UNIVERSIDADE PAULISTA

PROPENTOFILINA PREVINE O COMPORTAMENTO DOENTIO E O COMPORTAMENTO TIPO-DEPRESSIVO INDUZIDO POR LIPOPOLISSACARÍDEO EM RATOS

Dissertação apresentada ao Programa de Pós-graduação em Patologia Ambiental e Experimental da Universidade Paulista - UNIP, para obtenção do título de Mestre em Patologia Ambiental e Experimental

MÁRCIA MARIA TIVELLI MORAES

SÃO PAULO

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Área de concentração: Patologia Ambiental e Experimental

Orientador: Prof. Dr. Thiago Berti Kirsten

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Aprovado em : ___/___/_____

Banca Examinadora

Prof (a). Dr.(a) _____
Instituição:_____Julgamento_____

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Instituição:_____Julgamento_____

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Instituição:_____Julgamento_____

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PREFÁCIO

Este volume refere-se à dissertação de mestrado apresentada como requisito para a defesa de mestrado perante a banca examinadora no Programa de Pós-Graduação em Patologia Ambiental e Experimental, Universidade Paulista.

Segundo as normas do Programa, a dissertação poderá ser em forma de artigo científico de autoria do aluno e organizado de acordo com as exigências do veículo de publicação científica escolhido.

O periódico científico escolhido para a submissão foi o ***Brain, Behavior, and Immunity***, Elsevier, ISSN: 0889-1591, sob o título "**Propentofylline prevents sickness behavior and depressive-like behavior induced by lipopolysaccharide in rats**" e com tradução para português como "**Propentofilina previne o comportamento doentio e o comportamento tipo-depressivo induzido por lipopolissacarídeo em ratos**".

RESUMO

Estudos recentes têm demonstrado a relação entre a depressão e distúrbios imunológicos. Sabendo-se da eficácia limitada dos medicamentos antidepressivos existentes e os efeitos anti-inflamatórios da propentofilina, o objetivo deste estudo foi utilizar propentofilina como um possível tratamento para a depressão. Utilizamos um modelo de comportamento tipo-depressivo em ratos induzido por administrações repetidas de lipopolissacarídeo (LPS). Nós estudamos o comportamento doentio, avaliando o peso diário corporal, o comportamento em campo aberto e os níveis plasmáticos do fator de necrose tumoral alfa (TNF-α). Foram avaliados os níveis de ansiedade pelo teste do claro-escuro. A avaliação do comportamento tipo-depressivo foi realizada por meio do teste do nado forçado. Os níveis plasmáticos do fator neurotrófico derivado do cérebro (BDNF) também foram avaliados como biomarcador de depressão. A exposição ao LPS induziu a perda de peso corporal, prejuízos comportamentais no campo aberto (diminuição da locomoção e do levantar e aumento da imobilidade), e aumentou os níveis plasmáticos de TNF-α em ratos, em comparação com o grupo controle. Assim, o LPS induziu comportamento doentio 24 e 48 horas após sua exposição. Administrações repetidas de LPS também aumentaram a imobilidade e reduziram a escalada no teste do nado forçado, em comparação com o grupo de controle, isto é, o LPS induziu o comportamento tipo-depressivo em ratos. A propentofilina preveniu o comportamento doentio após quatro dias consecutivos de tratamento, bem como preveniu o comportamento tipo-depressivo após cinco dias consecutivos de tratamento. Nem o LPS nem a propentofilina influenciaram os níveis de ansiedade e de BDNF nos ratos. Concluindo, a administração de LPS induziu comportamento doentio e comportamento tipo-depressivo em ratos por meio de via inflamatória. A propentofilina preveniu tanto o comportamento doentio quanto o comportamento tipo-depressivo, incluindo para os parâmetros comportamentais e imunológicos. Os presentes achados podem contribuir para uma melhor compreensão e tratamento da depressão e doenças associadas.

Palavras-chave: Depressão; Ansiedade; Campo Aberto; Claro-Escuro; Nado forçado; TNF-α.

