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EFEITO FARMACOLÓGICO DO DISSELENETO DE *m*-TRIFLUORMETIL-FENILA NA COMORBIDADE ENTRE DOR E DEPRESSÃO EM CAMUNDONGOS

TESE DE DOUTORADO

César Augusto Brüning

Santa Maria, RS, Brasil

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César Augusto Brüning

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciências Biológicas, Área de Concentração em Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
Doutor em Bioquímica Toxicológica.

Orientadora: Prof. Dra. Cristina Wayne Nogueira

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Universidade Federal de Santa Maria
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Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica

A Comissão Examinadora, abaixo assinada,
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EFEITO FARMACOLÓGICO DO DISSELENETO DE *m*-TRIFLUORMETIL-FENILA NA COMORBIDADE ENTRE DOR E DEPRESSÃO EM CAMUNDONGOS

elaborada por
César Augusto Brüning

como requisito parcial para obtenção do grau de
Doutor em Bioquímica Toxicológica

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Santa Maria, 24 de julho de 2015

À minha família

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“A menos que modifiquemos a nossa maneira de pensar, não seremos capazes de resolver os problemas causados pela forma como nos acostumamos a ver o mundo.”

(Albert Einstein)

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

EFEITO FARMACOLÓGICO DO DISSELENETO DE *m*-TRIFLUORMETIL-FENILA NA COMORBIDADE ENTRE DOR E DEPRESSÃO EM CAMUNDONGOS

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ORIENTADORA: CRISTINA WAYNE NOGUEIRA
Data e Local da Defesa: Santa Maria, 24 de julho de 2015.

A dor crônica e a depressão geralmente coexistem, e inúmeros mecanismos estão envolvidos na patogênese desta comorbidade, o que a torna resistente ao tratamento. O sistema serotoninérgico é considerado um mecanismo central na díade dor-depressão, que também pode ser originada a partir de disfunções do sistema imune ou danos no tecido neuronal, envolvendo o processo de neuroinflamação. O composto orgânico de selênio disseleneto de *m*-trifluormetil-fenila (*m*-CF_3-PhSe)$_2$ apresenta efeito antinociceptico e do tipo antidepressivo em modelos agudos em camundongos e evidências comportamentais demonstraram que o seu efeito antidepressivo está relacionado com o sistema serotoninérgico. O objetivo deste estudo foi caracterizar melhor os efeitos farmacológicos do (*m*-CF_3-PhSe)$_2$ e investigar o efeito do mesmo na comorbidade entre dor e depressão em camundongos, abordando os mecanismos patogênicos desta condição. O projeto de pesquisa foi aprovado pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal de Santa Maria, sob o número 042/2012. Primeiramente foi demonstrado que o sistema serotoninérgico também está envolvido no efeito antinociceptivo do (*m*-CF_3-PhSe)$_2$ (1–50 mg/kg, p.o.) no teste da injeção de glutamato na pata de camundongos, uma vez que antagonistas dos receptores serotoninérgicos 5-HT_{1A} (WAY100635) e $5HT_{2A/2C}$ (ritanserina) bloquearam seu efeito. Além disso, o (*m*-CF_3-PhSe)$_2$ (10 e 50 mg/kg, p.o.) inibiu a recaptação de serotonina (5-HT) *ex vivo* em sinaptossomas. Também foi determinado a distribuição de selênio em diferentes tempos após a administração de (*m*-CF_3-PhSe)$_2$ (500 mg/kg, p.o.) em camundongos, o qual apresentou uma ampla distribuição em diferentes tecidos, incluindo o cérebro. Considerando que a inflamação pode estar intimamente relacionada com a depressão, avaliou-se também o efeito do (*m*-CF_3-PhSe)$_2$ (0.01–50 mg/kg, i.g.) no comportamento do tipo depressivo induzido pela administração intracerebroventricular (i.c.v.) da citocina pró-inflamatória fator de necrose tumoral-α (TNF-α) nos testes do nado forçado (TNF) e da suspensão da cauda (TSC). O tratamento agudo com (*m*-CF_3-PhSe)$_2$ em baixas doses (a partir de 0.1 mg/kg) preveniu o aumento do tempo imobilidade dos animais, o qual representa um comportamento do tipo depressivo, em ambos os testes, sem alterar a atividade locomotora dos camundongos. As doses que não foram efetivas (0.01 mg/kg no TNF e 0.1 mg/kg no TSC) no tratamento agudo bloquearam o efeito do TNF-α quando administradas subcronicamente por 2 semanas. Além disso, o (*m*-CF_3-PhSe)$_2$ apresentou efeito anti-inflamatório nos tratamentos agudo e subcrônico ao prevenir a ativação da proteína quinase ativada por mitógeno p38 (p38 MAPK) e o aumento dos níveis de fator nuclear-κB (NF-κB) induzidos pelo TNF-α no hipocampo e córtex pré-frontal de camundongos. Por fim, foi demonstrado que o (*m*-CF_3-PhSe)$_2$ (1 e 10 mg/kg, i.g.) apresentou efeito antinociceptivo e do tipo antidepressivo em um modelo de comorbidade entre dor e

depressão induzido pela ligação parcial do nervo ciático (LPNC) em camundongos, associado a um efeito anti-inflamatório. A LPNC induziu alodínia mecânica observada no teste dos filamentos de Von-frey e aumento do tempo de imobilidade dos animais no TNF e o (*m*-CF_3-PhSe)$_2$ tanto no tratamento agudo quanto no subcrônico em baixas doses foi efetivo em bloquear estas alterações. A LPNC causou também o aumento dos níveis de citocinas pro-inflamatórias no soro, no córtex cerebral e no hipocampo de camundongos, bem como do hormônio adrenocorticotrófico (ACTH) e da corticosterona no soro, a ativação da p38 MAPK, o aumento dos níveis de NF-κB e da cicloxigenase-2 (COX-2), a diminuição dos níveis do fator neurotrófico derivado do cérebro (BDNF) e o aumento da recaptação de 5-HT e a liberação de glutamato, no córtex e no hipocampo. De maneira geral, tanto o tratamento agudo (10 mg/kg, i.g.) quanto o subcrônico (0.1 mg/kg) com (*m*-CF_3-PhSe)$_2$ foram eficazes em bloquear essas alterações, embora os melhores resultados foram observados no tratamento subcrônico. Tendo em vista que a díade dor-depressão é uma condição multipatogênica e a inflamação pode ter um papel central nessa comorbidade, o (*m*-CF_3-PhSe)$_2$ poderia ser considerado uma interessante alternativa terapêutica para tratar a dor crônica associada à depressão.

Palavras-chave: dor, depressão, serotonina, citocinas, inflamação, selênio.

ABSTRACT

Thesis of Doctor's Degree
Professional Graduation Program in Biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria

PHARMACOLOGICAL EFFECT OF *m*-TRIFLUOROMETHYL-DIPHENYL DISELENIDE ON PAIN AND DEPRESSION COMORBIDITY IN MICE

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ADVISOR: CRISTINA WAYNE NOGUEIRA
Defense Place and Date: Santa Maria, July 24th, 2015.

Chronic pain and depression often coexist, and several mechanisms are involved in the pathogenesis of this comorbidity, which confer it resistance to treatment. The serotonergic system is considered a central mechanism in the pain-depression dyad, which could also be generated from dysfunctions in the immune system or injuries to neuronal tissue, involving the process of neuroinflammation. The organoselenium compound *m*-trifluoromethyl-diphenyl diselenide ($(m\text{-}CF_3\text{-}PhSe)_2$) shows antinociceptive and antidepressant-like effects in acute models in mice and behavioral evidence demonstrated that its antidepressant-like effect is related to the serotonergic system. The aim of this study was to further characterize the pharmacological effects of $(m\text{-}CF_3\text{-}PhSe)_2$ and investigate its effect on the comorbidity between pain and depression in mice, addressing the pathogenic mechanisms of this condition. The research project was approved by the ethics committee (CEUA) of the Federal University of Santa Maria (042/2012). First, it was demonstrated that the serotonergic system is involved in the antinociceptive effect of $(m\text{-}CF_3\text{-}PhSe)_2$ (1–50 mg/kg, p.o.) in the glutamate test, once antagonists of serotonergic receptors 5-HT_{1A} (WAY100635) and 5-$HT_{2A/2C}$ (ritanserin) blocked its effect. In addition, $(m\text{-}CF_3\text{-}PhSe)_2$ (10 and 50 mg/kg, p.o.) inhibited the serotonin (5-HT) reuptake *ex vivo* in synaptosomes. It was also assessed the selenium distribution at different time points after the administration of $(m\text{-}CF_3\text{-}PhSe)_2$ (500 mg/kg, p.o.), which demonstrated a wide distribution in different tissues, including the brain. Taking into account that inflammation could be closely related to depression, the effect of $(m\text{-}CF_3\text{-}PhSe)_2$ on the depressive-like behavior induced by intracerebroventricular (i.c.v.) injection of tumor necrosis-α (TNF-α) in the forced swimming test (FST) and tail suspension test (TST) was also evaluated. The acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ (from 0.1 mg/kg) at low doses prevented the increase in the immobility time of animals, which is an indicative of depressive-like behavior, in both tests, without altering the locomotor activity of mice. The doses that were not effective (0.01 mg/kg in the FST and 0.1 mg/kg in the TSC) in the acute treatment blocked the effect of TNF-α when chronically administered for 2 weeks to mice. In addition, $(m\text{-}CF_3\text{-}PhSe)_2$ demonstrated an anti-inflammatory effect in both acute and subchronic treatments, preventing the activation of p38 mitogen-activated protein kinase (p38 MAPK) and the increase of nuclear factor-κB (NF-κB) levels induced by TNF-α in the hippocampus and pre-frontal cortex of mice. Lastly, it was demonstrated that $(m\text{-}CF_3\text{-}PhSe)_2$ (1 and 10 mg/kg, i.g.) elicited antinociceptive and antidepressant-like effects in a model of pain and depression comorbidity induced by partial sciatic nerve ligation (PSNL) in mice, which was related to anti-inflammatory effect. PSNL induced mechanical allodynia observed in the Von-

frey hair test and increased the immobility time of animals in the FST and (*m*-CF_3-PhSe)$_2$ in both acute and subchronic treatments at low doses was effective in blocking these alterations. PSNL also induced an increase of pro-inflammatory cytokines in serum, cerebral cortex and hippocampus of mice. An increase of adrenocorticotropic hormone (ACTH) and corticosterone in the serum, activation of p38 MAPK, an increase in the NF-κB and cyclooxygenase-2 (COX-2) levels, a decrease of brain-derived neurotrophic factor (BDNF) levels and an increase of 5-HT reuptake and glutamate release in the cortex and hippocampus of mice were found in PSNL mice. In a general way, both acute (10 mg/kg, i.g.) and subchronic (0.1 mg/kg, i.g.) (*m*-CF_3-PhSe)$_2$ treatments were effective in preventing these alterations, although the best results were observed in the subchronic treatment. Considering that pain-depression dyad is a multi-pathogenic condition and inflammation could have a central role in this comorbidity, (*m*-CF_3-PhSe)$_2$ might be considered an interesting therapeutic alternative to treat chronic pain associated with depression.

Keywords: pain, depression, serotonin, cytokines, inflammation, selenium.

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(*m*-CF_3-PhSe)$_2$ – Disseleneto de *m*-trifluormetil-fenila

(PhSe)$_2$ – Disseleneto de difenila

5-HT – 5-Hidroxitriptamina, serotonina

ACTH – Hormônio adrenocorticotrófico

ALT – Alanina aminotransferase

ANVISA – Agência Nacional de Vigilância Sanitária

AST – Aspartato aminotransferase

BDNF – Fator neurotrófico derivado do cérebro

COX-2 – Cicloxigenase-2

CRH – Hormônio liberador de corticotrofina

FDA – *Food and Drug Administration*

HPA – Hipotálamo-pituitária-adrenal

i.c.v. – Intracerebroventricular

IASP –Associação Internacional para o Estudo da Dor

IDO – Indoleamina-2,3-dioxigenase

IFN-γ – Interferon-γ

IL-1β – Interleucina-1β

IL-6 – Interleucina-6

iMAO– Inibidores da monoaminoxidase

iNOS – Óxido nítrico sintase induzível

ISRN – Inibidores seletivos da recaptação de norepinefrina

ISRS – Inibidores seletivos da recaptação de serotonina

JNK – Quinase c-Jun N-terminal

LPNC – Ligação parcial do nervo ciático

LPS – Lipopolissacarídeo

MAO – Monoaminoxidase

MAPK – Proteína quinase ativada por mitógeno

NMDA – N-metil D-aspartato

OMS – Organização mundial da saúde

SNC – Sistema nervoso central

SNP – Sistema nervoso periférico

TNF – Teste do nado forçado

TNF-α – Fator de necrose tumoral-α

TSC – Teste da suspensão da cauda

VEGF – Fator de crescimento do endotélio vascular

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1 INTRODUÇÃO

1.1 Dor

A dor é um mecanismo protetor que ocorre sempre que qualquer tecido é lesionado, fazendo com que o indivíduo reaja para remover o estímulo doloroso. No entanto, a dor também pode ser um efeito secundário, incapacitante, de muitas condições médicas, e o controle da dor é uma das prioridades terapêuticas mais importantes. O reconhecimento de que a dor persistente é uma síndrome — ou mesmo uma doença (Bonica, 1953) — levou à fundação da Associação Internacional para o Estudo da Dor (IASP). A IASP define dor como "uma experiência sensorial e emocional desagradável associada a uma lesão tecidual real ou potencial ou descrita em termos de tal lesão" (Loeser e Treede, 2008).

Tipicamente a dor envolve um estímulo nocivo que ativa nervos sensoriais terminais nos tecidos corporais chamados nociceptores. O corpo celular dos nociceptores encontra-se fora do sistema nervoso central (SNC), nos gânglios da raiz dorsal. Os axônios periféricos inervam os tecidos superficiais e profundos, enquanto que o axônio central realiza sinapse com neurônios de segunda ordem no cordão espinhal, que por sua vez projetam-se para as regiões supraespinhais do SNC, como o tronco encefálico, hipotálamo e tálamo. Essa vias ascendentes fazem conexões com neurônios de terceira ordem que projetam-se para o córtex somatosensorial, ínsula, giro cingulado anterior e córtex pré-frontal (Millan, 1999; Almeida et al., 2004; Willis e Coggeshall, 2004) (Figura 1). Estudos em humanos demonstraram que todas estas estruturas são ativadas em associação à sensação de dor (Rainville et al., 2001; Almeida et al., 2004). A transmissão do impulso nociceptivo é mediada principalmente pelo glutamato (Guyton e Hall, 2006).

