHDA) is a proinflammatory and oxidative neurotoxin used in animal model of PD. Omega-3 (w-3) is related in literature as an anti-inflammatory bioactive with pharmacological applications. This study aimed to determine the effects of supplementation with w-3 in rats using an experimental model of PD induced by 6-OHDA unilateral injection into striatum.

Methods and Results: Male Wistar rats (200-250 g) were divided in four groups (n=10 per group): sham group, which received 0.9% saline; group injured by 6-OHDA and/or treated groups by w-3 (1.5 or 3.0 g/kg by gavage) at 28 days. On the 4th day of treatment was performed 6-OHDA injection into the right striatum. On the 25th day of treatment was observed behavioral tests through rotational test induced by apomorphine (3 mg/Kg, i.p.) and activity exploratory assay. In the 28th day, animals were euthanized and right striatum nucleus was removed to measure dopamine and nitrite levels. The results showed an increase in the number of apomorphine-induced rotations in the 6-OHDA group (226.9±28.8 contralateral rotations) compared to the sham group (0.2±0.1). A motor partial recovery was observed in animals treated with w-3, which reduced the number of rotations around 41 and 75% (w3 1.5: 97.56±15.1 and w3 3: 41.18±7.27 turns/h, respectively). The 6-OHDA group showed a decrease (70%) on locomotor activity as compared to sham and w-3 treated groups. We observed a reduction in the dopamine levels (61%) in the 6-OHDA group. However, groups treated with w-3 (1.5 and 3.0 g/Kg) showed a minor reduction (24 and 26%, respectively). Nitrite levels were decreased in animals treated with w-3 (1.5 and 3.0 g/Kg) at 66 and 77%, respectively, as compared to 6-OHDA group.

Conclusion: The results of this study suggest that supplementation with w-3 reversed the behavioral and neurochemical alterations induced by 6-OHDA, with effects potentially beneficial in the treatment of PD.

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EFFECTS OF ENVIRONMENTAL TOBACCO SMOKE IN BRAIN DURING A SYSTEMIC INFLAMMATORY RESPONSE

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Introduction: Environmental tobacco smoke (ETS) is associated with a great risk of diseases. Previous studies from our laboratory have shown that ETS induces changes in antioxidant enzyme and lipid peroxidation in CNS. Therefore, the aim of our study is to evaluate the effects of the exposure to ETS with and/or without a systemic inflammatory response induced by LPS.

Methods: C57BL/6 mice were exposed to 3R4F reference research cigarette twice a day during 15 days. After the last exposure (8 a.m.), the mice were challenged with saline or LPS iv. (0.1µg/animal). Animals were euthanized 2, 4 or 6h after challenged and the frontal cortex, striatum, cerebellum and hippocampus were isolated. The primers IL-6, IL-1β, TNF-α, TLR2, TLR4 and iNOS were analyzed by RT-PCR and the quantification of IL-6, IL-10 and TNF-α was performed by ELISA in the following groups (n=6): CO – control, ETS – ETS exposure, LPS – challenged with LPS and ETS/LPS – ETS and challenged with LPS.

Results: No differences were found on the RT-PCR analysis after ETS exposure alone. However, our results showed an increase in IL-1β expression in hippocampus after 2 (p<0.001) and 4 (p<0.01) hours in ETS/LPS compared with CO. In frontal cortex there was an increase in TNFα expression after 2 (p<0.001) and 4 (p<0.001) hours in both groups challenged with LPS compared with CO and ETS. Moreover, in striatum we observed an increase in IL-6 expression after 2 hours (p<0.01) in LPS group compared with CO, which was blocked by the tobacco smoke in ETS/LPS (p<0.01). After 4 hours it was also shown an increase in IL-1β expression (p<0.001) in LPS group compared with CO which was prevented in ETS/LPS group (p<0.05). In cerebellum there was an increase in IL-1β and TLR2 expression after 2 hours and an increase in TNFα and TLR4 expression after 4 hours in the groups LPS and ETS/LPS (p<0.01) when compared with CO. Furthermore, the ELISA assay demonstrated a decrease in IL-10 levels after 4 hours which is reversible after 6 hours in hippocampus, frontal cortex and cerebellum in all groups when compared with CO.

Conclusion: Although ETS alone did not produce any changes in gene transcription, it was able to increase IL-10 level in cerebellum 6h after the challenge. However, during a systemic inflammation ETS is capable to proceed as an inducer or a blocker of gene transcription depending on the primers and structures analyzed.

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EFFECTS OF SULFATED POLYSACCHARIDES FROM GRACILARIA CORNEA RED SEAWEED ON OXIDATIVE STRESS IN BRAIN AREAS OF MICE