ABSTRACT

Recent studies have demonstrated the relation between depression and immune disturbances. Knowing the efficacy limitation of existing antidepressants drugs and the anti-inflammatory effects of propentofylline, the objective of this study was to use propentofylline as a depression treatment. We used a rat model of depressive-like behavior induced by repetitive lipopolysaccharide (LPS) administration. We studied sickness behavior, evaluating daily body weight, open field behavior, and TNF-α plasmatic levels. Anxiety-like behavior (light-dark test), depressive-like behavior (forced swim test), and plasmatic levels of the brain-derived neurotrophic factor (BDNF, depression biomarker) were also evaluated. LPS induced body weight loss, open field behavior impairments (decreased locomotion and rearing, and increased immobility), and increased plasmatic TNF-α levels in rats, compared with control group. Thus, sickness behavior was observed after 24 and 48 hours of LPS exposure. Repetitive LPS administration also increased the immobility and reduced climbing in the forced swim test, when compared with the control group, i.e., LPS induced depressive-like behavior in rats. Propentofylline prevented sickness behavior after four days of consecutive treatment, as well as prevented the depressive-like behavior after five days of consecutive treatments. Neither LPS nor propentofylline has influenced the anxiety and plasmatic BDNF levels of rats. In conclusion, LPS administration induced sickness behavior and depressive-like behavior in rats via inflammatory pathway. Propentofylline prevented both sickness behavior and depressive-like behavior, in the light of behavioral and immune parameters. The present findings may contribute to a better understanding and treatment of depression and associated diseases.

Key-words: Depression; Anxiety; Open-field test; Light-dark test; Forced swim test; TNF-α.

SUMÁRIO

1 INTRODUÇÃO

A depressão é um transtorno de humor complexo, caracterizada pela perda de interesse ou prazer, anedonia, apatia, falta de concentração, baixa energia, distúrbios do sono e do apetite, redução do interesse social e sexual, entre outros sintomas (THOMPSON et al., 2001; CARROLL; CASSIDY; COTE, 2003). Estima-se que de 40 a 60% dos suicídios estão diretamente ligados à depressão (CLARK, 1990; LONNQVIST et al., 1995). Mais de 15% de todos os adultos devem experimentar um episódio de depressão em algum momento de sua vida, sendo que mulheres são mais acometidas do que os homens (20% *vs*. 10%) (MURRAY; LOPEZ, 1997; PARKER; BROTCHIE, 2010). Os custos relacionados com esta desordem representam um encargo econômico de dezenas de bilhões de dólares por ano (JENKINS; GOLDNER, 2012). Portanto, a depressão tem sido considerada como o mal do século.

Infelizmente, pouco se sabe sobre a etiologia e patofisiologia da depressão. É considerada como um distúrbio de causas multifatoriais, incluindo fatores genéticos, eventos estressores, doenças, desequilíbrio hormonal, e abuso de drogas (CARROLL; CASSIDY; COTE, 2003; COLMAN; ATAULLAHJAN, 2010). Embora a hipótese monoaminérgica (serotonina e noradrenalina) seja reconhecida e aceita, inclusive para a prescrição de antidepressivos, ela não consegue explicar e tratar vários aspectos da depressão (RAEDLER, 2011). Smith (1991) propôs a teoria dos macrófagos da depressão, que afirma que a secreção excessiva de interleucina (IL) -1 e outros produtos de macrófagos estão envolvidos na patogênese da depressão. Neste sentido, alguns pacientes diagnosticados com depressão têm níveis elevados de citocinas tais como o fator de necrose tumoral (TNF-α) e IL-6 no sangue (DOWLATI et al., 2010). Pacientes com hepatite C ou câncer tratados com interferon alfa (IFN-α) também desenvolveram depressão (LOFTIS; HAUSER, 2004). Além disso, mesmo doses baixas de lipopolissacarídeo (LPS) administradas em pacientes voluntários são capazes de aumentar os níveis séricos de citocinas pró-inflamatórias e induzir a anedonia, que é um dos principais sintomas de depressão (EISENBERGER et al., 2010). O LPS é uma endotoxina que mimetiza a infecção por bactérias gram-negativas por meio da ativação do sistema imunológico, induzindo síntese e liberação de citocinas, tais como TNF-α, IL-1β, e IL-6 (ADEREM; ULEVITCH, 2000; HAVA et al., 2006; KIRSTEN et al., 2013).