Os sinais enviados pelos nociceptores para o SNC são processados e geram múltiplas respostas fisiológicas e comportamentais. O processamento das informações sobre o ambiente corporal interno e externo geradas pelos nociceptores é chamado nocicepção (Sherrington, 1906). Dentre as respostas geradas pela estimulação nociva uma é a indução do "estado emocional desagradável". Outras respostas incluem o reflexo, aumento da frequência cardíaca e pressão sanguínea, entre outros parâmetros, que podem ocorrer sem a presença de dor. Desta forma, dor e nocicepção são conceitos distintos e algumas respostas nociceptivas não necessariamente indicam dor (Millan, 1999; Julius e Basbaum, 2001).

O organismo também possui mecanismos intrínsecos responsáveis por controlar a dor. A transmissão do impulso nociceptivo no corno dorsal da medula espinhal está submetida à

modulação pela via descendente (Fields e Basbaum, 1994; Millan, 2002) (Figura 1). Os sinais supraespinhais são integrados nas áreas periventricular e da substância cinzenta periaquedutal do mesencéfalo de onde neurônios projetam-se para o núcleo magno da rafe, localizado nas regiões inferior da ponte e superior da medula oblonga, e o núcleo paragigantocelular, localizado lateralmente na medula oblonga. A partir destas áreas, os sinais de segunda ordem são transmitidos para um complexo inibitório da dor localizado nos cornos dorsais da medula espinhal. Neste ponto, o impulso nociceptivo pode ser bloqueado antes de ser enviado ao encéfalo. Vários neurotransmissores estão envolvidos no sistema de analgesia pela via descendente, destacando-se em especial a serotonina (5-HT) e as encefalinas. De fato, as moléculas opióides interagem com neurônios serotoninérgicos no núcleo magno da rafe e da substância cinzenta periaquedutal, facilitando a via descendente antinociceptiva (Millan, 2002).

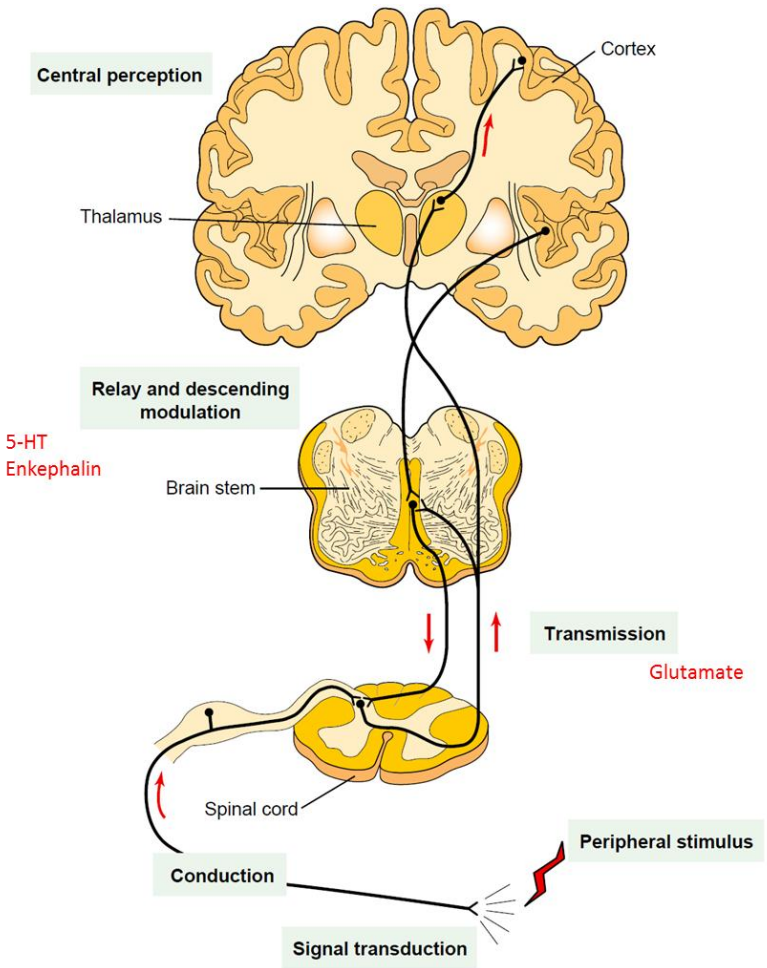

Figura 1. Representação esquemática da principal via neuronal responsável pela dor e o sistema de analgesia da via descendente. Adaptado de Siegel **—** *Basic Neurochemistry — Molecular, Cellular and Medical Aspects*7 Ed.

Em relação à duração, a dor pode ser classificada em transitória, aguda e crônica. Na dor transitória, os nociceptores da pele e outros tecidos são ativados sem que haja qualquer lesão, desempenhando um papel essencialmente protetor sem necessidade de atenção clínica. Por sua vez a dor aguda caracteriza-se por lesão tecidual provocada frequentemente por trauma, intervenção cirúrgica ou doença e pode durar de poucos dias a poucas semanas, desaparecendo com a resolução da lesão. Normalmente a intervenção clínica é útil no sentido de bloquear ou reduzir a dor ou acelerar o processo de restabelecimento do tecido lesado. Já a dor crônica permanece mesmo após a resolução da lesão e pode durar de meses a anos, causando muito sofrimento e incapacidade para o indivíduo. Na dor crônica a lesão tecidual excede a capacidade de reparação do organismo, devido à perda de parte do tecido, a extensão do trauma ou a lesão do próprio sistema nervoso. As terapias clínicas disponíveis normalmente não são efetivas e fornecem apenas alívio transitório sendo que fatores ambientais e afetivos, bem como o estresse podem contribuir significativamente para a intensidade e persistência da dor. Além disso, tanto a dor aguda quanto a crônica estão frequentemente associadas a processos inflamatórios, como resultado da lesão tecidual, reatividade imune anormal ou lesão nervosa (Loeser e Melzack, 1999; Stein et al., 2003).

A dor crônica resultante de lesão a um nervo do sistema nervoso periférico (SNP) ou SNC, devido a uma doença, lesão ou inflamação é chamada de dor neuropática. Segundo a IASP 7–8 % dos casos de dor crônica na população em geral apresentam característica neuropática. Esse tipo de dor geralmente é muito mais severa, debilitante e de difícil tratamento, principalmente porque os mecanismos patofisiológicos são pouco conhecidos (Harden e Cohen, 2003). Os sintomas da dor neuropática podem incluir alodínia (dor resultante de um estímulo que normalmente não é nocivo), hiperalgesia (uma resposta excessiva a um estímulo nocivo) e dor espontânea (Woolf e Mannion, 1999).

1.2 Depressão

A depressão maior é uma doença mental caracterizada por humor deprimido, perda de interesse ou prazer, diminuição de energia, sentimentos de culpa e fracasso, distúrbios de humor e apetite e incapacidade de concentração. Além disso, a depressão é geralmente acompanhada de sintomas de ansiedade. Esses problemas podem se tornar crônicos ou recorrentes e levar a substanciais prejuízos na qualidade de vida do indivíduo, seja na

capacidade de autocuidado ou em executar suas responsabilidades diárias (WHO, 2012). Em um cenário mais crítico a depressão pode levar ao suicídio. Segundo dados da Organização Mundial da Saúde (OMS), ao menos 350 milhões de pessoas vivem com depressão, considerada a principal causa de incapacidade na população em geral, e em torno de 1 milhão de pessoas a cada ano tiram suas próprias vidas, significando quase 3000 vidas perdidas a cada dia. Para cada pessoa que comete suicídio, 20 ou mais atentam contra sua própria vida (WHO, 2012).

Os aspectos patofisiológicos da depressão ainda não são completamente compreendidos e a hipótese mais aceita é a teoria monoaminérgica, na qual a deficiência dos neurotransmissores 5-HT, norepinefrina e dopamina estaria intimamente associada à patogênese da depressão (Coppen, 1967). Inúmeras evidências contribuem para a teoria monoaminérgica, sendo que dentre as diferentes monoaminas, a 5-HT é a mais extensivamente estudada. A 5-HT é sintetizada no neurônio pré-sináptico a partir do triptofano pela ação da enzima triptofano hidroxilase e armazenada em vesículas. Após sua liberação na fenda sináptica e interação com os receptores serotoninérgicos, a 5-HT é recaptada para o interior do neurônio pré-sináptico por um transportador específico, onde é armazenada nas vesículas pré-sinápticas ou degradada pela enzima monoamino oxidase (MAO) (Figura 2). Moléculas que bloqueiam a recaptação de 5-HT ou inibem a MAO, aumentam a disponibilidade deste neurotransmissor na fenda sináptica e têm apresentado substancial efetividade clínica como antidepressivos (Mann, 2005; Belmaker e Agam, 2008). A depleção experimental do triptofano causa recidiva no estado depressivo em pacientes tratados com inibidores seletivos da recaptação de 5-HT (ISRS). Da mesma forma, a depleção da tirosina hidroxilase, necessária à síntese de norepinefrina, causa recidiva nos pacientes tratados com inibidores seletivos da recaptação de norepinefrina (ISRN) (Ruhe et al., 2007). Além disso, a maioria dos neurônios serotoninérgicos, noradrenérgicos e dopaminérgicos localizados no mesencéfalo e no tronco encefálico projetam-se para inúmeras áreas cerebrais envolvidas na regulação de diversas funções que geralmente encontram-se alteradas na depressão, incluindo humor, atenção, sistema de recompensa, sono, apetite e cognição (Mann, 2005; Belmaker e Agam, 2008).

A hipótese da deficiência monoaminérgica é ainda considerada a teoria neurobiológica da depressão clinicamente mais relevante, de forma que os ISRS e ISRN são os principais antidepressivos prescritos. No entanto, muitos pacientes são resistentes a este tipo de tratamento e efeitos clínicos são perceptíveis apenas algumas semanas após o início da terapia

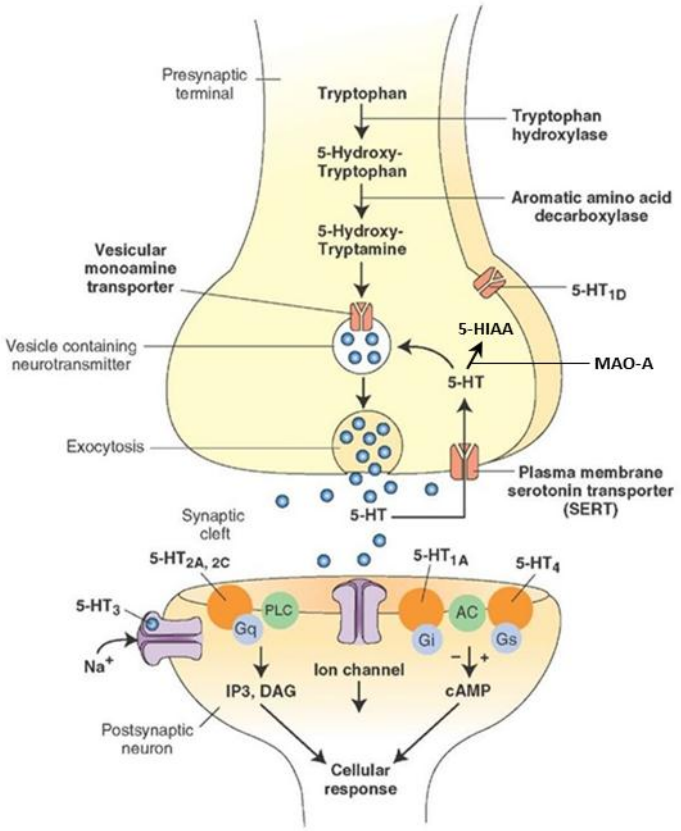

Figura 2. Representação esquemática da sinapse serotoninérgica. Adaptado de what-when-how.com/ neuroscience/neurotransmitters.

(Hegadoren et al., 2009; Dupuy et al., 2011). Além disso, estudos avaliando a eficácia dos ISRS têm demonstrado que grande parte da resposta antidepressiva é também vista nos grupos placebo (Lacasse e Leo, 2005; Kirsch et al., 2008). Desta forma, muitos pesquisadores acreditam que a disfunção do sistema monoaminérgico pode ser efeito secundário de outras alterações associadas à depressão (Belmaker e Agam, 2008).

O sistema opióide tem sido considerado um possível alvo no tratamento da depressão (Schreiber et al., 2002; Zomkowski et al., 2005) em adição ao seu importante papel na analgesia. Os receptores opióides μ e os opióides endógenos endorfinas são densamente distribuídos em diversas regiões cerebrais relacionadas à resposta a agentes estressores e estímulos emocionais (Drevets, 1998; Martin-Schild et al., 1999; Sheline, 2000; Zadina, 2002). Evidências demonstram que pacientes com depressão apresentam níveis diminuídos de β-endorfina (Darko et al., 1992; Djurovic et al., 1999) e uma pronunciada redução dos receptores opióides μ no tálamo posterior e no córtex cingulado anterior (Kennedy et al., 2006). Pacientes com depressão refratária têm apresentado melhora clínica com o uso de

agonistas de receptores opióides μ, oxicodona e oximorfona, e do agonista parcial buprenorfina (Bodkin et al., 1995; Stoll e Rueter, 1999) e em camundongos, o agonista de receptores opióides δ, KNT-127, também produz ação do tipo antidepressiva (Saitoh et al., 2011). Interessantemente, as endorfinas podem modular as transmissões serotoninérgicas (Tao e Auerbach, 2002; Hung et al., 2003), dopaminérgicas (Bujdoso et al., 2003; Huang et al., 2004) e noradrenérgicas (Al-Khrasani et al., 2003; Hung et al., 2003).