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Introduction: The seaweed have been mainly used in the medical and biochemical research and sulfated polysaccharides (SPs) comprise a complex group of macromolecules with a wide range of important biological properties. In recent years, SPs of algae were reported to be useful candidates in the search for an effective non-toxic substance and have been demonstrated to play an important role as free radical scavengers in vitro and antioxidants for the prevention of oxidative damage in living organisms. This study was designed to investigate the effect of SPs on oxidative stress in brain areas of mice. Methods and Results: The animals (mice, Swiss male, 28-32 g) were divided into 3 experimental groups and treated with SPs 5mg/kg (A), SPs 10mg/kg (B) or salina 0.9% (C) by intraperitoneal via (i.p.). After twenty-four hours, mice were sacrificed and cerebral prefrontal cortex (CPF), hippocampus (HC) and striatum (CE) was excised, homogenized and centrifuged. Finally, the supernatant was separated and used for evaluate lipid peroxidation levels, nitrite content and catalase activities. Statistical analyses were performed by one-way analysis of variance (ANOVA) and Student-Newman-Keuls test. It was observed that Thiobarbituric Acid Reactive Substances (TBARS) was increased (p<0.05) after the administration of SPs (5 or 10mg/kg) in CPF (C: 1.07 ± 0.17; A: 1.88 ± 0.22 and B: 3.65 ± 0.18) and CE (C: 1.37 ± 0.13, A: 2.65 ± 0.26 and B: 2.42 ± 0.24) and did not show any significant effect in HC (C: 1.17 ± 0.22; A: 1.17 ± 0.22 and B: 1.63 ± 0.12). Nitrite levels increased (p<0.05) in two groups treated with SPs (5 or 10mg/kg) in CPF (C: 5.85 ± 0.8, A: 7.14 ± 0.36 and B: 9.19 ± 1.23) and HC (C: 6.17 ± 0.85; A: 7.16 ± 0.46 and B: 11.94 ± 1.37), but in CE (C: 27.54 ± 4.9, A: 9.4 ± 0.91 and B: 9.3 ± 1.3) was observed a decrease compared with the control. Catalase activity test showed no statistical difference (p>0.05). Conclusion: Sulfated polysaccharides of Gracilaria cornea reduced oxidative stress probably acting on the nitric oxide pathway, however no improvement at levels of catalase, an enzyme that participates of endogenous oxidant system, was observed. Therefore, further investigations about other antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione redutase (GSH), may confirm the antioxidant effect of those substances.

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EXPOSURE TO METALS AND SERUM LEVELS OF PROLACTIN IN COMMUNITIES OF ABAETETUBA AND BARCARENA MUNICIPALITIES, PARÁ BRAZIL

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Introduction: Prolactin is a hormone-cytokine involved in the reproductive process also associated to the regulation of the immunological system, osmoregulation and angiogenesis. In the immune regulation in physiologic and pathological states PRL has been investigated in autoimmune diseases and also in exposed groups to metals that could influence the immune system. It was evaluated the serum levels of PRL and the blood concentrations of cadmium, lead and mercury in two communities in the municipal districts of Abaetetuba, and Barcarena, Pará, Brazil, this last one is an area under influence of industrial processes related to kaolin, bauxite and aluminum. Methods and Results: The epidemiological study was approved by the Institutional Committee of Ethics in Research with Human beings. Dosages of PRL were accomplished by enzyme immunoassay (ELFA) and the analyses of metals for Atomic Absorption Spectrometry by cold vapor (mercury) and for hydride generation (cadmium and lead). Preliminary results presented PRL average of 10.47±21.35 ng/ml in Maranhão Community (Abaetetuba) and 6.77±8.79 ng/ml in Bairro Industrial (Barcarena) (p=0.008). Lead average in Maranhão was of 7.91±3.06 µg/l and 22.85±13.51 µg/l in Bairro Industrial (p=0.000); Average levels of cadmium was of 0.41±0.24 µg/l in Maranhão and 0.81±0.96 µg/l in Bairro Industrial (p <0.0001); Mercury average levels in Maranhão was of 10.46 ug/l and 3.76 ug/l in Bairro Industrial (p <0.0001). PRL presented moderate correlation with the levels of cadmium in Bairro Industrial (r=0.40, p=0.005). Conclusion: Except in Maranhão that presented larger mercury levels (possibly due to the fish consumption for being a riverine community) the other metals were more elevated in Bairro Industrial, adjacent to the industrial area of Barcarena, that also presented the smallest concentrations of PRL. The study continues in those communities to evaluate the metal exposure and if it can influence the levels of circulating PRL.

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FATTY ACID AND GM1 TREATMENT IN NEUROBLASTOMA CELL CULTURE

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Introduction: The relationship between obesity and cancer has been identified epidemiologically. Although the biological links are not well-known, studies have showed that the causes range from chronic inflammation of the adipose tissue to metabolic disorders resulting from excess fat. The ganglioside GM1 (monosialotetrahexosylganglioside) induces neuritogenesis in tumor cells of the neuroblastoma (neuro2a) lineage causing the cells to become morphologically similar to neurons. Studies have shown that the actions of GM1 on tumors might be pro- or anti-carcinogenic in different situations. Besides the relationship between obesity and cancer, recent research has shown that high fat diets cause inflammation in the hypothalamus as well as neuronal apoptosis. In this context, the aim of this work was to study the action of fatty acids (FA) and GM1, either alone or together, on neuro2a cells.

Methods and Results: The cell clone neuro2a were plated per well in 24-well plates and maintained in DMEM and HAM-F12 culture medium (at a ratio of 1:1, with 10% FBS) in an incubator containing 5% CO² and at 37°C. The cells were divided into four groups: control cultures treated only with culture medium; cultures treated with 70 mM of GM1 (Sygen-GM1, TRB Pharma); cultures treated with 200 mM of a mixture of palmitate and stearate (fatty acid = FA, Sigma); and cultures treated with GM1 and fatty acids at a dose of 200 µM (palmitate:stearate, 1:1). It was performed morphometric quantify-cation and counting the number of cells, immunocytochemistry and analysis of TNFα expression by ELISA. Cultures that were treated with FA behaved similarly to control cultures in terms of the cell number, the cell size and the presence of neurites, as well as the expression of Ki67 and B cell lymphoma 2 (Bcl-2). Whereas GM1 treatment, either with or without FA, reduced all of these variables. Cultures treated only with FA, although not statistically different from control cultures, showed a trend to an increase in the studied variables. Only the levels of TNFα in FA treated cultures, showed significantly lower values compared to the other groups.