Com base nestes aspectos neuroimunes, muitas drogas têm sido testadas para o tratamento da depressão, em especial a utilização de fármacos anti-inflamatórios (RAEDLER, 2011). Por exemplo, o celecoxibe, inibidor ciclooxigenase-2, que inibe a produção de citocinas pró-inflamatórias, tem efeitos terapêuticos em pacientes depressivos tratados com reboxetina (MULLER et al., 2006). Resultados semelhantes foram encontrados com a associação do celecoxibe com fluexetina (AKHONDZADEH et al., 2009). Inibidores de TNF-α, tais como o etanercept e infliximab reduziram os sintomas depressivos em pacientes com psoríase e doença de Crohn e foram elencados como potenciais tratamentos para pacientes (PERSOONS et al., 2005; TYRING et al., 2006). Neste sentido, devido às características anti-inflamatórias da propentofilina, a propomos como uma candidata para o tratamento da depressão. Propentofilina (3,7-diidro-3-metil-1-(5-oxo-hexil)-7-propil-1H-purina-2,6-diona), um derivado de xantina, apresenta intenso efeito neuroprotetor, antioxidante e efeitos anti-inflamatórios (SWEITZER; DE LEO, 2011; BONDAN et al., 2014). Clinicamente, ela demonstrou eficácia no tratamento da demência vascular degenerativa, potencial tratamento adjuvante para a doença de Alzheimer, esquizofrenia e esclerose múltipla (SWEITZER; DE LEO, 2011). A propentofilina atua como um modulador de células da glia e inibe a produção macrofágica de TNF-α (JUNG et al., 1997).

Assim, o objetivo deste estudo foi utilizar a propentofilina como um tratamento para a depressão, devido à limitação da eficácia de fármacos antidepressivos existentes. Importante destacar que, até onde sabemos, nenhum estudo examinou a propentofilina para a depressão. Foi utilizado um modelo de comportamento tipo-depressivo em ratos por meio de administrações repetidas de LPS (YIRMIYA, 1996; FRENOIS et al., 2007; DANTZER et al., 2008; BAY-RICHTER et al., 2011). Primeiro, avaliamos a indução e a remissão do comportamento doentio com base no modelo de Dantzer (DANTZER et al., 2008), avaliando o peso corporal diario, o comportamento em campo aberto, e os níveis plasmáticos de TNF-α. Foram avaliados os níveis de ansiedade pelo teste do claro-escuro. A avaliação do comportamento tipo-depressivo foi realizada por meio do teste do nado forçado. Os níveis plasmáticos do fator neurotrófico derivado do cérebro (BDNF) também foram avaliados como biomarcador de depressão (HASHIMOTO; SHIMIZU; IYO, 2004; DRZYZGA; MARCINOWSKA; OBUCHOWICZ, 2009). O BDNF é uma pequena proteína encontrada por todo o cérebro, sistema nervoso central e sangue periférico.

É membro de uma família de proteínas conhecidas como neurotrofinas, com função de regular a sobrevivência, a morfologia, o desenvolvimento e a função neuronal, além de desempenhar papel crítico na sinaptogênese e na plasticidade sináptica (BINDER; SCHARFMAN, 2004). O BDNF parece estar envolvido na gênese de muitos casos de depressão, sendo que diversos pacientes depressivos apresentaram níveis reduzidos de BDNF (HASHIMOTO; SHIMIZU; IYO, 2004; DRZYZGA; MARCINOWSKA; OBUCHOWICZ, 2009). Inclusive, uma nova classe de medicamentos antidepressivos ligados a interferências na expressão de BDNF tem sido estudada (DRZYZGA; MARCINOWSKA; OBUCHOWICZ, 2009; LI et al., 2011).

2 ARTIGO

Propentofylline prevents sickness behavior and depressive-like behavior induced by lipopolysaccharide in rats

Márcia M. T. Moraes, Marcella C. Galvão, Danilo Cabral, Cideli P. Coelho, Nicolle Queiroz-Hazarbassanov, Maria M. Bernardi, Thiago B. Kirsten

Highlights

The objective was to use propentofylline (PRO) as a depression treatment.

Repetitive LPS administration induced sickness behavior and depressive-like behavior.

PRO prevented body weight loss and open field behavior impairments induced by LPS.