Alterações no eixo hipotálamo-pituitária-adrenal (HPA) também têm sido fortemente associadas à depressão. O hormônio liberador de corticotrofina (CRH) é liberado pelo hipotálamo em resposta à percepção de estresse psicológico por outras regiões do encéfalo. O CRH por sua vez induz a secreção do hormônio adrenocorticotrófico (ACTH), o qual estimula as glândulas adrenais a liberarem cortisol na corrente sanguínea, e por um mecanismo de retroalimentação negativa, o cortisol inibe a liberação de seus precursores (Figura 3). O CRH induz várias respostas fisiológicas e comportamentais, sendo que muitas delas estão associadas aos sintomas de depressão, como diminuição de apetite, distúrbios do sono e dimuição do libido (Nemeroff, 1996). Estudos demonstram que a exposição prolongada a elevados níveis de hormônios do estresse aumenta a atrofia e a susceptibilidade a danos e morte neuronal, especialmente no hipocampo, o qual é uma importante região de integração do humor (Sapolsky, 2000; Duman e Monteggia, 2006). Níveis elevados de CRH e cortisol

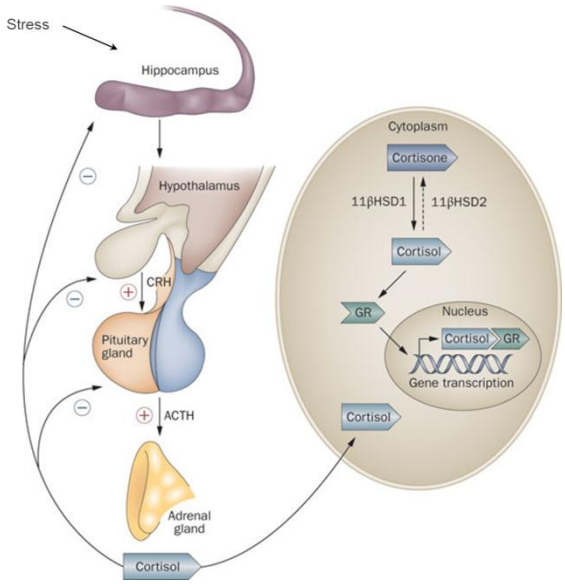

Figura 3. Representação esquemática do eixo hipotálamo-pituitária-adrenal (HPA). Adaptado de Strachan et al. (2011), *Nature Reviews Endocrinology.*

têm sido observados no líquor (Merali et al., 2004) e no plasma (Burke et al., 2005) de pacientes depressivos, respectivamente, bem como altos níveis de cortisol e ACTH foram detectados na urina (Rubin et al., 1987). Além disso, pesquisadores demonstraram que 50 % dos pacientes com depressão severa não respondem ao teste de supressão do cortisol pelo glicocorticoide dexametasona (Carroll et al., 2007) e a terapia com antidepressivos têm se mostrado eficaz na redução da atividade do eixo HPA (Carroll et al., 2007; Belmaker e Agam, 2008).

A redução de fatores neurotróficos, como o fator neurotrófico derivado do cérebro (BDNF) e o fator de crescimento do endotélio vascular (VEGF) também pode ter grande importância na patogênese da depressão. Esses fatores são extremamente importantes para o processo de neurogênese e plasticidade celular e encontram-se reduzidos em pacientes depressivos, especialmente em estruturas límbicas, como o hipocampo (Sapolsky, 2000; Duman e Monteggia, 2006). A ativação do eixo HPA pode exacerbar essa redução, enquanto que o tratamento com antidepressivos pode reverter esse processo aumentando os níveis de mRNA para o BDNF e/ou aumentando a expressão de seu receptor, trkB (Duman et al., 1997; 2000).

Outra linha de evidência sugere que uma disfunção do sistema glutamatérgico possa ter importante papel na depressão. O glutamato é o principal mediador da transmissão sináptica excitatória no cérebro de mamíferos (Orrego e Villanueva, 1993). Em condições fisiológicas, o glutamato possui importante papel na plasticidade sináptica, aprendizado e memória, mas em condições patológicas este neurotransmissor pode ser uma potente excitotoxina neuronal e contribuir para a diminuição da plasticidade sináptica e neuronal que é observada em pacientes com depressão severa ou resistente (Sanacora et al., 2008). Essa teoria ganha força considerando que uma única dose de quetamina, antagonista dos receptores glutamatérgicos N-metil-D-aspartato (NMDA), produz rápido e pronunciado efeito antidepressivo em pacientes resistentes a outros tratamentos (Zarate et al., 2006). Inibidores da liberação de glutamato, por exemplo lamotrigina e riluzole, também demonstraram efeito antidepressivo (Kendell et al., 2005) e níveis aumentados deste neurotransmissor foram detectados, por ressonância magnética, em pacientes depressivos (Hasler et al., 2007).

Estudos mais recentes têm demonstrado que existe uma comunicação entre o sistema imune e o sistema nervoso e que a depressão pode ser decorrente de processos inflamatórios (Safieh-Garabedian et al., 2002; Dantzer et al., 2008; Anisman, 2009). A inflamação periférica inicia uma cascata de eventos com liberação de substâncias imunoativas, como as

citocinas pró-inflamatórias, que podem culminar em neuroinflamação, com ativação dos macrófagos parenquimais cerebrais, conhecidos como micróglia, presentes no cérebro e no cordão espinhal. Essas células são mais quiescentes em comparação com macrófagos de outros tecidos, mas respondem aos estímulos inflamatórios produzindo mais citocinas pró-inflamatórias. Em adição, tanto as células cerebrais neuronais como as não-neuronais expressam receptores para esses mediadores (Dantzer, 2007). Nesse sentido, infecções virais e bacterianas que produzem um aumento na liberação de citocinas pró-inflamatórias podem levar a sintomas de depressão (Dantzer et al., 2008). Além disso, os níveis destas citocinas como a interleucina-1β (IL-1β), interleucina-6 (IL-6) e o fator de necrose tumoral-α (TNF-α) foram encontrados aumentados no soro de pacientes com depressão (Levine et al., 1999; Tuglu et al., 2003; Diniz et al., 2010). Em modelos animais, a injeção intracerebroventricular (i.c.v.) de TNF-α induz um comportamento do tipo depressivo (Kaster et al., 2012) e da mesma forma, a injeção sistêmica de lipopolissacarídeo (LPS) em camundongos induz comportamento do tipo depressivo via ativação do receptor de IL-1 (Zhu et al., 2010).

Existem diversos mecanismos descritos pelos quais as citocinas pró-inflamatórias podem estar envolvidas na patogênese da depressão (Figura 4), e todos eles estão diretamente relacionados às teorias descritas anteriormente. As citocinas pró-inflamatórias podem alterar o metabolismo e liberação de 5-HT no SNC (Cho et al., 1999). Citocinas como IL-1β e TNF-α ativam o transportador de 5-HT, aumentando a recaptação de 5-HT e consequentemente diminuindo a disponibilidade desta na fenda sináptica. O aumento da atividade deste transportador pelas citocinas é mediada pela ativação da proteína quinase ativada por mitógeno (MAPK) p38 (p38 MAPK) (Zhu et al., 2006; Malynn et al., 2013). As citocinas pró-inflamatórias, como o TNF-α e o interferon-γ (IFN-γ) também podem ativar a enzima indoleamina-2,3-dioxigenase (IDO), responsável pelo catabolismo do triptofano, diminuindo a disponibilidade deste para a síntese de 5-HT (Kim et al., 2012). Além disso, a degradação do triptofano gera quinurenina e o agonista glutamatérgico ácido quinolínico, que podem causar neurotoxicidade e desempenhar importante papel na indução dos transtornos de humor (Loftis et al., 2010; Kim et al., 2012). Tem sido demonstrado também que as citocinas pró-inflamatórias podem aumentar a liberação de glutamato e diminuir sua recaptação astrocitária (Tilleux e Hermans, 2007; Ida et al., 2008), aumentando os níveis deste neurotransmissor na fenda sináptica, o que contribui para a toxicidade glutamatérgica (Hardingham et al., 2002).

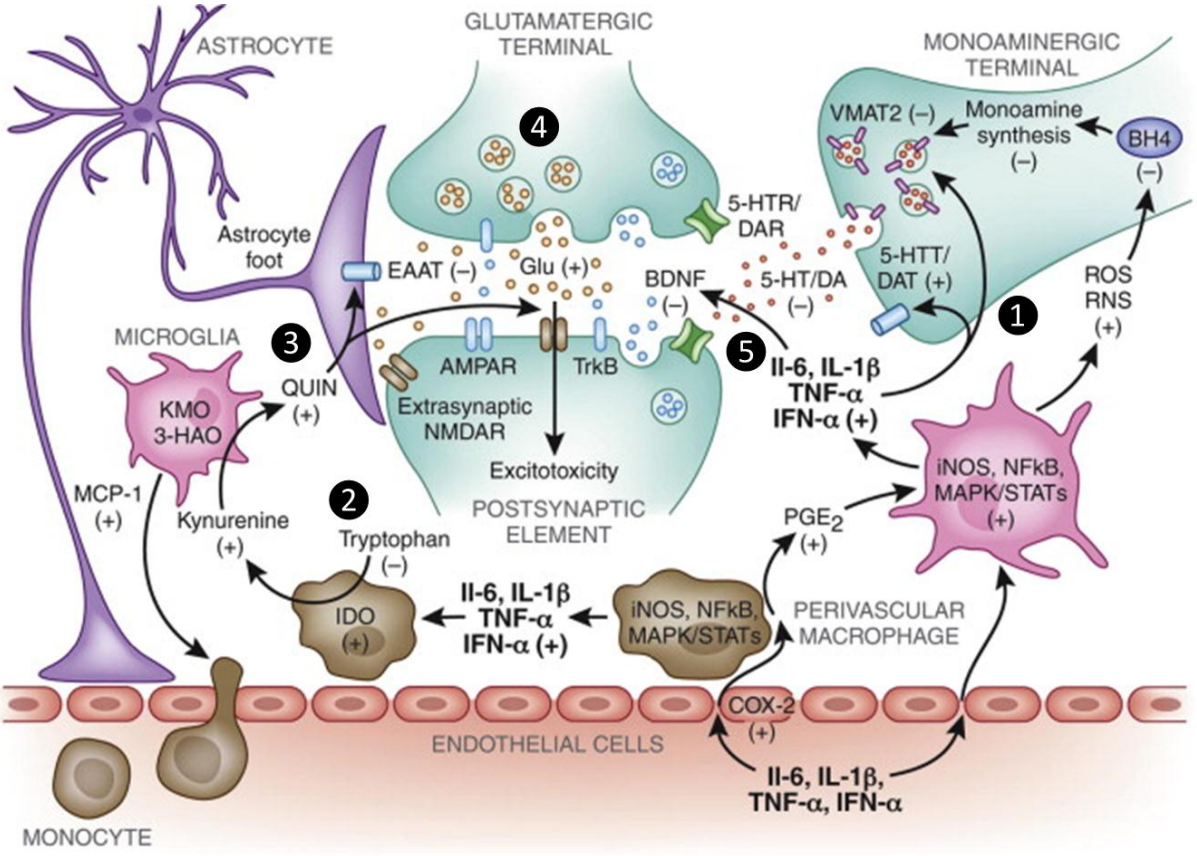

Figura 4. Efeito das citocinas pró-inflamatórias em diferentes vias neuronais. Os números indicam a recaptação de serotonina (1), atividade da IDO (2), níveis de ácido quinolínico (3), liberação de glutamato (4) e níveis de BDNF (5). Adaptado de Felger and Lotrich (2013), *Neuroscience.*

A hiperativação do eixo HPA, que como citado anteriormente pode estar intimamente relacionada à depressão, pode ocorrer devido ao aumento das citocinas pró-inflamatórias, uma vez que o TNF-α é um potente modulador do CRH (Anisman et al., 2002). Em condições fisiológicas, os glicocorticoides inibem a liberação de CRH por retroalimentação, porém na neuroinflamação as citocinas pró-inflamatórias causam resistência dos receptores glicocorticoides em células imunes e em outras células alvo através da indução de MAPKs como a quinase c-Jun N-terminal (JNK) e a p38, levando à continuada e excessiva produção de CRH. Devido à resistência dos receptores glicocorticoides, o efeito inibitório do cortisol na produção de citocinas pró-inflamatórias pelas células imunes periféricas e centrais também não ocorre levando a um aumento descontrolado da produção dessas citocinas (Raison e Miller, 2003; Irwin e Miller, 2007).

As citocinas pró-inflamatórias também podem afetar negativamente o processo de neurogênese e neuroplasticidade (Patel et al., 2003; Goshen e Yirmiya, 2009; Koo et al., 2010). Já foi demonstrado que as mesmas diminuem a proliferação celular no hipocampo através da ativação do receptor IL-1 e diminuem os níveis de BDNF (Kaneko et al., 2006; Guan e Fang, 2006; Tong et al., 2008; Cortese et al., 2011). Além disso, as citocinas influenciam a fosforilação do receptor trkB, interferindo ainda mais na via de sinalização do BDNF (Cortese et al., 2011).

1.3 Comorbidade entre dor e depressão

Como descrito anteriormente, a dor engloba componentes sensoriais, cognitivos e afetivos. Os componentes afetivos da dor incluem sensações de aborrecimento, tristeza, ansiedade e depressão em resposta ao estímulo nocivo. Em particular, a depressão e a dor compartilham um alto grau de comorbidade e muitos estudos têm demonstrado a estreita relação entre essas duas condições. Segundo dados clínicos, a prevalência de dor crônica em pacientes tratados para depressão atinge valores de 50–60% (von Knorring et al., 1983; Lee et al., 2009; Aguera-Ortiz et al., 2011) e estudos longitudinais apontam que a depressão é um fator de risco para o desenvolvimento de dor crônica (Gureje et al., 2001; Carroll et al., 2004). Reciprocamente, a prevalência de depressão em pacientes com dor crônica pode chegar a 85 % (Bair et al., 2003; Williams et al., 2003). De fato, a dor é um importante fator de risco para a depressão. Em um estudo coorte, a severidade e cronicidade da dor foram significantemente associadas com o desenvolvimento de depressão (Hilderink et al., 2012).

A dor também afeta o prognóstico e o tratamento da depressão e vice-versa. Existe uma forte correlação entre a severidade da dor e o grau de depressão (Fishbain et al., 1997) e a severidade da dor basal antes do início da administração de antidepressivos tem sido mostrada como um forte fator preditivo negativo da resposta ao tratamento (Bair et al., 2004). Ao mesmo tempo, a depressão pode afetar negativamente o prognóstico e o tratamento da dor crônica. Pacientes com dor crônica e depressão relatam maior duração e severidade dos sintomas de dor (Bair et al., 2004) e apresentam menor resposta ao tratamento em comparação a pacientes não depressivos (Dworkin e Gitlin, 1991). A depressão influencia significativamente a percepção da intensidade da dor, e pacientes depressivos frequentemente apresentam alodínia (Blackburn-Munro e Blackburn-Munro, 2001; Wilson et al., 2001). Na

prática clínica, a comorbidade entre dor e depressão tem sido rotulada como díade dor-depressão (Bair et al., 2004; Goldenberg, 2010). Isto implica que essas duas condições frequentemente coexistem e respondem a tratamentos de maneira similar, exacerbam uma a outra e compartilham as mesmas vias neurobiológicas.