Conclusion: We conclude that FA might influence the behavior of neuro2a cells and GM1 might significantly interfere with the development of these cultures by reducing the characteristics of tumor cells and by providing features that are similar to neural cells.
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Introduction: Chronic stress is a harmful agent and may lead to depression. On the other hand, moderate physical exercise is known to improve many health parameters and can attenuate stress effects. Little is known whether gender can interfere in these effects. The aim of this study was to investigate the effects of chronic variable stress or exercise on behavior, blood components and organ weights in male and female rats.

Methods and Results: Procedures were approved by the State University of Londrina Ethics Committee for Animal Research (proc: 24606.2012.58). Adult Wistar rats were used (24 males and 23 females). Each gender was subdivided into three groups: Exercised, Stressed and Control. The Exercised group was submitted to swimming sessions with a 4% of its body weight load, 5 times a week, 40 min/day (water temperature: 32±1°C). The Stressed group was submitted to a chronic variable stress regimen per 19 days (a different stressor per day). The Control group was continuously kept in the vivarium. Before and after the exercise or stress regimen, each animal was tested for sucrose preference in order to investigate possible changes in anhedonia (a depressive behavior). All the rats were anesthetized and their blood was collected by cardiac puncture for complete blood count. After that, they were euthanized and adrenal, spleen, thymus, heart and the peritoneal fat were weighed. Parametric or nonparametric statistical analyses were run (significance level: P<0.05). Within Control animals, males showed more red blood cells and hemoglobin than females; such gender differences were not found in the Exercised or Stressed groups. Within the Stressed group, females presented enlarged adrenals in comparison to males. Stressed males were the only group which showed a decrease in sucrose consumption in the second evaluation. Exercised females (as compared to their controls) showed decreased peritoneal fat and enlarged hearts. They also, as compared to Exercised males, presented enlarged adrenals. Conclusion: Exercise was effective in relation to its purpose (decreasing peritoneal fat in the females and; increasing cardiac mass in both, males and females) and did not affect behavior (sucrose preference test). On the other hand, stress led to a decrease in the sucrose preference test in males. In general, results showed distinguishable effects from chronic stress or exercise.

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IMPACT OF PERIPHERAL INFLAMMATION ON BLOOD BRAIN BARRIER: OESTROGEN RESOLVING ACTION

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Introduction: Sex differences have been observed in neurological disorders with an inflammatory component. Studies have particularly focused on oestrogen, the main sex hormone in females. This molecule has well-known neuroprotective and anti-inflammatory properties, although its mechanism of actions is not fully clear. Recent evidence suggests a direct action of oestrogen on the microvascular endothelium of the blood brain barrier as well as in modulating anti-inflammatory mediators such as Annexin A1.

Methods and Results: We have used a combined in vivo/ in vitro methodology represented by wild-type and AnxA1 null mice (male/female). Our in vivo data suggest an inherent sex difference in the response of the blood brain barrier to peripheral inflammatory stimulation (i.p. lipopolysaccharide, 3 mg/kg body weight) in young adults (2 months old), which appears to be lost in reproductively senescent, and consequently estrogen-deficient, females (15 months old). Furthermore, using the human brain microvascular endothelial cell line hCMEC/D3, we show that oestrogen up-regulates the transcription and the expression of annexin A1 and of its putative receptor FPR2 (formyl peptide receptor 2, ALXR), followed by increased expression of the main tight junction proteins of the blood brain barrier. Additionally we see a striking protective effect of oestrogen under pro-inflammatory conditions (TNF-α and IFN-γ), where the hormone blocks the cytokine-induced increase in paracellular permeability.

Conclusions: Our findings open up a new avenue for the development of therapeutic tools for cerebrovascular disorders, additionally suggesting mechanisms which may have implications for sex dimorphism in the manifestations of neurological diseases associated with neuroinflammation.

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Introduction: Cytokines are important immune response modulators that may be influenced by hypoxia exposure (Immunol Invest. 39:219-234, 2010). This influence may be responsible for worsening sleep. Our hypothesis is that worsening of sleep can be consequences of increased levels of proinflammatory cytokines in hypoxia condition. Thus, the aim of the present study was to evaluate the effects of hypoxia about cytokine levels and the relationship with the sleep. Methods and results: Twelve healthy men were randomly divided into 2 groups: (Normoxia, n=6 and Hypoxia, n=6). The mean values were: age (years - 22 ± 2), body mass (kg - 68 ± 8), height (m - 1.77 ± 6) and BMI (kg/m2 - 21 ± 2). Polysomnography was conducted from 22:00 to 6:00. Blood samples for analysis (IL-1β, IL-1ra, IL-6, and IL-10, all kits provided by the R&D systems) were collected in 4 moments, before sleep and 20min after awakening on the two consecutive days. The hypoxia group was exposed to hypoxic condition for 29 hours, equivalent to 4.500 m (Colorado Altitude Training™/12 CAT-Air Unit). This study was approved by the Ethics Committee of the Universidade Federal de São Paulo/ Hospital São Paulo (1110/08). The comparisons were performed using ANOVA followed by the Duncan Post Hoc and the level of significance was set at p<5%. The results showed that the hypoxia group significantly reduced the total sleep time (279.0 ± 49min.), sleep efficiency (61.3 ± 9%), the latency to sleep (39.5 ± 9min.) and apnea index/hipopnea increased (19.4 ± 29.4 per h) in the second night compared to the first (343.8 ± 22.1min.; p<0.05), (87.2 ± 6.2%; p<0.05), (17.6 ± 7.2min.; p<0.05) and (0.58 ± 0.7 per h; p<0.05). There is no difference between the first and second night for the normoxia group in relation to sleep. In parallel, it was observed increased IL-1β levels (2.78±1.71 pg/mL) in the second day in hypoxia group compared to the second night in hypoxia (1.10±0.52pg/mL; p<0.05). IL-6 levels was increased (2.35±0.53 pg/mL) in the second night in hypoxia group compared to the first night in hypoxia (1.71±0.25 pg/mL; p<0.05). There was no difference for the normoxia group between the second day and second night in relation to cytokines. Conclusion: Hypoxia increases proinflammatory cytokines and worsens sleep. These results suggest that changes in sleep can be modulated by cytokine levels in hypoxia condition.