PRO also prevented the increased TNF-α levels and immobility in the forced swim test.

Thus, PRO prevented both sickness behavior and depressive-like behavior.

Propentofylline prevents sickness behavior and depressive-like behavior induced by lipopolysaccharide in rats

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Abstract

Recent studies have demonstrated the relation between depression and immune disturbances. Knowing the efficacy limitation of existing antidepressants drugs and the anti-inflammatory effects of propentofylline, the objective of this study was to use propentofylline as a depression treatment. We used a rat model of depressive-like behavior induced by repetitive lipopolysaccharide (LPS) administration. We studied sickness behavior, evaluating daily body weight, open field behavior, and TNF-α plasmatic levels. Anxiety-like behavior (light-dark test), depressive-like behavior (forced swim test), and plasmatic levels of the brain-derived neurotrophic factor (BDNF, depression biomarker) were also evaluated. LPS induced body weight loss, open field behavior impairments (decreased locomotion and rearing, and increased immobility), and increased plasmatic TNF-α levels in rats, compared with control group. Thus, sickness behavior was observed after 24 and 48 hours of LPS exposure. Repetitive LPS administration also increased the immobility and reduced climbing in the forced swim test, when compared with the control group, i.e., LPS induced depressive-like behavior in rats. Propentofylline prevented sickness behavior after four days of consecutive treatment, as well as prevented the depressive-like behavior after five days of consecutive treatments. Neither LPS nor propentofylline has influenced the anxiety and plasmatic BDNF levels of rats. In conclusion, LPS administration induced sickness behavior and depressive-like behavior in rats via inflammatory pathway. Propentofylline prevented both sickness behavior and depressive-like behavior, in the light of behavioral and immune parameters. The present findings may contribute to a better understanding and treatment of depression and associated diseases.

Keywords: LPS; Depression; Anxiety; Open field test; Light-dark test; Forced swim test; TNF-α.

1. Introduction

Depression is a complex mood disorder, characterized by loss of interest or pleasure, anedonia, apathy, poor concentration, low energy, disturbed sleep and appetite, reduced social and sexual interest, among other symptoms [1, 2]. It is estimated that 40 to 60% of suicides are directly linked to depression [3, 4]. Over 15% of all adults will experience an episode of major depression at some point in their lifetime, being women more affected than men (20% vs. 10%) [5, 6]. The costs related to this disorder represent an economic burden of tens of billions of dollars per year [7]. Therefore, depression has been considered as the plague of this century.

Unfortunately, little is still known about the etiology and pathophysiology of depression. It is considered as a disorder of multifactorial causes, including genetic factors, stressful events, diseases, hormonal imbalance, and drug abuse [2, 8]. Although the monoaminergic (serotonin and noradrenaline) hypothesis is recognized and accepted, and is the basis for supporting antidepressants prescription, it fails to explain and treat many aspects of depression [9]. Smith [10] proposed the macrophage theory of depression, which states that the excessive secretion of interleukin (IL)-1 and other products of macrophages are involved in the pathogenesis of depression. In this sense, some patients diagnosed with depression have increased levels of cytokines such as tumor necrosis factor (TNF-α) and IL-6 in the blood [11]. Hepatitis C or cancer patients treated with interferon alpha (IFN-α) also developed depression [12]. Moreover, even low doses of lipopolysaccharide (LPS) administered to volunteer patients are able to increase serum levels of

proinflammatory cytokines and induce anedonia, which is one of the main symptoms of depression [13]. LPS is an endotoxin that mimics infection by gram-negative bacteria by activating the immune system to release cytokines, such as TNF-α, IL-1β, and IL-6 [14-16].

Based on these neuroimmune aspects, many drugs have been tested for the treatment of depression, especially the use of anti-inflammatory drugs [9]. For example, the cyclooxygenase-2 inhibitor celecoxib, that inhibits proinflammatory cytokines production, has therapeutic effects in depressive patients treated with reboxetine [17]. Similar results were found with the association of celecoxib with fluexetina [18]. TNF-α inhibitors, such as etanercept and infliximab reduce depressive symptoms in patients with psoriasis and Crohn's disease and have been examined as potential treatments for depressive patients [19, 20]. In this sense, due to the anti-inflammatory characteristics of propentofylline, we proposed it as a candidate for depression treatment. Propentofylline (3-methyl-1-(5'-oxohexyl)-7-propylxanthine), a xanthine derivative, presents profound neuroprotective, antioxidant and anti-inflammatory effects [21, 22]. Clinically, it has shown efficacy in degenerative vascular dementia and as a potential adjuvant treatment to Alzheimer's disease, schizophrenia, and multiple sclerosis [21]. Propentofylline acts as a glial modulator and inhibits macrophagic TNF-α production [23].