Estudos recentes têm apontado que o desenvolvimento da díade dor-depressão pode estar intimamente relacionado a eventos inflamatórios, e a neuroinflamação seria o mecanismo comum desta comorbidade (Walker et al., 2014). Como mencionado anteriormente, existe uma comunicação entre o sistema imune e o sistema nervoso central e condições inflamatórias podem afetar o ambiente neuronal e induzir sintomas depressivos (Dantzer et al., 2008), mas ao mesmo tempo também podem levar ao desenvolvimento e manutenção da dor crônica (Vallejo et al., 2010). Interessantemente, embora múltiplas condições possam gerar a dor neuropática, um mecanismo comum é a presença de inflamação no local do nervo lesionado, causando hipersensibilidade e alodínia transitórias. No entanto, em alguns casos, essa condição torna-se crônica. As citocinas pró-inflamatórias contribuem para este processo através da hipersensibilização das fibras aferentes transmissoras do estímulo doloroso e também sensibilizando nervos vicinais que não sofreram dano (Sommer e Kress, 2004; McMahon et al., 2005). A lesão a um nervo periférico também leva à ativação da micróglia com liberação de citocinas pró-inflamatórias, indução da cicloxigenase-2 (COX-2) e de óxido nítrico sintase induzível (iNOS) no sistema nervoso central (SNC) (DeLeo e Yezierski, 2001; Watkins et al., 2001). A ativação da micróglia pode ocorrer devido à fosforilação da p38 MAPK (Tikka et al., 2001; Koistinaho et al., 2002). O mecanismo pelo qual a p38 MAPK é ativada no SNC após a lesão a um nervo periférico ainda não foi completamente elucidado. Sabe-se porém que a regulação da ativação da p38 MAPK ocorre em resposta aos níveis intracelulares de Ca^{2+} (Farber e Kettenmann, 2006). A lesão a um nervo periférico induz a liberação de neurotransmissores como glutamato e ATP a partir da fibras nervosas aferentes (Ji e Suter, 2007). O glutamato liberado ativa os receptores NMDA da micróglia, que por sua vez despolarizam a membrana celular causando a abertura dos canais de Ca^{2+} dependentes de voltagem. O grande influxo de íons Ca^{2+} ativa a p38 MAPK e sua via de sinalização com consequente ativação da micróglia (Kim e Ko, 1998; Lohr e Deitmer, 2006; Chiang et al., 2013), a qual desempenha um importante papel no desenvolvimento e potenciação da dor neuropática (Watkins et al., 2001; McMahon et al., 2005; Vallejo et al., 2010). De fato, inúmeros estudos com modelos animais têm demonstrado a ocorrência tanto da dor neuropática como depressão após a constrição de um nervo

periférico, como o nervo ciático (Jesse et al., 2010; Chiang et al., 2013) e a administração de minociclina, inibidor da ativação da micróglia, é capaz de prevenir o desenvolvimento de dor neuropática, sem alterar a dor aguda e os eventos inflamatórios iniciais (Padi e Kulkarni, 2008).

Os sinais inflamatórios podem ter diversos efeitos sobre a função neuronal e alterar diferentes vias de neurotransmissores, como descrito anteriormente. A inflamação crônica pode levar a permanente reestruturação dessas vias e à transição de mal-estar inicial à depressão e da dor aguda à dor crônica, mesmo que a resposta inflamatória inicial tenha sido dissipada (Walker et al., 2014). Alterações no metabolismo da 5-HT, no sistema glutamatérgico e no eixo HPA, pela ação das citocinas pró-inflamatórias, além de induzirem o estado depressivo, também podem ter estreita relação com o desenvolvimento da dor neuropática. De fato, em um modelo animal de artrite inflamatória, a indução de alodínia mecânica e térmica, bem como o comportamento do tipo depressivo, foi associada à ativação da IDO hipocampal, culminando no aumento na taxa quinurenina/triptofano e por consequência na diminuição dos níveis de 5-HT. Corroborando com estes dados, também foi demonstrado que em pacientes com comorbidade entre dor e depressão há um aumento plasmático da taxa quinurenina/triptofano (Kim et al., 2012). Conforme discutido previamente, baixos níveis de 5-HT estão diretamente relacionados aos sintomas depressivos e também diminuem a inibição da transmissão do impulso nociceptivo pela via descendente. O sistema glutamatérgico também desempenha um papel importante na díade dor-depressão. Além do glutamato ser o principal neurotransmissor das fibras aferentes nociceptivas primárias, ele também contribui para a sensibilização central e desenvolvimento da dor crônica (Ji et al., 2003; Coull et al., 2003; Inquimbert et al., 2012). De acordo, o antagonista dos receptores NMDA quetamina possui tanto efeito antidepressivo como analgésico (Sigtermans et al., 2009), e de fato bloqueia o comportamento do tipo depressivo associado à dor neuropática (Wang et al., 2011). Não menos importante, a ativação do eixo HPA parece contribuir significativamente para a comorbidade entre dor e depressão (Blackburn-Munro e Blackburn-Munro, 2001). A dor crônica pode ser considerada uma forma crônica de estresse, sendo que muitos estímulos nociceptivos podem ativar o eixo HPA e vários componentes deste eixo estão envolvidos na resposta à dor (Clauw e Chrousos, 1997; Taylor et al., 1998). Em conformidade com o descrito anteriormente, em diferentes condições de dor crônica como fibromialgia e artrite reumatoide, onde eventos inflamatórios também estão presentes, ocorre aumento da liberação de hormônios do estresse (Lentjes et al., 1997; Shanks et al., 1998;

Harbuz et al., 1999). Inicialmente altos níveis de glicocorticoides resultam em aumento dos disparos dos neurônios serotoninérgicos do núcleo da rafe, mas à medida que o estresse se torna crônico pode ocorrer a depleção de 5-HT, o que leva ao desenvolvimento da depressão e facilitação da via nociceptiva ascendente (Mason, 1999). Os altos níveis de glicocorticoides e as citocinas pro-inflamatórias induzem também a supramencionada resistência dos receptores glicocorticoides e mineralocorticoides, especialmente no hipocampo, onde há uma maior concentração desses receptores (Blackburn-Munro e Blackburn-Munro, 2001; Lathe, 2001). Isso prejudica o mecanismo de retroalimentação negativa do eixo HPA e também induz atrofia nos dendritos apicais hipocampais (Magarinos et al., 1999; Maletic et al., 2007). O hipocampo é considerado uma região chave na comorbidade entre dor e depressão, uma vez que é uma região comum à diferentes vias e neurotransmissores que regulam tanto a dor crônica quanto a depressão (Fasick et al., 2015).

1.4 Terapias farmacológicas

Os principais analgésicos utilizados no tratamento da dor crônica incluem os anti-inflamatórios não esterodais, os opióides e também anticonvulsivantes e antidepressivos. No entanto, essas terapias frequentemente apresentam eficácia passageira e induzem complicações ao longo prazo (Blackburn-Munro e Blackburn-Munro, 2001). Um importante objetivo no tratamento da dor é evitar a cronicidade e reduzir a incapacidade funcional do paciente. Isso requer que o tratamento previna o desenvolvimento de sensibilização central e minimize o estresse físico e emocional causado pela dor (Martelli et al., 2004). Dentre os antidepressivos, os mais utilizados na clínica são os fármacos que agem no sistema monoaminérgico, como os ISRS, ISRN, antidepressivos tricíclicos e inibidores da MAO (iMAO). Entretanto, esses fármacos apresentam eficácia somente em 60-70 % dos pacientes com depressão, o início do efeito é tardio, e fornecem pouca proteção à recaída após o término do tratamento (Millan, 2004, 2006).

Embora os mecanismos ainda não sejam de fato muito bem estabelecidos, é comum na prática clínica o uso dos mesmos medicamentos para tratar tanto a dor crônica quanto a depressão. As vias neuronais e as regiões cerebrais afetadas compartilhadas por essas duas condições permitem, por exemplo, que antidepressivos também tenham certa eficácia na dor crônica. Dessa forma, atualmente os ISRS, ISRN e os antidepressivos tricíclicos são

aprovados pela *Food and Drug Administration* (FDA) para o tratamento de diversos tipos de dor crônica (Doan et al., 2015). No entanto, a eficácia destes antidepressivos bem como os tradicionais analgésicos da classe dos opióides para o tratamento da comorbidade entre dor e depressão tem-se mostrado inferior a 50% (Fava e Davidson, 1996; Kroenke et al., 2009). Além disso, os efeitos dos antidepressivos tradicionais sob o componente afetivo da dor são ainda menos convincentes sob condições inflamatórias, as quais podem estar no ponto central da interseção da dor e depressão (Boyce-Rustay et al., 2010). De fato, níveis aumentados de citocinas têm sido detectados no plasma de pacientes que não respondem ao tratamento com os antidepressivos (Maletic et al., 2007; O'Brien et al., 2007). Dessa forma, têm-se intensificado a busca por novas terapias mais efetivas, e que possam tratar a dor e a depressão simultaneamente.

Conforme descrito anteriormente, os sistemas opióide e monoaminérgico interagem no controle da dor e também estão envolvidos na patofisiologia da depressão. Desta forma a modulação de ambos os sistemas através de um fármaco ou combinação de fármacos poderia ser eficaz no tratamento da díade dor-depressão (Berrocoso e Mico, 2009). Um exemplo é o tramadol, o qual é um agonista fraco dos receptores μ opióides e inibidor dual da recaptação de 5-HT e norepinefrina. Este fármaco é amplamente utilizado como analgésico e têm demonstrado efeito antidepressivo em estudos pré-clínicos e clínicos (Rojas-Corrales et al., 1998; Shapira et al., 2001; 2002). Além disso, a combinação de codeína, um agonista fraco de receptores opióides, com inibidores da recaptação de 5-HT demonstrou efeito antidepressivo superior em camundongos quando comparado ao efeito destas moléculas separadamente (Berrocoso e Mico, 2009).

Considerando o fato de que os níveis de TNF-α têm-se mostrado elevados em pacientes com dor crônica e depressão, estudos pré-clínicos e clínicos apontam que o bloqueio da via de sinalização desta citocina pró-inflamatória alivia os sintomas de ambas as condições (Mathias et al., 2000; Sommer et al., 2001; Lichtenstein et al., 2002; Krugel et al., 2013). Em modelos animais, a administração i.c.v. de anticorpos anti-TNF-α em ratos é eficaz em bloquear a hiperalgesia induzida pela lesão ao nervo ciático (Ignatowski et al., 1999) e reduzir o comportamento do tipo depressivo (Reynolds et al., 2004). Clinicamente, a administração peri-espinhal de etanercepte, um antagonista dos receptores de TNF-α TNFR2, apresentou resultados positivos em pacientes com dor crônica (Tobinick e Davoodifar, 2004) e pacientes com isquemia ou trauma encefálico que receberam etanercepte relataram diminuição da dor e melhora do humor (Tobinick et al., 2012). Da mesma forma, a administração intravenosa de

infliximabe, um anticorpo anti-TNF-α, demonstrou-se eficaz em aliviar a dor crônica (Hess et al., 2011) e reduzir sintomas de depressão em pacientes com altos níveis de marcadores inflamatórios (Raison et al., 2013).

Moléculas que modulam o sistema glutamatérgico também têm despertado interesse para o tratamento da dor crônica e depressão. Como mencionado anteriormente, o antagonista dos receptores NMDA quetamina apresenta tanto efeito antidepressivo como analgésico. Recentemente a quetamina emergiu como um rápido, duradouro e potente antidepressivo. Uma única dose subanestésica de quetamina diminuiu os sintomas depressivos em pacientes resistentes aos tratamentos clássicos com início de resposta entre 1-2 horas e em alguns casos durando até 7 dias (Berman et al., 2000; Zarate et al., 2006; Machado-Vieira et al., 2009). Além disso, a quetamina demonstrou certa eficácia no tratamento de pacientes com dor neuropática refratária associada a sintomas depressivos (Ushida et al., 2002; Sigtermans et al., 2009; Schwartzman et al., 2009). Os inibidores da liberação de glutamato lamotrigina e riluzole também têm demonstrado eficácia no tratamento da depressão refratária (Kendell et al., 2005; Sanacora, 2009).

Outras possíveis alternativas terapêuticas da díade dor-depressão incluem indutores de neurogênese, estimuladores da síntese de BDNF, moduladores do eixo HPA e inibidores da IDO. Inibidores duais da recaptação de 5-HT e norepinefrina apresentam efeito antidepressivo não só por aumentar a disponibilidade destes neurotransmissores na fenda sináptica, mas também por aumentar a expressão de fatores neurotróficos necessários à sobrevivência neuronal, como o BDNF e seu receptor, trkB (Chen et al., 2001; Sairanen et al., 2005; Maletic et al., 2007). De fato, a administração do potente estimulador da síntese de BDNF, 4-metilcatecol, mostrou-se eficaz em reduzir a dor crônica associada à depressão em um modelo animal (Fukuhara et al., 2012). Antidepressivos como a fluoxetina também podem agir no eixo HPA, reduzindo a liberação de CRH e regulando positivamente a expressão de receptores glicocorticoides. Como consequência deste último, os antidepressivos podem restaurar o efeito inibitório dos glicocorticoides sobre o sistema imune (Blackburn-Munro e Blackburn-Munro, 2001; Raison e Miller, 2003). Inibidores da IDO também podem ter eficácia clínica da díade dor e depressão, uma vez que esta enzima tem importante papel nesta comorbidade. Como previamente descrito, citocinas pró-inflamatórias aumentam a atividade da IDO e por consequência diminuem a disponibilidade de 5-HT, além de aumentar os níveis do agonista glutamatérgico ácido quinolínico (Kim et al., 2012).

Apesar das possíveis alternativas terapêuticas listadas acima, as opções farmacológicas para o tratamento da comorbidade entre dor e depressão ainda são bastante limitadas e apresentam baixa eficácia. Considerando a origem multifatorial e o complexo perfil clínico desta comorbidade, de fato é improvável que todos os sintomas sejam controlados por um fármaco possuindo um único mecanismo de ação e desta forma o conceito de um tratamento multimodal tem atraído a atenção. No que se refere à farmacoterapia, duas estratégias centrais podem ser aplicadas. Primeiro, o uso de fármacos que tenham dois ou mais mecanismos complementares e, segundo, a coadministração de dois ou mais fármacos diferentes. Ambas as estratégias poderiam aumentar a eficácia, acelerar a ação e tratar simultaneamente os diversos sintomas desta complexa díade (Millan, 2014). No entanto, a administração de diversos fármacos pode ser bastante inconveniente, diminuir a adesão ao tratamento e provocar diversas reações adversas (Prudent et al., 2008). Dessa forma, o desenvolvimento de novas moléculas que possam agir em diferentes alvos, aumentando a janela terapêutica e reduzindo os efeitos colaterais torna-se bastante interessante.