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LEPTIN ACTIVATES EOSINOPHIL LEUKOTRIENE C4 SYNTHESIZING MACHINERY: ROLE OF PI3K AND ENDOGENOUS RANTES

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Introduction: Leptin is an adipocytokine involved not only in the control of body weight but also in the neuro-immuno-endocrine modulation. Several leukocytes involved in immune and inflammatory conditions express functional leptin receptors, including human eosinophils. Here, we investigated potential leptin effect on eliciting eosinophil LTC₄ synthesis.

Methods and results: In vitro direct stimulation of human blood eosinophils with leptin elicited a rapid activation of human eosinophils. Specifically, identical to RANTES stimulation, leptin induced priming for enhanced synthesis of LTC₄, but not of PGE₂ and dose-dependent lipid body biogenesis. Mouse eosinophils were also directly activated in vitro by leptin stimulation. Similar to in vitro data, leptin administration in actively sensitized mice induced both increased lipid body assembly within infiltrating eosinophils and LTC₄ production. PI3K activation within eosinophils represents part of downstream signaling involved in leptin-induced lipid body-driven LTC₄ synthesis, since PI3K inhibitors blocked both lipid body biogenesis, LTC₄ synthesis and RANTES release triggered by in vitro leptin stimulation. In vivo, PI3K deficient mice in response to leptin administration displayed reduced eosinophilic inflammation with reduced in situ levels of RANTES. As both in vitro and in vivo leptin-driven eosinophil activation appear to be associated with RANTES secretion, we analyzed whether endogenous RANTES mediated leptin effect. Indeed, leptin-induced activation of human eosinophils was found to be mediated by an autocrine activity of endogenous CCR3-acting RANTES, since the effects of leptin were blocked by neutralizing anti-RANTES and anti-CCR3 antibodies. Similarly, in vivo lipid body biogenesis within infiltrating eosinophils and LTC₄ production of leptin-challenged mice were significantly inhibited by pre-treatment with anti-RANTES antibody.

Conclusions: Altogether, our findings unveiled leptin role in activating either human or mouse eosinophil lipid body-driven LTC₄ synthesizing machinery, a phenomenon that involves signaling through PI3K activation and RANTES mediation. Our results establish eosinophil activation by leptin as a connection between obesity and allergy disorders.

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MODULATION OF DEXTRAN SULFATE SODIUM-INDUCED COLITIS BY HYDROGEN SULFIDE

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Introduction: Pathomechanism and treatment of inflammatory bowel disease are still unresolved scientific clues. Major involvement of transient receptor potential ankyrin 1 (TRPA1) receptors in animal models of ulcerative colitis has been reported. It has been shown recently that endogenous gaseous mediator hydrogen sulfide (H$_2$S) activates TRPA1 receptors. H$_2$S is known to modulate inflammation, but precise mechanism of its action remains to be clarified. In the present study we investigated the role of exogenously applied and endogenous H$_2$S as well as TRPA1 receptors in dextran sulfate sodium (DSS)-induced murine colonic inflammation. Preceding results suggest a marked pro-inflammatory role of TRPA1 signalling in this model. However, no data are available on the involvement of H$_2$S-evoked TRPA1 receptor activation regarding colitis.

Methods: Colitis was evoked by adding 2% DSS to the drinking water of TRPA1 WT and KO mice for 7 days. Some animal groups consumed water supplemented with H$_2$S donor NaHS (100 mg/L). Other animal groups were injected with D,L-propargylglycine (50 mg/kg, i.p. daily) to inhibit endogenous H$_2$S synthesis. Clinical status of the animals was scored throughout the experiment. Myeloperoxidase activity, interleukin-16 and soluble intercellular adhesion molecule 1 content of colon samples were determined. Histological evaluation of colon samples was performed.

Results and conclusion: Our results indicate anti-inflammatory influence of TRPA1 receptors on the clinical condition in DSS-induced colitis. Our data regarding cellular inflammatory events reveal intricate regulation by endogenous and exogenous H$_2$S. The gasotransmitter might exert both pro- and anti-inflammatory actions that might be mediated via TRPA1 receptor activation or different pathways. However, changes in neutrophil accumulation and cytokine secretion evoked by exogenous H$_2$S or lack of endogenous H$_2$S did not change clinical manifestation of colitis in TRPA1 WT mice.

This study contributes to the understanding of the participation of H$_2$S and TRPA1 receptors in colonic inflammation and emphasizes vast complexity of the effects of H$_2$S.

Financial support: This study was funded by grants 34039/KA-OTKA/11-16 and TAMOP-422A-11/1/KONV-2012-0024.
MODULATION OF EXPERIMENTAL COLITIS BY THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IS NOT RESTRICTED TO THE EFFECTS OF GLUCOCORTICOIDS

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Introduction: Inflammatory immune responses may be modulated by the hypothalamic-pituitary-adrenal axis (HPA) through neuroimmunoendocrine interactions and cortisol secretion. However, even in the presence of intact adrenal glands patients may develop chronic diseases such as Inflammatory Bowel Disease (IBD), which may be caused by an imbalance between regulatory and effector responses in the intestinal mucosa. Then, our objective was to investigate the role of the adrenal glands and endogenous glucocorticoid in experimentally induced IBD.