The objective of this study was to use propentofylline as a depression treatment, because of the efficacy limitation of existing antidepressants drugs. We used a rat model of depressive-like behavior induced by repetitive LPS administration [24-27]. First, we evaluated sickness behavior induction and remission based on Dantzer et al. [26] model, evaluating daily body weight, open field behavior, and TNF-α plasmatic levels. Anxiety-like behavior was evaluated with the light-dark test.

Depressive-like behavior was evaluated with the forced swim test. Besides TNF-α [11], the brain-derived neurotrophic factor (BDNF) has also been considered as a depression biomarker [28, 29] and its plasmatic levels was evaluated.

2. Materials and Methods

2.1. Ethics statement

The present study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Paulista University, Brazil (Permit Number: 296/14). All efforts were made to minimize suffering, reduce the number of animals used, and utilize alternatives to in vivo techniques when available. The experiments were also performed in accordance with good laboratory practice protocols and quality assurance methods.

2.2. Animals

A total of 40 Wistar male rats with 95-115 days of age and weighing 305-375 g were used. They were housed in polypropylene cages (45.5 X 34.5 X 20 cm; 5 rats per cage) with microisolator system (Tecniplast, Buguggiate, Italy), controlled temperature (22°C ± 2°C) and humidity (55–65%) with artificial lighting (12-hr light/12-hr dark cycle, lights on at 7:00 AM). The animals had free access to irradiated rodent chow (BioBase, Águas Frias, Brazil) and filtered water. Sterilized and residue-free wood shavings were used for animal bedding.

2.3. Treatments and groups

Rats were treated with propentofylline solution and/or LPS solution and/or their vehicle, as described below. Propentofylline was administered at 12.5 mg/kg/day dose (Agener União Química, Sao Paulo, Brazil, 20 mg/mL solution) by intraperitoneal (i.p.) route [22]. Rats received propentofylline for five consecutive days. LPS (from Escherichia coli; Sigma, St. Louis, USA, serotype 0127: B8) was dissolved in sterile saline (1 mg/mL LPS in a 0.9% NaCl solution) and administered i.p. at a dose of 1 mg/kg/day, based on Bay-Richter et at. [24] studies. This dose is considered able to induce sickness behavior for at least 24 hours, without sepsis [24]. Rats received LPS for two consecutive days, on days 3 and 4 of propentofylline treatment. Sterile saline solution (0.9% NaCl) was administered as vehicle/control groups. Each rat schedule with saline treatment received a 0.1 mL/100 g, i.p., of saline solution.

The rats were randomly divided into four groups (n = 10 per group). (1) SAL+SAL (control group), rats that received saline solution for five consecutive days. On days 3 and 4 they also received an additional saline dose 1 hour after the first injection. (2) SAL+LPS (LPS group), rats that received saline solution for five consecutive days. On days 3 and 4 they also received a LPS dose 1 hour after the saline injection. (3) PRO+LPS, rats that received propentofylline solution for five consecutive days. On days 3 and 4 they also received a LPS dose 1 hour after the propentofylline injection. (4) PRO+SAL (propentofylline group), rats that received propentofylline solution for five consecutive days. On days 3 and 4 they also received a saline dose 1 hour after the propentofylline injection.

2.4. Sickness behavior

Sickness behavior is normally a temporary state characterized by adaptive behavioral- and neuroimmune-specific changes orchestrated by the host to fight the invading microorganism and heal more quickly, as well as to reduce exposure of the sick animal to predation and contamination of their colony [30, 31]. Some of the most typical symptoms of the sickness behavior are prostration, decreases in exploratory activity and in feeding behavior, weight loss, and increase of peripheral proinflammatory cytokines levels (such as TNF-α) [32, 33]. Thus, we evaluated the open field general activity and body weight of rats daily, as well as measured the plasmatic TNF-α levels.