1.5 Modelos animais para o estudo da dor e depressão

Como discutido anteriormente a dor é um fenômeno perceptual. Ela é gerada pelos nociceptores teciduais, modificada por mecanismos espinhais e supraespinhais e integrada em uma experiência sensorial com um componente afetivo no encéfalo. Dessa forma, o estudo da dor em modelos animais *in vivo* permite que a natureza multidimensional da dor seja examinada, possibilitando o desenvolvimento de novos alvos terapêuticos e triagem de novas moléculas com efeito antinociceptivo (Gregory et al., 2013). Alguns pesquisadores defendem a teoria de que somente humanos podem sentir dor e que animais apresentariam apenas nocicepção, sem o componente afetivo, enquanto outros argumentam que todos os vertebrados e até mesmo invertebrados podem ter a sensação de dor (Bateson, 1991; Carruthers, 1996; Sherwin, 2001). No entanto, de fato a dor em animais não pode ser monitorada diretamente, podendo apenas ser estimada avaliando uma resposta a um estímulo nocivo. Esses estímulos podem ser elétricos, térmicos, mecânicos ou químicos, sendo que este último tipo é o que provavelmente mais se aproxima da dor aguda clínica (Le Bars et al., 2001). Os principais testes nociceptivos são realizados em roedores e destacam-se o teste da retirada da cauda após um estímulo térmico (Janssen et al., 1963), o teste de contorção

abdominal induzida por ácido acético (Correa et al., 1996), o teste de injeção de glutamato (Beirith et al., 2002) ou formalina (Hunskaar e Hole, 1987) na pata e o teste da chapa quente (Woolfe, 1944), dentre outros. Em relação aos modelos de dor crônica, os mais utilizados são os relacionados à lesão a um nervo periférico (Jaggi et al., 2011), os quais variam em relação à forma como é realizada esta lesão (Bennett e Xie, 1988; Seltzer et al., 1990; Decosterd e Woolf, 2000). Esses modelos são bastante úteis uma vez que o comportamento dos animais mimetiza os sintomas de dor neuropática em pacientes (Bennett e Xie, 1988; Bennett, 1993). A alodínia ou hiperalgesia que frequentemente ocorrem da dor neuropática usualmente são monitoradas em roedores através do teste dos filamentos de Von-frey, aplicados na pata do animal.

Da mesma forma, a utilização de modelos animais para o estudo da etiologia da depressão, bem como o desenvolvimento de novos alvos terapêuticos têm sido de extrema importância. Embora seja bastante difícil avaliar a depressão em animais, uma vez que sentimentos de tristeza, culpa e pensamentos suicidas são principalmente limitados aos humanos (DellaGioia e Hannestad, 2010), existem diversos fenótipos da depressão que podem ser reproduzidos e avaliados independentemente (Hasler et al., 2004). Um modelo animal de depressão deve ser análogo à sintomatologia clínica (validade facial), induzir mudanças comportamentais que podem ser medidas objetivamente e revertidas pelos tratamentos que são efetivos em humanos (validade preditiva) e também ser reprodutível entre pesquisadores (McKinney e Bunney, 1969). Neste contexto, o paradigma mais amplamente utilizado para avaliar o comportamento do tipo depressivo é o teste do nado forçado (TNF) em roedores (Porsolt et al., 1979). Neste teste, os animais são colocados em um cilindro com água, representando uma situação adversa, e após tentativas iniciais de escape os mesmos adotam uma postura imóvel. A imobilidade é interpretada como um comportamento do tipo depressivo (Cryan et al., 2005). Essa mesma abordagem também é utilizada no teste da suspensão da cauda (TSC) em roedores (Steru et al., 1985). Modelos de depressão a longo prazo, como a exposição de animais ao estresse crônico (Willner, 2005), eventos traumáticos (Heim e Nemeroff, 2001), privação maternal (Anisman et al., 1998) e privação do sono (McEwen, 2006) também são bastante utilizados, uma vez que são capazes de gerar mudanças comportamentais semelhantes aos sintomas depressivos, os quais são revertidos pelo tratamento com antidepressivos. Devido ao amplamente discutido papel do sistema imune na patogênese da depressão, modelos de estimulação do sistema imune através da administração de endotoxinas ou citocinas pró-inflamatórias também têm sido utilizados recentemente

(DellaGioia e Hannestad, 2010; Kaster et al., 2012). Estas substâncias imunoativas induzem sintomas em roedores similares ao observados em pacientes depressivos (Dantzer, 2009), e também podem ser revertidos pela administração de antidepressivos (Yirmiya et al., 2001; Kaster et al., 2012).

Devido a grande comorbidade entre dor e depressão, diversos modelos animais também têm sido utilizados no estudo da patogênese desta díade, bem como no desenvolvimento de novos alvos terapêuticos (Yalcin et al., 2014). Os modelos de dor neuropática são os que melhor mimetizam a relação de dor crônica e depressão em humanos (Doan et al., 2015). A constrição do nervo ciático em camundongos, por exemplo, induz a alodínia, hiperalgesia e também o comportamento do tipo depressivo (Goncalves et al., 2008; Jesse et al., 2010; Goffer et al., 2013). Por outro lado, a depleção de monoaminas, pela administração de reserpina, induz o comportamento depressivo e também alodínia e hiperalgesia (Nagakura et al., 2009; Arora et al., 2011).

1.6 Selênio e compostos orgânicos de selênio

O selênio é um elemento traço amplamente distribuído pelo corpo humano, fazendo parte da composição química de selenoproteínas como a glutationa peroxidase, tioredoxina reductase e selenoproteína P, as quais são conhecidas pelo seu importante papel na proteção do organismo contra a peroxidação lipídica e danos celulares oxidativos (Steinbrenner e Sies, 2013). A ingestão diária de selênio recomendada pela Agência Nacional de Vigilância Sanitária (ANVISA) é de 70 µg (Dumont et al., 2006). Interessantemente, dados clínicos e pré-clínicos têm apontado uma possível ligação entre a deficiência de selênio e sintomas depressivos. Um estudo conduzido com 585 participantes em uma região dos Estados Unidos demonstrou que altos níveis de selênio na água subterrânea foram associados com menos sintomas de depressão (Johnson et al., 2013). Maiores níveis de selênio também foram relacionados com menores índices de depressão em pacientes idosos (Gao et al., 2012) e com a prevenção de depressão pós-parto, a qual tem prevalência de aproximadamente 6.5–12.9 % (Gavin et al., 2005). Em adição, foi demonstrado em um estudo caso-controle que a baixa ingestão de selênio está associada com alto risco de recaída à depressão (Pasco et al., 2012). De fato, o selênio pode ser importante para as funções cerebrais uma vez que quando há deficiência deste elemento no organismo o cérebro recebe uma oferta prioritária (Buckman et

al., 1993; Whanger, 2001). Além disso, baixos níveis de selênio também foram relacionados ao déficit cognitivo e ao agravamento de doenças neurodegenerativas (Schweizer et al., 2004), além de alterar a taxa de *turnover* de neurotransmissores (Castano et al., 1997).

Na natureza, o selênio encontra-se nas formas orgânica (selenocisteína, selenocistina e selenometionina) e inorgânica (selenito e selenato), sendo que as formas orgânicas apresentam menor toxicidade e maior biodisponibilidade (Nakamuro et al., 2000; Narajji et al., 2007). Os compostos orgânicos de selênio sintéticos têm recebido bastante atenção ultimamente devido a diversas propriedades farmacológicas que os mesmos têm apresentado (Nogueira et al., 2004; Nogueira e Rocha, 2011). Interessantemente, alguns desses novos compostos têm demonstrado tanto efeito antinociceptivo como antidepressivo, como é o caso do protótipo disseleneto de difenila $(PhSe)_2$ (Savegnago et al., 2007; 2008) e o derivado disseleneto de *m*-trifluormetil-fenila (*m*-CF_3-$PhSe)_2$ (Brüning et al., 2010; 2011) (Figura 5). Em relação ao protótipo, este último não apresenta o efeito pró-convulsivante em altas doses, sugerindo que a introdução do grupamento CF_3 na molécula do $(PhSe)_2$ possa reduzir sua toxicidade (Nogueira et al., 2003a).

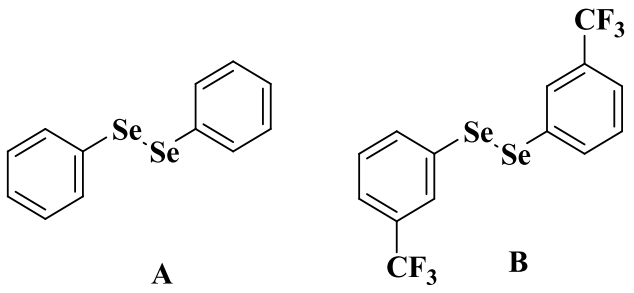

Figura 5. Estrutura química do disseleneto de difenila $(PhSe)_2$ (A) e do disseleneto de *m*-trifluormetil-fenila (*m*–CF_3–$PhSe)_2$ (B).

O efeito antinociceptivo do (*m*-CF_3-$PhSe)_2$ foi demonstrado em diversos testes em camundongos como o teste da retirada da cauda após estímulo térmico, a constrição abdominal induzida por ácido acético, a chapa quente e a injeção de capsaicina na pata, sendo que este efeito foi relacionado à interação com os receptores opióides centrais μ e δ (Brüning et al., 2010). Em relação ao efeito antidepressivo do (*m*-CF_3-$PhSe)_2$, observado no TNF em camundongos, evidências farmacológicas demonstraram o envolvimento tanto do sistema opióide quanto do sistema serotoninérgico, mais especificamente dos receptores 5-HT_{1A}, 5-

$HT_{2A/2C}$ and 5-HT_3, neste efeito (Brüning et al., 2011). Também relacionado ao sistema serotoninérgico, este composto apresenta efeito ansiolítico em camundongos, e inibe seletivamente a atividade da MAO-A cerebral *ex vivo*, responsável pelo catabolismo da 5-HT (Brüning et al., 2009). Além disso, um estudo *in vitro* demonstrou que o (*m*-CF_3-PhSe)$_2$ inibe a recaptação de 5-HT em sinaptossomas de ratos (Borges et al., 2009). Notavelmente, diferente análogos do (*m*-CF_3-PhSe)$_2$ demonstram efeito anti-inflamatório e imunomodulador, como o próprio protótipo (PhSe)$_2$ (Nogueira et al., 2003b; Rupil et al., 2012) e o disseleneto bis-3-hidroxifenila (Shin et al., 2009).

Tendo em vista (i) os múltiplos aspectos patofisiológicos da comorbidade entre dor e depressão discutidos anteriormente, (ii) a necessidade do desenvolvimento de terapias mais efetivas que tratem simultaneamente os diferentes sintomas dessa díade, (iii) os efeitos antinociceptivo e antidepressivo do (*m*-CF_3-PhSe)$_2$ já demonstrados e (iv) as evidências de que este composto possa agir em diferentes sistemas associadas tanto à dor quanto à depressão, torna-se interessante a investigação do possível efeito farmacológico do (*m*-CF_3-PhSe)$_2$ na comorbidade entre dor e depressão, melhor caracterizando sua ação no sistema serotoninérgico bem como um possível efeito anti-inflamatório.

2 OBJETIVOS

2.1 Objetivo geral

Considerando os aspectos mencionados anteriormente, o principal objetivo deste estudo foi avaliar o efeito farmacológico do composto orgânico de selênio (*m*-CF_3-PhSe)$_2$ na comorbidade entre dor e depressão em camundongos.

2.2 Objetivos específicos

- Analisar se o sistema serotoninérgico está envolvido no efeito antinociceptivo do (*m*-CF_3-PhSe)$_2$ no teste de injeção de glutamato na pata;
- Avaliar se o (*m*-CF_3-PhSe)$_2$ inibe a recaptação de [^{3}H]5-HT *ex vivo*;
- Determinar se o (*m*-CF_3-PhSe)$_2$ desloca a ligação específica de [^{3}H]5-HT a seus receptores;
- Analisar a distribuição de selênio em diferentes órgãos e tecidos após a administração de (*m*-CF_3-PhSe)$_2$ em camundongos;
- Investigar se os tratamentos agudo e subcrônico com (*m*-CF_3-PhSe)$_2$ possuem efeito do tipo antidepressivo no modelo de injeção de TNF-α i.c.v. em camundongos;
- Estudar o efeito dos tratamentos agudo e subcrônico com (*m*-CF_3-PhSe)$_2$ na ativação da p38 MAPK e nos níveis de NF-κB no córtex e no hipocampo de camundongos submetidos à injeção de TNF-α i.c.v.;
- Investigar se os tratamentos agudo e subcrônico com (*m*-CF_3-PhSe)$_2$ possuem efeito antinociceptivo e do tipo antidepressivo no modelo de ligação parcial do nervo ciático em camundongos, bem como o efeito destes tratamentos em diversos parâmetros relacionados à inflamação.

3 RESULTADOS

Os resultados que fazem parte desta tese de doutorado estão apresentados na forma de três artigos científicos. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências encontram-se nos próprios artigos, os quais estão estruturados de acordo com as normas de cada revista onde foram publicados. Em anexo a esta tese encontram-se as autorizações da editora para reprodução dos artigos científicos, bem como a aprovação do projeto de pesquisa pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal de Santa Maria.

3.1 Artigo 1

Serotonergic systems are implicated in antinociceptive effect of *m*-trifluoromethyl diphenyl diselenide in the mouse glutamate test

César Augusto Brüning, Bibiana Mozzaquatro Gai, Suelen Mendonça Soares, Franciele Martini, Cristina Wayne Nogueira.

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Serotonergic systems are implicated in antinociceptive effect of *m*-trifluoromethyl diphenyl diselenide in the mouse glutamate test

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ABSTRACT

The organoselenium compound *m*-trifluoromethyl diphenyl diselenide (m-CF_3-PhSe)$_2$ has antinociceptive actions in several animal models, which are mediated by interaction with endogenous opioid systems. It also shows antidepressant-like action mediated by both opioid and serotonergic systems. Considering that serotonin (5-HT) plays an important role in the descending control of pain, this study further investigated the role of serotonergic systems in the antinociceptive action of (m-CF_3-PhSe)$_2$ in the glutamate-induced licking behavior model in mice. (m-CF_3-PhSe)$_2$ (1–50 mg/kg, p.o.), morphine (2.5 mg/kg, s.c.) or paroxetine (5 mg/kg, i.p.) reduced glutamate-induced nociception. Selective 5-HT_{1A} and 5-HT_{2A} receptor antagonists, WAY100635 (0.7 mg/kg, i.p.) and ketanserin (0.3 mg/kg, i.p.), but not the selective 5-HT_3 receptor antagonist, ondansetron (0.5 mg/kg, i.p.), prevented the antinociceptive effect of (m-CF_3-PhSe)$_2$ (10 mg/kg) in the glutamate test. In biochemical studies, (m-CF_3-PhSe)$_2$ (10 and 50 mg/kg) decreased [^{3}H]5-HT uptake in crude synaptosomes of mouse brains and slightly inhibited *in vitro* [^{3}H]5-HT binding. In kinetic studies, the selenium (Se) distribution was determined at different time points after the administration of (m-CF_3-PhSe)$_2$ (500 mg/kg, p.o.) to mice. After 30 min, a high amount of Se was found in liver and kidneys, followed by the lung, red blood cells, serum and brain. A significant amount of Se accumulated in fat over the course of 8 h. Urine was an important route of Se excretion originating from (m-CF_3-PhSe)$_2$. Collectively, results of this study indicate an involvement of the serotonergic systems in the antinociceptive effect of (m-CF_3-PhSe)$_2$ and a wide distribution of Se derived from this compound.