Methods and Results: C57BL/6 mice were subjected to bilateral adrenalectomy and after a 15 day-surgery recovery period the colitis was induced by oral intake of water containing 3% Dextran Sulfate Sodium (DSS) continuously for survival analysis or for 6 consecutive days to collect samples. Animals were followed daily for weight loss and clinical signs of disease. Replacement of glucocorticoids was performed with 1 mg/kg/day i.p. of Dexamethasone, from the third day of colitis. Mice were sacrificed on the 6th day of colitis induction and colon samples were collected for HE evaluation, EPO, MPO and NAG activities by enzymatic assay and cytokine production by ELISA. Our results demonstrated that the absence of endogenous glucocorticoids led to increased susceptibility to colitis, with lower survival against the continuous stimulus with DSS 3%, higher clinical score of the disease and greater weight loss. Furthermore, it was observed that both in the presence or absence of adrenal glands, animals with colitis had greater activity of EPO, MPO and NAG enzymes as well as increased production of proinflammatory cytokines (TNF-α, IL-1β, IL-17, IFN-γ), which were even more pronounced in the absence of endogenous glucocorticoids. Moreover, although the replacement of glucocorticoids in adrenalectomized mice subjected to colitis was not able to reduce susceptibility to disease, there was a decrease in the score post-morten, in the local infiltrate of neutrophils and in the levels of proinflammatory cytokines, especially IL-17 and IFN-γ in the intestine of adrenalectomized treated mice.

Conclusions: Taken together, our results indicated that the control of intestinal inflammation by the HPA axis may not be restricted to the effects of glucocorticoids and other hormones produced by the adrenal glands may also be involved in this neuroimmunoendocrine modulation. Financial support: CAPES/FAPESP/NAPDIN (Núcleo de Apoio à Pesquisa em Doenças Inflamatórias).
NEUROPROTECTIVE ACTIVITY OF A SULFATED AGARAN ISOLATED FROM RED SEAWEED GRACILARIA CORNEA IN PARKINSON’S DISEASE MODEL INDUCED BY 6-HYDROXYDOPAMINE IN RATS: BEHAVIORAL AND NITRITE LEVELS ANALYSES

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Introduction: Neuroinflammation is implicated in the Parkinson’s disease (PD) progression. In the animal model of PD induced by the neurotoxin 6-hydroxydopamine (6-OHDA), occurs neurodegeneration through oxidative and inflammatory process. In this model we observed motor problems, behavioral alterations and increase of nitrite levels in cerebral areas. Sulfated agaran is a polysaccharide with sulfate groups found in the red seaweed Gracilaria cornea (SA). Recently, a study with SA showed anti-inflammatory effects and absence of toxic effects in vivo. The aim of this work is to evaluate the neuroprotective effects of SA in the modulation of behavioral and motor alterations and cerebral nitrite levels induced by 6-OHDA intrastriatal injection in rats. Methods and Results: The seaweed was collected from Flecheiras beach, Brazil. SA was obtained as previously described by Coura et al (2012). Male Wistar rats (250-300 g) were randomly divided in five groups (n=10 animals per group). Rats were anesthetized and treated with SA (15, 30 or 60 µg) + 6-OHDA (20 µg); 6-OHDA (20 µg) only; or saline (sham group). All treatments were realized through stereotaxic injection into right striatum and solutions were prepared in saline (0.9%; with 0.01% ascorbic acid). Animals were maintained under ad libitum feeding conditions. This study was approved by Ethics Committee of Animal Research of the Federal University of Ceará - CEPA (nº 45/13). 14 days later, rats were submitted to behavioral evaluation by open-field test and rotational test induced by apomorphine (3 mg/Kg). After, animals were euthanized and hippocampus, cortex and striatum were removed to measure nitrite levels. The open-field test showed an increase (p<0.01) in the motor activity in groups treated with SA (30 and 60 µg) in 63.1±5.7 and 74.9±5.8 (numbers lines crossing), respectively, in relation to 6-OHDA group (47.4±3.7). The rats treated with SA (15, 30 or 60 µg) had the rotation number reduced (p<0.001) in 80, 83 and 91%, respectively, in comparison to 6-OHDA group. Nitrite levels were significantly increased by 6-OHDA, its concentration was brought toward normality in animals treated with SA (60 µg). Conclusion: Sulfated agaran from seaweed G. cornea presented neuroprotective effects against motor alterations induced by 6-OHDA injection into striatum and recovered the nitrite levels in the rat cerebral tissues.

This work was support by CAPES and CNPq.
Introduction: Temporal lobe epilepsy (TLE) encompasses epileptic syndromes that arise in the temporal lobes and mesial temporal lobe epilepsy, the main subtype of TLE. The TLE is the commonest presentation of epilepsy in humans. Inflammation is generally regarded as harmful to the brain because local and infiltrated immune cells, as well as proinflammatory cytokines, exacerbate the neuronal damage in various neurodegenerative diseases, including TLE. Costimulatory molecules are key factors required for full activation of the primed T cells, especially the naive T cells. The aim of this work was to characterize the immune cells and their activation state in TLE patients compared with control group (CG) for better understanding the neuroimmunology related to the illness.

Methods and Results: It was included in the present study 21 TLE medicated patients and 22 controls matched by gender and age. B and T lymphocytes from whole peripheral blood from TLE and CG derived were evaluated in ex vivo condition using flow cytometry. Our preliminary results showed that the expression of HLA-DR, CD69, CTLA-4, CD25, IFN-γ, TNF and IL-17 in CD4+ lymphocytes from TLE is higher when compared with CG. Granzyme A, CTLA-4 and IL-17 expression was higher in CD8+ T cells in TLE patients compared with CG. We demonstrated that TLE patients an increased frequency of HLA-DR in CD19+ B cells and regulatory T cells CD4+CD25+Foxp3+ producing IL-10 when compare with CG. To better understanding the association of inflammatory markers and clinical parameters, we performed correlation analysis. Interestingly, a positive correlation between CD4+CD69+ and CD8+CD69+ lymphocytes with age were found. We also demonstrated that CD4+CTLA-4+ were negatively correlated with lengths of illness.