Body weight (g) was evaluated daily throughout the five days of treatments. The open field behavior was evaluated three times, 1 hour after the LPS/vehicle injections (days 3 and 4) and 24 hours after the last LPS/vehicle injection (day 5). The open-field apparatus is used to evaluate exploratory/motor behaviors [34]. It consisted of a round wooden arena (96 cm diameter, 29 cm high walls) that was painted gray with an acrylic washable cover and subdivided into 25 parts. Each rat was individually placed in the center of the apparatus, and the following parameters were evaluated over a period of 5 min: locomotion frequency (number of floor units entered with both feet), rearing frequency (number of times the rodents stood on their hind legs), and total immobility time (s). The testing room, which was isolated from experimenter, was a small room with dim lighting. A video camera mounted above the arena was used to collect the data. The apparatus was washed with a 5% alcohol/water solution before placement of the animals to obviate possible biasing effects from odor cues left by previous rats.

2.5. Anxiety-like behavior

Immediately after the last open field test (day 5), rats were observed in a light-dark apparatus to evaluate anxiety-like behavior [35]. This model is based on the innate aversion of rodents to bright places, generating an inherent conflict between their exploratory drive to a novel place and their avoidance of the lit compartment [35, 36]. The apparatus consisted of an acrylic box (80 cm length, 40 cm width, 30 cm high) containing two compartments (separated by a door with 13 x 8 cm): dark room with black walls and floor (34 cm length), and light room, with white walls and floor (44 cm length) and illuminated with white electronic lamp (15W, 4100K). Each rat was individually placed in the center of the light room, facing the wall opposite to the door. The following parameters were evaluated over a period of 5 min: dark side entry latency (s), total time (s) spent in the dark side, total time (s) spent in the light side, and rearing frequency. The testing room, which was isolated from experimenter, was a small room with dim lighting. A video camera mounted above the arena was used to collect the data. The apparatus was washed with a 5% alcohol/water solution before placement of the animals to obviate possible biasing effects from odor cues left by previous rats.

2.6. Depressive-like behavior

Immediately after the light-dark test (day 5), rats were observed in the forced swim test to evaluate depressive-like behavior. This test is the most widely used tool for assessing antidepressant activity preclinically [37]. It is based on the observation that rats, following initial escape-oriented movements, develop an immobile posture when placed in an inescapable cylinder of water. The immobility is thought to reflect a failure of persistence in escape-directed behavior (i.e., behavioral despair) [37]. In

this model, the more time rats spend immobile and not trying to escape (such as climbing), the more depressive-like behavior it represents. The apparatus consisted of round transparent acrylic arena (46 cm height, 20 cm diameter) containing 30 cm water at 23°C ± 1°C. Each rat was individually and gently placed on the water surface, and the following parameters were evaluated over a period of 7 min: first immobility latency (s), total immobility (s), and total time (s) spent climbing. Immobility was considered the absence of active behavior, i.e., when the rat was not swimming or climbing, remaining passively floating, or performing only minimal movements necessary to keep the nose above the water. The water in the cylinder was changed after each animal observation to avoid olfactory cues left by the previous animal.

2.7. Plasmatic evaluations

Immediately after the forced swim test (day 5), the rats were decapitated, and trunk blood was collected in conical tubes that contained 10% ethylenediaminetetraacetic acid (EDTA). The samples were centrifuged (3.500 RPMs, 15 min, 15°C), and plasma was obtained. Plasma samples of each animal were aliquoted and stored in different conical tubes for separate analyses of TNF-α and BDNF using enzyme-linked immunosorbent (ELISA) commercial kits in duplicate and according to the manufacturer's instructions.

TNF-α was quantified using the DuoSet R&D Systems kit (cat. no. DY510, Minneapolis, USA). TNF-α is considered a biomarker of sickness behavior [33, 38] and depression [11]. BDNF levels were determined using a Promega kit (cat. no. G7610, Madison, USA). BDNF has also been considered as a depression biomarker [28, 29]. In both cases, the results are expressed in pg/ml.