1. Introduction

Pain is an important clinical problem, and the use of analgesic agents for pain relief is generally followed by undesirable side effects (Katzung, 2009). In view of this, compounds showing antinociceptive effect have emerged as attractive therapeutic sources for the development of new relevant drugs for the management of several painful conditions.

Interest in organoselenium biochemistry and pharmacology has increased over the last years due to numerous reports showing the biological activity of organoselenium compounds (Nogueira et al., 2004). *m*-Trifluoromethyl diphenyl diselenide [(m-CF_3-PhSe)$_2$] has shown pharmacological properties, including antinociception (Brüning et al., 2010). (m-CF_3-PhSe)$_2$ reduced chemical and thermal nociception in mice through mechanisms that seem to involve an interaction with central opioid systems, specifically μ-opioid and δ-opioid receptors (Brüning et al., 2010). (m-CF_3-PhSe)$_2$, a lipophilic compound, crosses the blood brain barrier and reaches the central nervous system (CNS) where it exerts its pharmacological properties. Besides its antinociceptive actions, (m-CF_3-PhSe)$_2$ displays other beneficial activities such as anxiolytic and antidepressant-like (Brüning et al., 2009, 2011). The involvement of serotonergic systems in both anxiolytic and antidepressant-like actions of (m-CF_3-PhSe)$_2$ was previously demonstrated (Brüning et al., 2009, 2011).

The neurotransmitter 5-HT plays an important role in the modulation of nociceptive responses (Bardin, 2011; Granados-Soto et al., 2010; Millan, 2002). Spinal dorsal horn neurons are an important site for pain transmission and are subject to descending modulation from supraspinal sites (Heinricher et al., 2009). In this way, the spinally projecting brain stem serotonergic neurons are involved in the transmission of acute pain and the antinociceptive activity of various analgesics depends on the integrity of descending serotonergic pathways (Dogrul and Seyrek, 2006; Millan, 2002). 5-HT affects nociception *via* seven families of 5-HT receptors (5-HT_{1-7}) (Millan, 2002). Particular roles of 5-HT_{1A}, 5-HT_{2A}, and 5-HT_3 receptors in descending inhibition of pain are described (Dogrul and Seyrek, 2006; Millan, 2002).

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The first aim of the present study was to extend previous findings on antinociceptive action of $(m\text{-}CF_3\text{-}PhSe)_2$ by investigating the involvement of serotonergic systems, through behavioral and neurochemical analyses. Considering the importance of knowing the pharmacokinetic aspects of a drug, the second aim of this study was to assess the systemic distribution of this compound by measuring the selenium levels in different biological samples.

2. Material and methods

2.1. Animals

Adult female Swiss mice (25–35 g) were maintained at 22–25 °C with free access to water and food on a 12:12 h light/dark cycle (lights on at 07:00 a.m.). Female mice were randomly selected without monitoring the estrous cycle (Gomes et al., 2005). All manipulations were carried out between 08:00 a.m. and 04:00 p.m. All experiments were performed on separate groups of animals and each animal was used only once in each test. Adult male Wistar rats (200–300 g) were used to perform the *in vitro* assays. Animal care and all experimental procedures were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 80-23, revised in 1996) and in accordance to the guidelines of the Committee on Care and Use of Experimental Animal Resources, from the Federal University of Santa Maria, Brazil (#042/2012). All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs

$(m\text{-}CF_3\text{-}PhSe)_2$ was prepared and characterized in our laboratory by the method previously described (Paulmier, 1986). Analysis of the ^1H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of $(m\text{-}CF_3\text{-}PhSe)_2$ (99.9%) was determined by GC/MS. N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2pyridinylcyclohexanecarboxamide (WAY100635) and ketanserin were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). [^{3}H]5-HT (23 Ci/mmol) was purchased from LabEx Inc. (Hinsdale, Illinois, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers. For behavioral experiments all drugs were dissolved in saline except for $(m\text{-}CF_3\text{-}PhSe)_2$ that was dissolved in canola oil. Mice received all drugs in a constant volume of 10 mL/kg body weight. Appropriate vehicle-treated groups were also assessed simultaneously. Pretreatment time of 30 min for administration of $(m\text{-}CF_3\text{-}PhSe)_2$ was determined based on a previous study (Brüning et al., 2010).

2.3. Behavioral tests

2.3.1. Glutamate-induced nociception

The glutamate test was chosen for this study to further extend the antinociceptive effect of $(m\text{-}CF_3\text{-}PhSe)_2$ to another non-studied test, and because glutamate test is largely used to screen new drugs with antinociceptive effect. The method used for glutamate-induced licking was similar to that described previously (Beirith et al., 2002). After an adaptation period, mice were pretreated with a single oral dose (0.5–50 mg/kg, p.o.) of $(m\text{-}CF_3\text{-}PhSe)_2$ 30 min before the intraplantar (i.pl.) administration of glutamate (20 μmol/paw, 20 μL) in the ventral surface of the right hindpaw. The amount of time spent licking the injected paw was recorded with a chronometer for 15 min following glutamate injection and was considered as indicative of nociception. A separate group of animals was administered with morphine (2.5 mg/kg, s.c.) or paroxetine (5 mg/kg, i.p.) 30 min and 60 min before the glutamate test, respectively, as positive controls (Duman et al., 2004; Longhi-Balbinot et al., 2011).

2.3.2. The role of the serotonergic systems in the antinociceptive action of $(m\text{-}CF_3\text{-}C_6H_4Se)_2$ in the glutamate test

To address the role of the serotonergic systems in the antinociceptive action of $(m\text{-}CF_3\text{-}C_6H_4Se)_2$ in the glutamate test distinct groups of animals were treated with 5-HT receptor antagonists. For this purpose, mice were pretreated with WAY100635, a selective 5-HT_{1A} receptor antagonist (0.7 mg/kg, i.p.), ketanserin, a selective 5-HT_{2A} receptor antagonist (0.3 mg/kg, i.p.) or ondansetron, a selective 5-HT_3 receptor antagonist (0.5 mg/kg, i.p.). Fifteen minutes after antagonists' administration, $(m\text{-}CF_3\text{-}PhSe)_2$ (10 mg/kg, p.o.) or canola oil was administered and 30 min later the glutamate test was carried out. The doses of drugs were selected on the basis of literature (Longhi-Balbinot et al., 2011; Pietrovski et al., 2006).

2.4. Ex vivo assays

2.4.1. Tissue distribution of selenium (Se)

To examine the tissue distribution of the organoselenium compound $(m\text{-}CF_3\text{-}PhSe)_2$, selenium (Se) levels were measured in different biological samples at specific times after treatment. Mice were treated with 500 mg/kg of $(m\text{-}CF_3\text{-}PhSe)_2$ or canola oil (p.o.). This high dose was necessary to achieve detectable Se levels in different organs. After treatment, animals were housed in metabolic cages (1 mouse per cage) and urine and feces were collected at 0.5 h, 1 h, 4 h and 8 h (each time represents a separate group of animals). At the same specific times after treatment, whole blood samples were collected from anesthetized animals by cardiac puncture and plasma and red blood cells (RBC) were separated by centrifugation (2400 ×*g*, 10 min). The brain, liver, lung, kidney and adipose tissue were also excised. All samples were kept at −20 °C until analysis of the Se levels. The determination of Se levels was performed by inductively coupled plasma atomic emission spectrometry (ICPE-9000; Shimadzu Scientific Instruments). All samples were digested with distillated nitric acid (1/1, w/v) at 100 °C for 12 h. Afterwards, they were diluted 10 times with deionized water and analyzed on ICPE. Sample solutions were fed with a peristaltic pump into a manifold and then into a reaction coil where the Se was vaporized as free Se and swept by the inert carrier gas (ultrapure argon). Se concentrations were expressed as μg Se/g or mL of sample.

2.4.2. Synaptosomal [^{3}H]5-HT uptake

To test the hypothesis that the antinociceptive action of $(m\text{-}CF_3\text{-}PhSe)_2$ is mediated through an inhibition of 5-HT uptake, mice were pretreated with $(m\text{-}CF_3\text{-}C_6H_4Se)_2$ (10 and 50 mg/kg, p.o.) or vehicle (canola oil, p.o.), and after 30 min the animals were killed by decapitation and had their whole brain removed. Crude synaptosomes were obtained as described previously (Gray and Whittaker, 1962) with some modifications. The brain (except the cerebellum) was placed into ice-cold sucrose solution (0.32 M, pH 7.4/1:20 w/v), cut into small pieces and homogenized using a glass Potter–Elvehjem tube with a Teflon pestle. Homogenate solution was centrifuged at 1000 ×*g* at 4 °C for 10 min in a refrigerated centrifuge. The pellet was discarded and the supernatant was subsequently centrifuged at 12,000 ×*g* at 4 °C for 20 min. The final pellet was suspended in ten volumes of ice-cold sucrose solution (0.32 M, pH 7.4) and then used as a crude synaptosome preparation in the [^{3}H]5-HT uptake assay. [^{3}H]5-HT uptake into synaptosomes was carried out as described previously (Rocha et al., 2007), with some modifications. The synaptosomal suspension (100 μg of protein) was pre-incubated at 37 °C for 10 min in physiological salt solution (pH 7.4, adjusted with phosphoric acid 1%) of the following composition: 115 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1.2 $MgSO_4$, 1.2 mM KH_2PO_4, 1 mM $CaCl_2$, 11 mM glucose, and 0.01 mM pargyline plus ascorbic acid (0.1%). After pre-incubation, [^{3}H]5-HT uptake was initiated by the addition of 8 nM [^{3}H]5-HT. Synaptosomes were incubated for a further 6 min at 37 °C. [^{3}H]5-HT uptake was stopped by the immediate placement of assay tubes into ice, followed by centrifugation at 12,000 ×*g* at 4 °C for 5 min. Final pellets

were washed with cold incubation buffer. Radioactivity present in pellet was measured in a scintillation counter. Non-specific activity was obtained in the presence of 75 μM imipramine at 4 °C. Specific [^{3}H]5-HT uptake was indirectly estimated by subtracting the non-specific uptake from the total uptake determined at 37 °C. Results were expressed as *f* mol of [^{3}H]5-HT uptake·mg of protein^{-1}·min^{-1}.

2.5. In vitro 5-HT receptor binding

To test the hypothesis that the antinociceptive action of (*m*-CF_3-PhSe)$_2$ could be mediated through specific interaction with 5-HT receptors, we carried out an *in vitro* [^{3}H]5-HT binding assay in rat brain membranes.

2.5.1. Membrane preparation

The membrane preparation was carried out as described previously (Espinosa et al., 2006) with some modifications (Masuda et al., 2011). Rats were killed by decapitation and the brain without pons and cerebellum was quickly dissected and homogenized in 3 volumes (v/w) 50 mM Tris–HCl buffer at pH 7.4 containing 2.0 mM EDTA in ice. The homogenate was then diluted in 12 volumes (v/w) of the same buffer, incubated for 15 min at 37 °C to remove endogenous ligands, and centrifuged (40,000 ×*g*, 4 °C, 20 min). The resulting pellet was re-suspended in 10 volumes of the same buffer, incubated for 15 min at 37 °C and centrifuged as described above. The pellet was washed two additional times, re-suspended in the incubation buffer (50 mM Tris–HCl buffer pH 7.4, containing 0.1% ascorbic acid, 10 μM pargyline, 4 mM $CaCl_2$) and frozen at −80 °C until the binding assay.

2.5.2. Competitive [^{3}H]5-HT binding assay

Rat brain membranes (125 μg of protein) were incubated in a 50 mM Tris–HCl buffer, pH 7.4, containing 0.1% ascorbic acid, 10 μM pargyline and 4 mM $CaCl_2$ for 60 min at 25 °C with [^{3}H]5-HT 15 nM in microtubes. The incubation was performed in the presence or absence of (*m*-CF_3-PhSe)$_2$ (0.01 μM to 100 μM). [^{3}H]5-HT binding was stopped by centrifugation at 12,000 ×*g* at 4 °C for 10 min. Final pellets were washed with cold incubation buffer. Radioactivity present in pellet was measured in a scintillation counter. Non-specific activity was obtained in the presence of 10 μM non-labeled 5-HT at 25 °C. Specific [^{3}H]5-HT binding was indirectly estimated by subtracting the non-specific binding from the total binding. Results were expressed as CPM.

2.6. Protein determination

The protein content in synaptosomal suspension and rat brain membrane preparation was measured using the Bradford assay (Bradford, 1976).

2.7. Statistical analysis

Results are presented as the mean ± S.E.M. Comparisons between experimental groups were performed by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test for post-hoc comparison when appropriate. Positive controls in the glutamate test were compared with control group by Student-T-test. Maximal inhibition (I_{max}) value was calculated at the most effective concentration used. Probability values less than 0.05 ($P < 0.05$) were considered as statistically significant.

3. Results

3.1. Glutamate-induced nociception

Results presented in Fig. 1 show that (*m*-CF_3-PhSe)$_2$, at doses of 1 mg/kg to 50 mg/kg, caused a significant inhibition of glutamate-induced nociception ($P < 0.05$, Student–Newman–Keuls). Furthermore, the licking response of animals treated with positive control agents, morphine and paroxetine, was significantly reduced.

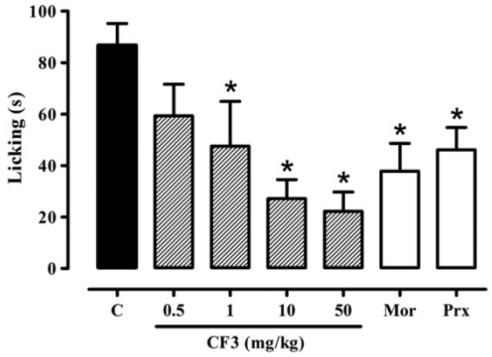

Fig. 1. Effect of (*m*-CF_3-PhSe)$_2$ in the glutamate-induced paw licking. Mice were treated with (*m*-CF_3-PhSe)$_2$ at doses of 0.5–50 mg/kg (p.o.) 30 min before the glutamate test. Morphine (2.5 mg/kg, s.c.) and paroxetine (5 mg/kg, i.p.) were used as positive controls given 30 and 60 min before the glutamate test, respectively. Each column represents the mean with S.E.M. for 7–8 mice in each group. Statistical analysis was performed by one-way ANOVA followed by the Student–Newman–Keuls test to compare (*m*-CF_3-PhSe)$_2$ with vehicle treated control, and by the Student T-test to compare positive controls with vehicle treated control group. (*) $P < 0.05$ when compared to control group. C: control; CF3: (*m*-CF_3-PhSe)$_2$; Mor: morphine; and Prx: paroxetine.