Conclusions: Taken together ours preliminary results, suggest that a proinflammatory condition involving specially effectors T cells that producing cytokine are present in TLE and may serve as an important marker for disease development or severity. These results could contribute to understanding this chronicle illness and the cellular/intracellular mechanisms involved in epileptogenic process.
Introduction: Bipolar disorder (BD) is a severe psychiatric disorder of unknown physiopathology that has been associated with a pro-inflammatory state. The aim of the present study was to investigate intracellular pathways involved with inflammatory signaling, assessing the phosphorylation levels of transcription factor nuclear factor kappa B (NF-kB) and mitogen-activated protein kinase (MAPKs) in peripheral blood mononuclear cells of euthymic BD patients and healthy controls. Methods and Results: Fifteen BD euthymic type I patients, and 12 healthy controls matched by age and gender were enrolled in this study. All subjects were assessed by the Mini-International Neuropsychiatry Interview and the patients also by the Young Mania Rating Scale and the Hamilton Depression Rating Scale. Phosphorylation levels of p65 NF-kB subunit, and MAPK ERK1/2, and p38 were assessed by Western blot and by flow cytometry. Plasma cytokines (IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, and IL-17A) were measured using cytometric bead arrays (CBA). BD patients presented increased pro-inflammatory cytokines levels in comparison with controls. Western blot analysis of PBMC showed that BD patients in comparison with controls presented a 7.2 fold-increase in phosphorylated p65 NF-kB protein levels (p = 0.01); 5.6 fold-increase in phosphorylated ERK1/2 (p = 0.004), and, 3.7 fold-increase in phosphorylated p38 protein levels (p = 0.005). Flow cytometry analysis of total PBMC of BD patients in comparison with controls showed the same pattern according to the increased phosphorylated levels of p65 NF-kB protein levels (p = 0.03); increased phosphorylated ERK1/2 levels (p = 0.02), and, increase phosphorylated p38 levels (p = 0.03). TNF-α correlated with the levels of phosphorylated p65 NF-kB. BD patients presented increased pro-inflammatory cytokines levels in comparison with controls, and TNF-α correlated with the levels of phosphorylated p65 NF-kB. Conclusion: The present study found increased activation of MAPK and NF-kB pathways in BD patients, which are in line with a pro-inflammatory status.

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RELATIONSHIP BETWEEN THE PATTERN OF MOLECULES EXPRESS BY MICROGLIA CELLS AND MODULATION OF T CELL PROLIFERATION.

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Introduction: Currently microglial cells have received great attention within the immune response. It was shown that microglial cells are capable of expressing suppressor molecules such as indoleamine-2,3-dioxygenase (IDO), which is an enzyme capable of suppressing the proliferation of T cells. Those cells are able to secrete large amounts of IL-12, IL-17, IL-21, IFN-γ and GM-CSF having a fundamental importance in the pathogenesis of multiple sclerosis (MS) and its murine model, EAE.

Objective: This study aims to elucidate the relationship between the expression of immune molecules in microglial cells and its effect on T cells

Methods: Two models were used for this study in vitro cell culture microglia from lineage cells (C8B4), or primary culture obtained from the nervous system of C57BL/6 mouse and in vivo, in which C57BL/6 mouse were immunized with MOG35-55 and treated or not with pellet 1-methyltryptophan (1-MT) the IDO blocker. The immune profile of microglia culture was evaluated using cytokine gene transcription by RT-PCR and activating adhesion molecule expression by flow cytometry. The evaluation of the activity of the enzyme IDO was performed by its expression when cells were activated or not with lipopolysaccharide (LPS) or IFN-γ. The protocol was evaluated for in vivo functionality of IDO and its relationship with EAE through the proliferation of T CD4 lymphocytes specific for MOG35-55.

Results: Our results demonstrate that both cultures (lineage and primary) have the ability to express various immune molecules in both pro and anti-inflammatory agents. Among these observed TLR-4, TLR-2, IL-6, IL-10 and TGF-β, as well as IDO. Both experiments, in vivo and in vitro, can demonstrate the decrease in CD4 T lymphocyte proliferation when there is blockage of IDO, and in the in vivo model this effect can be observed through the deterioration degree of the disease as well as in non-regression EAE.

Conclusions: Our results suggest that microglial cells have critical role in the modulation of immune response, being able to express molecules both pro- and anti-inflammatory properties. Through our experiments we suggest that IDO interferes with the control of proliferation of T CD4 exerted by microglia, once your block is responsible for the increased proliferation of the same, and its action is necessary for the control of EAE.

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ROLE OF ENDOGENOUS PGD2 ON EOSINOPHILIC INFLAMMATION INDUCED BY LEPTIN

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INTRODUCTION: Eosinophils are granulocytes classically associated with allergic diseases and helminth infections. Recently it has been also described an immunomodulatory role of eosinophils, such as the regulatory role of resident eosinophils found in adipose tissue, capable of regulating macrophage functions and tissue hemostasis. Leptin, a hormone/cytokine produced by adipocytes, is a survival factor for eosinophils which are known to express the leptin receptor Ob-Rb. Inasmuch as we just had identified eosinophils as producers of prostaglandin D2 (PGD2) which function in an autocrine/paracrine fashion regulating eosinophil activation (J Immunol. 187(12):6518-26, 2011), the aim of this study was to evaluate the role of eosinophil-derived PGD2 in leptin-induced eosinophilic inflammation.