2.8. Statistical analysis

Homogeneity and normality was verified using a Bartlett's test. One-way analysis of variance (ANOVA) followed by Newman-Keuls's multiple comparison test was used to compare the parametric data among the four groups. For analysis that includes evaluations in consecutive days, two-way ANOVA followed by Newman-Keuls's multiple comparison test was used. The results are expressed as the mean ± SEM. In all cases, the results were considered significant if $p < 0.05$.

3. Results

LPS induced body weight loss, open field behavior impairments (decreased locomotion and rearing, and increased immobility), and increased plasmatic TNF-α levels in rats, compared with control group. Thus, sickness behavior was observed after 24 and 48 hours of LPS exposure. Repetitive LPS administration also increased the immobility and reduced climbing in the forced swim test, when compared with the control group, i.e., LPS induced depressive-like behavior in rats. Propentofylline prevented sickness behavior after four days of consecutive treatment, as well as prevented the depressive-like behavior after five days of consecutive treatments. Neither LPS nor propentofylline has influenced the anxiety and plasmatic BDNF levels of rats.

Este volume de Dissertação de Mestrado apresenta somente o resumo dos principais resultados obtidos com este estudo. Para maiores detalhes, consultar a publicação em artigo científico, ou contatar o orientador deste estudo via e-mail: Prof. Dr. Thiago B. Kirsten, thik@outlook.com

4. Discussion

Este volume de Dissertação de Mestrado apresenta somente as conclusões do trabalho, sem sua discussão. Para maiores detalhes, consultar a publicação em artigo científico, ou contatar o orientador deste estudo via e-mail: Prof. Dr. Thiago B. Kirsten, thik@outlook.com

5. Conclusions

In conclusion, LPS administration induced sickness behavior and depressive-like behavior in rats via inflammatory pathway. Propentofylline prevented both sickness behavior and depressive-like behavior, including for the behavioral and immune parameters. The present findings may contribute to a better understanding and treatment of depression and associated diseases.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Figure Legends

Figure 1. Body weight: Effects of LPS (1 mg/kg/day) and propentofylline (12.5 mg/kg/day) on the body weight of adult male rats. SAL+SAL, saline injection at days 1-5 and another saline injection 1 h later at days 3-4; SAL+LPS, saline injection at days 1-5 and LPS injection 1 h later at days 3-4; PRO+LPS, propentofylline injection at days 1-5 and LPS injection 1 h later at days 3-4; PRO+SAL, propentofylline injection at days 1-5 and saline injection 1 h later at days 3-4 (n = 10 per group). *p < 0.05, SAL+LPS vs. SAL+SAL; #p < 0.05, SAL+LPS vs. PRO+LPS; +p < 0.05, SAL+SAL vs. PRO+SAL (two-way ANOVA followed by the Newman-Keuls test). The data are expressed as the mean ± SEM.

Figure 2. Open-field behavior: Effects of LPS (1 mg/kg/day) and propentofylline (12.5 mg/kg/day) on the open-field behaviors in adult male rats. SAL+SAL, saline injection at days 1-5 and another saline injection 1 h later at days 3-4; SAL+LPS, saline injection at days 1-5 and LPS injection 1 h later at days 3-4; PRO+LPS, propentofylline injection at days 1-5 and LPS injection 1 h later at days 3-4; PRO+SAL, propentofylline injection at days 1-5 and saline injection 1 h later at days 3-4 (n = 10 per group). ***p < 0.001, SAL+LPS vs. SAL+SAL; #p < 0.05 and ##p < 0.01, SAL+LPS vs. PRO+LPS; ++p < 0.01, SAL+SAL vs. PRO+SAL (two-way ANOVA followed by the Newman-Keuls test). The data are expressed as the mean ± SEM.

Figure 3. Anxiety-like behavior: Effects of LPS (1 mg/kg/day) and propentofylline (12.5 mg/kg/day) on the light-dark test in adult male rats. SAL+SAL, saline injection

at days 1-5 and another saline injection 1 h later at days 3-4; SAL+LPS, saline injection at days 1-5 and LPS injection 1 h later at days 3-4; PRO+LPS, propentofylline injection at days 1-5 and LPS injection 1 h later at days 3-4; PRO+SAL, propentofylline injection at days 1-5 and saline injection 1 h later at days 3-4 (n = 10 per group). **p < 0.01 (one-way ANOVA followed by the Newman-Keuls test). The data are expressed as the mean ± SEM.