3.2. The role of serotonergic systems in the antinociceptive action of (*m*-CF_3-PhSe)$_2$

(*m*-CF_3-PhSe)$_2$, at 10 mg/kg, significantly reduced glutamate-induced nociception and the systemic administration of the selective 5-HT_{1A} receptor antagonist, WAY100635 (0.7 mg/kg, i.p.), prevented this action (Fig. 2A). Ketanserin (0.3 mg/kg, i.p., a 5-HT_{2A} receptor antagonist) also significantly prevented antinociception caused by (*m*-CF_3-PhSe)$_2$ (Fig. 2B). The systemic treatment of animals with ondansetron (0.5 mg/kg, i.p., a 5-HT_3 receptor antagonist) did not affect antinociception elicited by (*m*-CF_3-PhSe)$_2$ (10 mg/kg, p.o.) in the glutamate test (Fig. 2C).

3.3. Tissue distribution of Se

Se levels after administration of (*m*-CF_3-PhSe)$_2$ (500 mg/kg, p.o) range from 10 μg/g to 26 μg/g in the liver, kidney and lung, and from 3 μg/mL to 12 μg/mL in serum and RBC. Se levels in the brain were lower, and ranged from 1 μg/g to 3 μg/g (Fig. 3A). There was no significant statistical difference in Se levels among the times of 0.5 h to 8 h after (*m*-CF_3-PhSe)$_2$ administration in the liver, kidney, lung, serum, RBC and brain. However, there was an increase in the Se levels in the adipose tissue after 4 h (87.37 μg/g ± 33.34 μg/g) and 8 h (106.97 μg/g ± 7.20 μg/g) of (*m*-CF_3-PhSe)$_2$ administration. Fig. 3B shows that urinary level of Se during the first 0.5 h was 195 μg/mL, while the highest concentration was achieved over the course of 1 h (1851 μg/g). After this time the Se levels decreased gradually. In feces, 12.55 μg/g and 2.55 μg/g of Se were excreted at 0.5 h and 1 h after (*m*-CF_3-PhSe)$_2$ administration, respectively. At 4 h, Se concentration was 413 μg/g and the highest concentration was achieved at 8 h (1101 μg/g).

3.4. [^{3}H]5-HT uptake

(*m*-CF_3-PhSe)$_2$, at doses of 10 and 50 mg/kg, decreased significantly [^{3}H]5-HT uptake in crude synaptosomal preparations of the whole brain when compared to that of the control group; there was no difference between the two doses (Fig. 4).

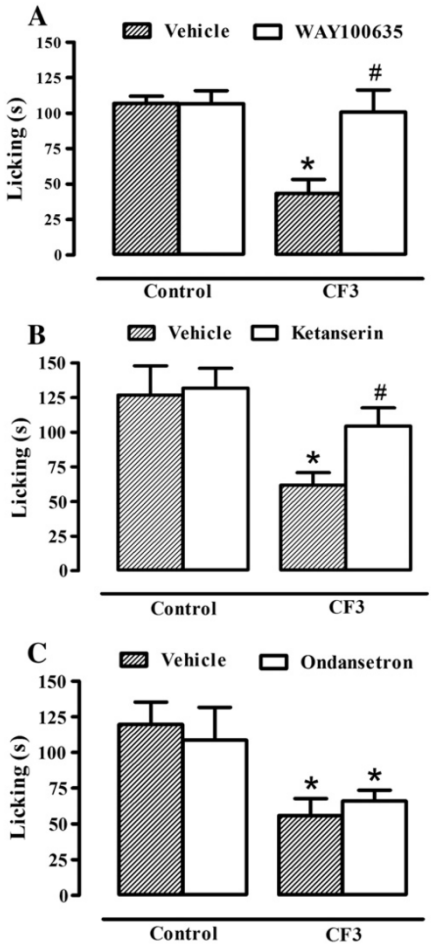

Fig. 2. Effect of pretreatment of mice with WAY100635 (0.7 mg/kg, i.p.) (A), ketanserin (0.3 mg/kg, i.p.) (B) or ondansetron (0.5 mg/kg, i.p.) (C) in the antinociceptive effect of $(m\text{-}CF_3\text{-}PhSe)_2$ (10 mg/kg, p.o.) against the glutamate-induced paw licking. WAY100635, ketanserin or ondansetron was administered 15 min before $(m\text{-}CF_3\text{-}PhSe)_2$ and the glutamate test was performed 30 min after $(m\text{-}CF_3\text{-}PhSe)_2$ administration. Each column represents the mean ± S.E.M. from 6–7 animals in each group. Statistical analysis was performed by one-way ANOVA followed by Student-Newman–Keuls test. (*) $P < 0.05$ as compared with the vehicle treated control. (#) $P < 0.05$ as compared with the same group pretreated with vehicle. CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

3.5. [^{3}H]5-HT binding

$(m\text{-}CF_3\text{-}PhSe)_2$ slightly inhibited the [^{3}H]5-HT binding from concentration of 1 μM. The IC_{50} (concentration that inhibited 50% of binding) was not calculated because the I_{max} was only 29% (Fig. 5).

4. Discussion

In this study we extended our results on antinociceptive actions of $(m\text{-}CF_3\text{-}PhSe)_2$ and provided behavioral and neurochemical evidence for the involvement of serotonergic systems in this action. The systemic administration of $(m\text{-}CF_3\text{-}PhSe)_2$ reduced the nociceptive response induced by intraplantar glutamate injection and antagonists of 5-HT_{1A} and 5-HT_{2A} receptors blocked the effect of $(m\text{-}CF_3\text{-}PhSe)_2$.

Excitatory amino acids such as glutamate in the peripheral endings of small diameter afferent fibers can contribute to the development and maintenance of pain. Nociception induced by intraplantar injection of glutamate seems to involve peripheral, spinal and supra-spinal sites, and this action can be mediated by N-methyl-D-aspartic acid (NMDA) and non-NMDA mechanisms (Beirith et al., 2002; Miller et al., 2011). The results demonstrated here indicated that $(m\text{-}CF_3\text{-}PhSe)_2$ at a dose range of 1 to 50 mg/kg produced an antinociceptive effect in the glutamate-induced paw licking in mice. Similarly, the positive controls, morphine (a non-selective opioid agonist) and paroxetine (a selective serotonin reuptake inhibitor, SSRI), reduced the licking time in the glutamate test.

It is well known that the sensation of pain is modified by descending inhibitory pathways in the CNS and 5-HT is the major transmitter of these pathways. Activation of descending pathways from the raphe nucleus to the dorsal horn mediated by 5-HT inhibits nociceptive transmission (Fields and Basbaum, 1994). In view of this, antidepressant drugs that modulate serotonergic systems are widely used in the treatment of pain, principally chronic pain states, as an adjuvant or alone (Fishbain et al., 2000). While the antidepressant effect of these drugs appears generally after several weeks of treatment, the analgesic action can be achieved after an acute single dose administration (Coquoz et al., 1993; Duman et al., 2004), as demonstrated here with the SSRI paroxetine.

One notable finding of this study was that selective antagonists of 5-HT_{1A} and 5-HT_{2A} receptors, WAY100635 and ketanserin, respectively, blocked the reduction in the paw-licking response elicited by $(m\text{-}CF_3\text{-}PhSe)_2$ in the glutamate test, without changing the locomotor activity of animals. This result indicates that serotonergic systems are involved in the antinociceptive action of $(m\text{-}CF_3\text{-}PhSe)_2$, through 5-$HT_{1A}$ and 5-HT_{2A} receptors, while the lack of effect of the selective 5-HT_3 antagonist, ondansetron, suggests that 5-HT_3 receptors are not involved in the effect of $(m\text{-}CF_3\text{-}PhSe)_2$. Accordingly, the activation of spinal cord and peripheral serotonin subtype receptors 5-HT_{1A} has been reported to reduce nociception (Jeong et al., 2012; Millan, 2002). By contrast, the role of 5-HT_2 receptors in the control of nociception is sometimes controversial. Some authors reported that activation of 5-HT_2 receptors at peripheral level produces nociceptive responses (Cervantes-Duran et al., 2012, 2013), while at spinal level activation of 5-HT_2 receptors causes antinociception (Bardin et al., 2000; Obata et al., 2001; Seyrek et al., 2010).

Taking into account the interaction of $(m\text{-}CF_3\text{-}PhSe)_2$ with serotonergic systems, the anxiolytic and antidepressant-like actions of this compound were previously demonstrated, and these actions were associated to the inhibition of MAO-A activity and consequently to the increase of 5-HT cerebral levels (Brüning et al., 2009, 2011). Increasing synaptic availability of monoamines, like 5-HT, is a well-established mechanism of action of many antidepressants, including their antinociceptive action (Bourin et al., 2001; Jung et al., 1997). The synaptic serotonergic transmission is primarily regulated by the action of the 5-HT transporter (SERT) (Lesch and Mossner, 1998), target of SSRIs, like paroxetine. In this way, a previous *in vitro* study showed that $(m\text{-}CF_3\text{-}PhSe)_2$ inhibits [^{3}H]5-HT uptake in rat synaptosomes at concentrations higher than 1 μM, with IC_{50} of 19.62 μM and I_{max} of 96% observed at 100 μM (Borges et al., 2009). In view of these data, this study attempted to evaluate the *ex vivo* effect of $(m\text{-}CF_3\text{-}PhSe)_2$ on mouse synaptosomal [^{3}H]5-HT uptake. Results showed that a single administration of $(m\text{-}CF_3\text{-}PhSe)_2$ at doses of 10 and 50 mg/kg, given 30 min before assay, reduced the [^{3}H]5-HT uptake by 13% and 12% in brain synaptosomal preparations of mice. This finding, in association with the MAO-A activity inhibition caused by $(m\text{-}CF_3\text{-}PhSe)_2$ provided neurochemical evidence for the involvement of serotonergic systems in the antinociceptive action of this organoselenium compound. Furthermore, we determined if the antinociceptive action of $(m\text{-}CF_3\text{-}PhSe)_2$ could be mediated through a specific interaction with 5-HT receptors. However, $(m\text{-}CF_3\text{-}PhSe)_2$ slightly inhibited *in vitro* [^{3}H]5-HT binding, with a maximal inhibition of 29% between 10 and 100 μM. Compared with the previous results on *in vitro* [^{3}H]5-HT uptake (Borges et al., 2009), it is most likely that the modulation of serotonergic systems by $(m\text{-}CF_3\text{-}PhSe)_2$ is not due to a specific interaction with 5-HT receptors.

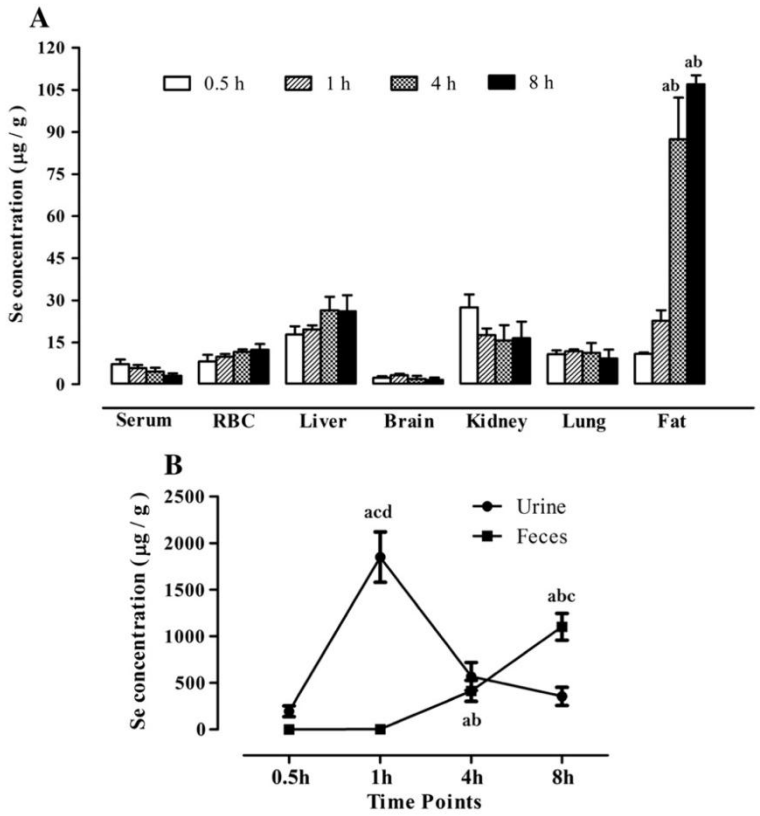

Fig. 3. Amount of selenium (Se) in different biological samples (A) and in urine and feces (B) after a single administration of 500 mg/kg of $(m\text{-}CF_3\text{-}PhSe)_2$ (p.o.) to mice. Each time-point represents the mean ± S.E.M. of 5–6 animals. (a, b, c and d) $P < 0.05$ as compared with 0.5 h (a), 1 h (b), 4 h (c) and 8 h (d) time-points, in the same biological sample (one-way ANOVA followed by Student–Newman–Keuls). RBC: red blood cells.

Considering the pharmacological properties of $(m\text{-}CF_3\text{-}PhSe)_2$, the knowledge of its pharmacokinetic aspects is essential. In this study, the distribution and excretion profile of $(m\text{-}CF_3\text{-}PhSe)_2$ were demonstrated through the determination of Se levels in different biological samples. It should be noted that Se can be derived from non-biotransformed $(m\text{-}CF_3\text{-}PhSe)_2$ or its possible metabolites. The results indicate a wide distribution of Se in different tissues. Thirty minutes after administration of $(m\text{-}CF_3\text{-}PhSe)_2$, a high amount of Se was found in the liver and kidneys, followed by the lung, RBC and serum. In spite of the lowest levels of Se being observed in the brain, this result confirms that $(m\text{-}CF_3\text{-}PhSe)_2$ or its metabolite crosses the blood brain barrier, which can help us explain its central actions, such as anxiolytic, antidepressant and antinociceptive actions (Brüning et al., 2009, 2010, 2011). Since $(m\text{-}CF_3\text{-}PhSe)_2$ inhibits synaptosomal [^{3}H]5-HT uptake in both *in vitro* and *ex vivo* assays, we believe that it reaches the CNS without suffering biotransformation, although the hypothesis that its pharmacological actions could be due to an active metabolite cannot be ruled out. The results further demonstrated that a large amount of $(m\text{-}CF_3\text{-}PhSe)_2$, or some metabolites, are stored in fat. In fact, over the period of 8 h there was a significant increase in the Se levels in this tissue. Similar results for Se distribution were found with diphenyl

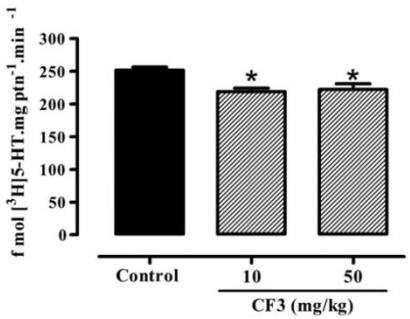

Fig. 4. Effect of $(m\text{-}CF_3\text{-}PhSe)_2$ (10 and 50 mg/kg, p.o.) on synaptosomal [^{3}H]5-HT uptake after 30 min of treatment. Each column represents the mean ± S.E.M. of 3–5 animals. (*) $P < 0.05$ when compared to the vehicle treated control (one-way ANOVA followed by Student–Newman–Keuls). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

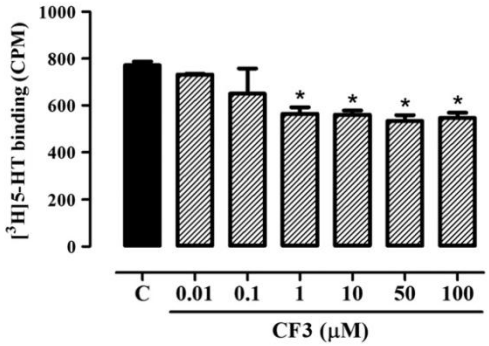

Fig. 5. Effect of $(m\text{-}CF_3\text{-}PhSe)_2$ on [^{3}H]5-HT binding in rat brain membranes. Results are expressed as mean ± S.E.M. for 3 independent experiments performed in triplicate, in different days, using different animals. (*) $P < 0.05$ when compared to the vehicle (one-way ANOVA followed by Student–Newman–Keuls). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

diselenide, an organoselenium compound analog of $(m\text{-}CF_3\text{-}PhSe)_2$. Diphenyl diselenide has low aqueous solubility and high octan-1-ol-water partition coefficients (>3) and similar to $(m\text{-}CF_3\text{-}PhSe)_2$ it has high affinity to the adipose tissue (Prigol et al., 2012). Indeed, lipophilic compounds are known to accumulate in adipose tissue and to be eliminated slowly (Nagahori et al., 2010).