METHODOLOGY AND RESULTS: For in vivo assays, eosinophilic pleurisy was triggered by intrapleural injection of leptin (1 mg/Kg) in Balb/c mice. After 24 h of in vivo leptin stimulation, in parallel to eosinophil influx and activation (characterized by lipid body biogenesis in infiltrating eosinophils and increased CysLT levels), we found increased levels of PGD2 in the pleural cavity compared to saline-stimulated animals (n = 6; p<0.05). The pre-treatment with HQL-79 (10 µM), an inhibitor of hematopoetic PGD synthase, inhibited both lipid body biogenesis and LTC4 synthesis, indicating that an eodogenous PGD2 mediate leptin-induced eosinophilic inflammation. Accordingly, in vitro, using bone marrow-differentiated mouse eosinophils, leptin (50 nM) was also able to trigger PGD2 synthesis as detected in eosinophil supernatants within 1 h of stimulation. Similarly, using human eosinophils purified from healthy donors as cell model, the pre-treatment with HQL-79 (10 µM), besides inhibiting PGD2 production, inhibited both lipid body biogenesis and LTC4 synthesis elicited by in vitro leptin stimulation (50 nM), demonstrating an autocrine/paracrine function of leptin-induced eosinophil-derived PGD2.

CONCLUSIONS: Our data demonstrated clearly the ability of leptin to activate PGD2 synthesizing machinery both in vivo during eosinophilic inflammation and in vitro by either mouse or human eosinophils. Moreover, our results with human cells unveil an autocrine/paracrine regulatory activity by endogenous PGD2 on eosinophil activation stimulated by leptin. Inasmuch as PGD2 is now emerging as a immunomodulatory molecule, its role in leptin-driven effects may indicate potential functions on obesity and other inflammatory disorders.

FINANCIAL SUPPORT: CNPq, FAPERJ, CAPES, FIOCRUZ, INPeTAm

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FINANCIAL SUPPORT: CNPq, FAPERJ, CAPES, FIOCRUZ, INPeTAm
STATINS THERAPY IMPROVES THE OUTCOME AND AMELIORATES RESPONSE TO GLUCOCORTICOID DURING EXPERIMENTAL COLITIS

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Introduction: Glucocorticoid (GC) therapy during Inflammatory Bowel Disease (IBD) is controversial. These anti-inflammatory drugs act by reducing the uncontrolled immune response, but the exacerbated immunosuppression caused by them may be harmful. Currently, statins have been studied to exert pleiotropic effects, primarily anti-inflammatory. Concomitant use of statins and steroids seems to have good prospects in several autoimmune and inflammatory diseases such as IBD. Therefore our aim was to evaluate if the use of statins alone or concomitant with GC improves the clinical outcome of experimental colitis.

Methods and Results: Male C57BL/6 mice were subjected to experimental colitis by 3% (w/v) dextran sodium sulfate (DSS) in drinking water. Mice received simple or combined treatment with dexamethasone (DEX; 1mg/kg; i.p.) and/or atorvastatin (ATO; 5mg/kg; i.p.). One independent group was treated with ATO alone orally (10mg/kg). Therapy was introduced when the first clinical signs of colitis were observed and performed daily. Mice were followed during eleven days for clinical disease aspects, weight loss and survival evaluation. The control groups without colitis under therapy with drugs alone or in different combinations were not different from each other in all evaluations. DEX-treated colitis group presented the worst outcome with increased weight loss, augmented clinical disease score and higher mortality. However, the concomitant use of DEX/ATO led to a general improvement of sick animals, which presented similar weight to DEX-treated group, but lower clinical score and reduced mortality, indicating a better disease prognosis. The treatment with ATO alone induced a sharply improvement of all the clinical aspects of the disease with marked reduction of clinical signs and diminished weight loss, besides absence of mortality during the experiment, indicating a putative regulatory role of statins on the immune response. In addition, the oral ATO administration induced the most prominent amelioration of disease in comparison to the other statin therapies for colitis.

Conclusion: Statins present a potential immunomodulatory role and may constitute an important alternative therapy for IBD, alone or in conjunction with GC, thus improving the pharmacological use of this drug.

Financial support: CAPES; FAPESP; NAPDIN.
STUDY OF THE SIGNALING PATHWAYS INVOLVED IN THE INHIBITION OF HIV-1 REPLICATION IN MACROPHAGES BY THE NEUROPEPTIDES VIP AND PACAP.

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FIOCRUZ/IOC, RIO DE JANEIRO - RJ - BRASIL.