Figure 4. Depressive-like behavior: Effects of LPS (1 mg/kg/day) and propentofylline (12.5 mg/kg/day) on the forced-swim test in adult male rats. SAL+SAL, saline injection at days 1-5 and another saline injection 1 h later at days 3-4; SAL+LPS, saline injection at days 1-5 and LPS injection 1 h later at days 3-4; PRO+LPS, propentofylline injection at days 1-5 and LPS injection 1 h later at days 3-4; PRO+SAL, propentofylline injection at days 1-5 and saline injection 1 h later at days 3-4 (n = 10 per group). *p < 0.05, **p < 0.01, ***p < 0.001 (one-way ANOVA followed by the Newman-Keuls test). The data are expressed as the mean ± SEM.

Figure 5. TNF-α and BDNF: Effects of LPS (1 mg/kg/day) and propentofylline (12.5 mg/kg/day) on TNF-α and BDNF plasma levels in adult male rats. SAL+SAL, saline injection at days 1-5 and another saline injection 1 h later at days 3-4; SAL+LPS, saline injection at days 1-5 and LPS injection 1 h later at days 3-4; PRO+LPS, propentofylline injection at days 1-5 and LPS injection 1 h later at days 3-4; PRO+SAL, propentofylline injection at days 1-5 and saline injection 1 h later at days 3-4 (n = 10 per group). **p < 0.01 (one-way ANOVA followed by the Newman-Keuls test). The data are expressed as the mean ± SEM.

Supplementary Table 1. Statistical values of F and p of two-way analysis of variance of body weight and open field general activity

	Body weight	Locomotion	Rearing	Immobility
Interaction				
F	2.37	2.90	1.37	3.13
p	0.0343 *	0.0115 *	0.2349	0.0073 **
Treatment				
F	13.62	10.88	19.05	19.09
p	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***
Days				
F	6.94	13.69	19.97	19.96
p	0.0015 **	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Supplementary Table 2. Statistical values of F and p of one-way analysis of variance of light-dark test, forced-swim test, and plasmatic evaluations

	F	*p*
Light-dark test		
Dark side entry latency	2.06	0.1233
Dark side total time	5.68	0.0027 **
Light side total time	5.68	0.0027 **
Rearing frequency	5.20	0.0044 **
Forced-swim test		
First immobility latency	0.44	0.7223
Immobility time	4.34	0.0104 *
Climbing time	11.60	< 0.0001 ***
Plasmatic evaluations		
TNF-α	4.37	0.0100 *
BDNF	2.82	0.0524

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3 CONSIDERAÇÕES FINAIS

Concluindo, a administração de LPS induziu comportamento doentio e comportamento tipo-depressivo em ratos por meio de via inflamatória. A propentofilina preveniu tanto o comportamento doentio quanto o comportamento tipo-depressivo, incluindo para os parâmetros comportamentais e imunológicos. Os presentes achados podem contribuir para uma melhor compreensão e tratamento da depressão e doenças associadas.

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ANEXO: Certificado de aprovação do Comitê de Ética em Pesquisa no Uso de Animais

Vice-Reitoria de Pós-Graduação e Pesquisa

C E R T I F I C A D O

CERTIFICAMOS, que o protocolo nº 296/14 CEUA/UNIP, sobre o projeto de pesquisa intitulado "Propentofilina previne o comportamento doentio e o comportamento tipo depressivo induzido por lipopolissacarídeo em ratos'', ''Espécie utilizada: rato heterogênico '' ''Número de animais utilizados: 40 '' sob a responsabilidade de '' THIAGO BERTI KIRSTEN e MÁRCIA MARIA TIVELLI MORAES'' , está de acordo com os Princípios Éticos, seguindo as diretrizes e normas regulamentadoras de pesquisa envolvendo animais, conforme a Lei Federal nº 11.794/08 que institui o Código de Proteção aos Animais do Estado de São Paulo e foi aprovado por este Comitê de Ética em Pesquisa.
Universidade Paulista, em São Paulo-SP, aos 10 dias do mês de dezembro de 2014.

Juliana Guizi
Secretária da Comissão de Ética no Uso de Animais – CEUA
Universidade Paulista – UNIP

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