Introduction: The Vasoactive Intestinal Peptide (VIP) and the Pituitary Adenylate Cyclase Activating Peptide (PACAP) are molecules with potent immunoregulatory functions, including: the regulation of inflammatory responses, Th1/Th2 differentiation and cell migration. VIP and PACAP binds to receptors coupled to G protein (VPAC1, VPAC2 and PAC1) and have a wide tissue distribution. Recently, we demonstrated that HIV-1-infected macrophages exposed to VIP and PACAP have a diminished viral production (Temerozo et al, 2013). Now we continue the study on the inhibitory potential of these neuropeptides aiming to identify the signaling pathways involved in this phenomenon. Methods and Results: At first, monocye-derived macrophages, obtained from healthy donors by density gradient centrifugation, were infected in vitro with HIV-1 and treated with VIP and PACAP at 1 or 10 nM, in a regime of one or three doses. We evaluated the replication of HIV-1 at different time-points (ELISA for HIV-1 p24 Ag in supernatants) and observed that the three-dose treatment at 1 nM has the same HIV-1 inhibitory potential of the one-dose treatment with 10 nM for each peptide (47% ± 8 versus 48% ± 13 for VIP, and 63% ± 15 versus 51% ± 17 for PACAP – n=4). After, we treated infected macrophages with VIP and PACAP in the presence of a PKA inhibitor (H89). After 12 days, replication was evaluated and we found that the inhibition of PKA reduced the HIV-1 inhibition promoted by VIP (48% to VIP versus 23% to PKAi/VIP) and PACAP (58% ± 18 to PACAP versus 34% ± 14 to PKAi/PACAP – n=3). Parallel to this we treated macrophages with either VIP or PACAP for 24 hours, and infected with HIV-1. In this scenario, we observed that HIV-1 replication was inhibited 44% ± 11 to VIP, and by 39% ± 13 to PACAP (n=3). Conclusions: At the moment, the inhibition of viral replication achieved with the consecutive treatment at low doses of VIP and PACAP, together with the replication-resistant profile induced by pre-treatment, reinforces the HIV-1-inhibitory potential of these neuropeptides in macrophages. And, the data obtained from PKA-inhibition experiments indicates that key components of their signaling are involved in inhibition of HIV-1 by VIP and PACAP. Financial support: IOC/Fiocruz, CNPq and Faperj.
THE IMMUNOMODULATORY EFFECT OF VAGUS NERVE STIMULATION IN ZYMOSAN-INDUCED MONOARTHRITIS IS NOT DEPENDENT ON THE SPLEEN

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INTRODUCTION. The central nervous system (CNS) is involved in the control of humoral and cellular immune responses. Recently, a complex passympathetic “cholinergic anti-inflammatory pathway” (CAP; also known as anti-inflammatory reflex) has been described and is activated by efferent vagus nerve stimulation sending signals that terminate in the spleen (the target organ). In the present study, we investigated the role of vagus nerve stimulation (VNS) in the knee joint neutrophil migration in zymosan-induced mono-arthritis model.

METHODS AND RESULTS.

VNS: The platinum electrode attached to the stimulation device (STM 150) controlled by AcqKnowledge software (Biopac Systems) was placed across the right vagus nerve in anesthetized Balb/c male mice. Right vagus nerve stimulation was then applied for 2 min at 5 hz, 0,1 ms, 1 V. Sham animals underwent right neck dissection without stimulation.

Zymosan-induced arthritis: All groups were subjected to intra-articular (i.a.) injection of 30 μg of zymosan suspended in sterile saline into their right knee joints. Control animals received only saline.

Assessment of cell influx and chemokine/cytokine release in the joint lavage: At 6 h after injection of zymosan, the animals were euthanized and the synovial cavity of the knee joints was then washed with saline containing EDTA. The synovial exudates were collected by aspiration and total cell counts were performed using a Neubauer chamber. After centrifuging, the supernatants were used for measuring the concentrations of TNF-α, IL-1β, IL-10 and MIP-2 using ELISA technique.

Additionally, in order to investigated the components involved in the CAP, we performed VNS in splenectomized animals or pre-treated with i.a. guanetidine (sympatholytic drug) administration. In the present study, we observed that VNS decreased neutrophil migration and chemokines/cytokines production in the knee joint after zymosan administration. Additionally, the effect was dependent on release on catecholamines by terminal adrenergic sympathetic nerves joint knee but not on intact spleen.

CONCLUSION. Our results suggest that VNS is able to control excessive neutrophil migration toward the knee joint and suggest new therapies to the chronic inflammatory conditions including autoimmune diseases.

FINANCIAL SUPPORT. FAPESP, CNPq
TRIMETAZIDINE EXERTS PROTECTION AGAINST SEIZURES INDUCED BY PILOCARPINE IN MICE

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Introduction: Trimetazidine (TMZ), a novel anti-ischemic agent, is used in the therapy of angina, vertigo and chorioretinal diseases. It has also been examined for its effect on nociception, inflammation and neuroprotection in various animal models. Trimetazidine inhibits the initiation of glutathione production and improves thiol component level in neuronal cells therefore, has shown beneficial effects on antioxidant enzymes in brain. Additionally, it has shown protection against animal models of inflammation, nociception, gastric injury and iron-induced epilepsy. The present study was designed to investigate the effect of trimetazidine on pilocarpine induced seizures in mice.

Methods and Results: Male Swiss mice, 25-30g received TMZ (5, 10 and 20 mg/kg, i.p.), fenobarbital (FEN 10 and 30 mg/Kg, i.p.), association (TMZ 5 + FEN 10 mg/kg, i.p.) or saline - NaCl 0.9%, i.p. during seven days. 60 minutes after the last injection, pilocarpine (320 mg/Kg, s.c.) was administered and behavioral tests were performed. After behavioral tests animals were euthanized and cerebral areas were removed for neurochemical analysis. The results showed that pretreatment with TMZ decreases animal’s death and significantly raised the seizure-threshold as compared to control group. However the highest dose (20 mg/kg) showed best effect, increased the latency of seizures in 41% (11.76±0.75 (8)) compared with control (8.36±0.41 (10)) led to absence seizures in 17% of the animals, reduced the occurrence of status epilepticus in 33% and 40% of animals protected from death. Further, co-administration of per se ineffective dose of TMZ (5 mg/kg) with subanticonvulsant dose of FEN (10 mg/kg) offered significant protection, increased the latency of seizures in 109% (16.35 ± 0.90(6)) compared to control, led to absence seizures in 30% of the animals, reduced the occurrence of status epilepticus (50%).

Conclusion: These results indicate that TMZ possesses anticonvulsant activity against pilocarpine-induced seizure in mice. However, more studies are required to explore the exact mechanism of action of TMZ in epilepsy.

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