Regarding the excretion patterns, the results showed that urine is an important elimination route of $(m\text{-}CF_3\text{-}PhSe)_2$ or its metabolites. A large amount of Se was found in the urine in the first 30 min, which means that $(m\text{-}CF_3\text{-}PhSe)_2$ is rapidly absorbed by the gastrointestinal tract. The highest urine Se levels were observed at 1 h after the p.o. compound administration and decreased gradually over the course of 8 h. Moreover, the fecal route also contributes largely to the elimination of $(m\text{-}CF_3\text{-}PhSe)_2$ or its metabolites, since at 4 h and 8 h high levels of Se were observed in feces, which can originate from hepatic or even non-biliary intestinal elimination due to the lipophilic nature of $(m\text{-}CF_3\text{-}PhSe)_2$. To the best of our knowledge, this is the first study describing the pharmacokinetics of $(m\text{-}CF_3\text{-}PhSe)_2$, and it provides information about the tissue distribution and excretion of this compound. However, the data are limited since only Se levels were measured, and this measure does not help in clarifying if $(m\text{-}CF_3\text{-}PhSe)_2$ was biotransformed or not. Therefore, more studies are necessary to elucidate the pharmacokinetic profile of $(m\text{-}CF_3\text{-}PhSe)_2$.

In conclusion, this study provides evidence for the involvement of serotonergic transmission in the antinociceptive action of $(m\text{-}CF_3\text{-}PhSe)_2$, since selective antagonists of 5-HT_{1A} and 5-HT_{2A} receptors prevented its effect. Additionally, a single dose of $(m\text{-}CF_3\text{-}PhSe)_2$ inhibited [^{3}H]5-HT uptake *ex vivo* reinforcing its interaction with serotonergic systems. For the first time, pharmacokinetic data demonstrated that $(m\text{-}CF_3\text{-}PhSe)_2$ can be widely distributed among tissues. Finally, considering the importance of serotonergic systems in nociceptive mechanisms, as well as opioid systems, $(m\text{-}CF_3\text{-}PhSe)_2$ could constitute an attractive molecule for the development of new therapeutic strategies for pain treatment.

Acknowledgments

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3.2 Artigo 2

Depressive-like behavior induced by tumor necrosis factor-α is attenuated by *m*-trifluoromethyl-diphenyl diselenide in mice

César Augusto Brüning, Franciele Martini, Suelen Mendonça Soares, Lucielli Savegnago, Tuane Bazanella Sampaio, Cristina Wayne Nogueira

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Depressive-like behavior induced by tumor necrosis factor-α is attenuated by *m*-trifluoromethyl-diphenyl diselenide in mice

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ABSTRACT

A growing body of evidence associates activation of immune system with depressive symptoms. Accordingly, pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), have been shown to play a pivotal role in the pathophysiology of depression. The aim of this study was to evaluate the effectiveness of acute and subchronic treatments with $(m\text{-}CF_3\text{-}PhSe)_2$ to prevent the depressive-like behavior induced by intracerebroventricular injection of TNF-α in mice. TNF-α induced depressive-like behavior in the forced swimming test (FST) and tail suspension test (TST) (0.1 and 0.001 ƒg/5 μL/site, respectively) without changing locomotor activity, performed in the locomotor activity monitor (LAM). Acute (0.01–50 mg/kg; intragastric (i.g.); 30 min) and subchronic (0.01 and 0.1 mg/kg; i.g.; 14 days) treatments with $(m\text{-}CF_3\text{-}PhSe)_2$ at low doses were effective against the effect of TNF-α in the FST and TST. Nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase (p38 MAPK), important proteins in TNF-activated signaling, were determined in the prefrontal cortex and hippocampus of mouse. TNF-α (0.1 ƒg/5 μL/site) increased NF-κB levels and p38 MAPK activation in both brain areas and acute (10 mg/kg; i.g.) and subchronic (0.01 mg/kg; i.g.) treatments with $(m\text{-}CF_3\text{-}PhSe)_2$ were effective in attenuating this increase. Although more studies are necessary to indicate this compound as a therapeutic alternative to depression, the antidepressant-like and anti-inflammatory effects of $(m\text{-}CF_3\text{-}PhSe)_2$ demonstrated herein may support it as an interesting molecule in the search for new drugs to treat depressive disorders that have been largely linked to immune process and inflammation.

1. Introduction

Depression is one of the severe psychiatric disorders and has been estimated as the second biggest contributor of the global load of neurological diseases and disability for the next years (Pitchot et al., 2010). Although a large number of experimental and clinical studies indicate important roles for monoaminergic systems in the pathophysiology and treatment of depression, the etiology of this disease is still not fully understood (Elhwuegi, 2004). It has become increasingly clear that there is abundant crosstalk between the peripheral immune system and the central nervous system (CNS). This communication is mediated largely by cytokines produced by immune cells found both centrally and peripherally. In fact, patients with major depression have increased serum levels of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) (Himmerich et al., 2008; Lanquillon et al., 2000; Tuglu et al., 2003).

Both central and peripheral administration of recombinant pro-inflammatory cytokines in rodents can induce a spectrum of symptoms collectively known as "sickness behavior" that has been linked to symptoms of major depressive disorder (Bluthe et al., 2000; Dantzer, 2004; Kaster et al., 2012). TNF-α is particularly interesting with respect to major depression because improvement of the disorder correlates with a decrease in serum levels of this cytokine (Lanquillon et al., 2000) and deletion of TNF-α receptors, TNFR1 and TNFR2, produces antidepressant-like profile in rodents (Simen et al., 2006). Moreover, some antidepressants have been shown to decrease IL-1β and TNF-α production (Himmerich et al., 2010; Nickola et al., 2001).

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The organoselenium compound *m*-trifluoromethyl-diphenyl diselenide [(*m*-CF_3-PhSe)$_2$] has been shown to have antidepressant-like effect in mice, which is related to interaction with the serotonergic system (Brüning et al., 2011). Remarkable, analogs of (*m*-CF_3-PhSe)$_2$, such as diphenyl diselenide and *p*-methoxyl-diphenyl diselenide, have been shown to have antiinflammatory effects, reducing the activation of macrophage and microglia and the levels of pro-inflammatory cytokines *in vitro* and *in vivo* (Brüning et al., 2012; Luchese et al., 2012; Pinton et al., 2013; Rupil et al., 2012).

Given recent data that immune system can be involved in etiology of depression, the aim of this study was to evaluate the effectiveness of acute and subchronic treatments with (*m*-CF_3-PhSe)$_2$ to prevent the depressive-like behavior induced by intracerebroventricular injection of TNF-α in mice. The levels of proteins related to inflammation, nuclear factor kappa B (NF-κB) and p38 mitogen-activated protein kinase (p38 MAPK), in the prefrontal cortex and hippocampus, two important brain areas involved in antidepressant response, were also determined.

2. Methods

2.1. Animals

The experiments were conducted using male adult Swiss mice (25–35 g) from our own breeding colony. Animals were housed in polypropylene cages (41 × 34 × 16 cm) in groups of 15 mice per cage with free access to tap water and food (Guabi, Campinas, São Paulo, Brazil). They were kept in a separate animal room, on a 12-h light/12-h dark cycle, with lights on at 7:00 a.m., in a controlled temperature environment (22 ± 2 °C). Mice were acclimatized to the laboratory for at least 1 h before testing. All manipulations were carried out between 08.00 a.m. and 04.00 p.m. The present experimental study was approved by the Ethical Research Committee of the Federal University of Santa Maria, affiliated to the National Council for the Control of Animal Experimentation (CONCEA), and registered under the number #042/2012.

2.2. Chemicals

(*m*-CF_3-PhSe)$_2$ was synthesized in our laboratory by the method of Paulmier (1986). ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure and the chemical purity (99.9%) was determined by GC/MS. Recombinant TNF-α from mouse expressed in *Escherichia coli*, tris (hydroxymethyl) aminomethane–hydrochloride (Tris–HCl), ethylene diaminetetraacetic acid (EDTA), magnesium chloride ($MgCl_2$), potassium acetate (KAc), protease inhibitor cocktail, sodium dodecyl sulfate (SDS), acrylamide, bis-acrylamide, glycine, 2-β-mercaptoethanol, bromophenol blue, NP-40, pre stained molecular weight standards and serum bovine albumin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Rabbit anti-NF-κB p65 (nuclear factor-κB) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit anti-p-p38 MAPK antibody (phosphorilated p38 mitogen-activated protein kinase; Thr180/Tyr182) and rabbit anti-p38 MAPK were purchased from Cell Signaling Technology (Danvers, USA). Mouse anti-α-tubulin was purchased from abcam (San Francisco, USA). Secondary antibodies conjugated with horseradish peroxidase were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Chemiluminescence kit was purchased from Amersham (São Paulo/Brazil).

2.3. Drug administration procedure

(*m*-CF_3-PhSe)$_2$ was dissolved in canola oil and administered to each mouse by the intragastric (i.g.) route at a volume of 10 mL/kg (*m*-CF_3-PhSe)$_2$ and vehicle were administered by using a gastro-esophageal probe that releases them directly into the stomach. A 22 G ball tip needle was used to prevent damage to the esophagus and from passing through the glottal opening into the trachea. The conscious mice were manually restrained firmly by gripping a fold of skin from the scruff of neck down the back, immobilizing the head with the neck extended in the vertical position. The needle was passed gently through the mouth and pharynx into the esophagus and the compound or canola oil was then administered slowly. TNF-α was dissolved in sterile saline and administered by the intracerebroventricular (i.c.v.) route. The i.c.v. injection of TNF-α was performed using a "free hand" method, without stereotaxic setup, under light ether anesthesia (just the necessary for the loss of the postural reflex) as originally described by Haley and McCormick (1957) and modified by Laursen and Belknap (1986) with the bregma fissure as a reference. The asepsis of the injection site was carried out using gauze embedded in 70% ethanol. Bregma was found by lightly rubbing the point of the needle over the skull until the suture was felt through the skin. Each animal was gently restrained by hand at the neck with the thumb and forefinger and a 0.4 mm external diameter hypodermic needle, which was linked to a 10 μL Hamilton syringe, was inserted unilaterally 1 mm to bregma and perpendicular to the plane of the skull and no more than 2 mm into the brain (a retainer was attached to the needle). A volume of 5 μL of sterile saline containing TNF-α was injected gradually into the ventricle over 1 min and the needle remained in place for more 30 s in order to avoid the reflux of the TNF-α. After the experiments the brains were dissected and results from mice presenting misplacement of the injection site or any sign of cerebral hemorrhage were excluded (less than 5%).

2.4. Experimental design

The depressant-like effect of TNF-α was evaluated in the forced swimming test (FST) and the tail suspension test (TST). TNF-α was injected 1 h before the behavioral tests. The effective concentrations of TNF-α to induce mouse depressive-like behavior in the FST and TST were 0.1 *f*g/5 μL/site and 0.001 *f*g/5 μL/site, respectively (Kaster et al., 2012). Mice were acutely and subchronically treated with (*m*-CF_3-PhSe)$_2$ to investigate its effect on depressive-like behavior induced by TNF-α (Fig. 1). In the acute treatment, mice received vehicle or (*m*-CF_3-PhSe)$_2$ at the dose range of 0.01–50 mg/kg 30 min after TNF-α injection. After 30 min, mice were submitted to the locomotor activity monitor (LAM) and the FST (n = 87; 6–9/group). Another set of animals were evaluated in the LAM and the TST (n = 86; 8–10/group). The LAM was performed to evaluate possible changes in mouse locomotion. In the subchronic treatment, mice received vehicle or (*m*-CF_3-PhSe)$_2$ once a day during two weeks at doses of 0.01 and 0.1 mg/kg (subeffective doses in the FST and TST, respectively). In the 14th day, TNF-α was injected in mice 30 min before the last administration of 0.01 mg/kg (*m*-CF_3-PhSe)$_2$ and/or TNF-α at 0.1 *f*g/5 μL/site and after 30 min the mice were submitted to the LAM and the FST (n = 36; 8–10/group). Another set of animals received 0.1 mg/kg (*m*-CF_3-PhSe)$_2$ and/or TNF-α at 0.001 *f*g/5 μL/site and after 30 min the mice were submitted to the LAM and the TST (n = 39; 9–11/group).

2.5. Behavioral testing

2.5.1. Spontaneous locomotor activity

The locomotor activity of each mouse was evaluated in the LAM, which consist of a clear acrylic plastic box (45 × 45 × 45 cm) with a removable plastic lid perforated with holes for ventilation. The monitor is surrounded by a frame consisting of 32 photocells mounted on opposite walls (16 L × 16 W, spaced 2 cm apart) that

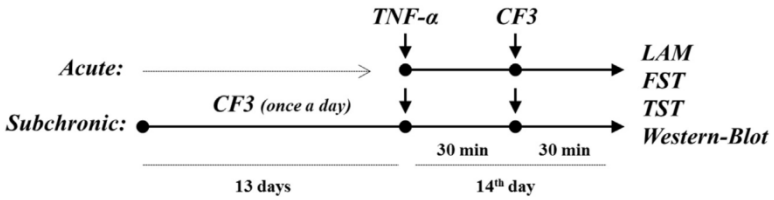

Fig. 1. Schematic representation of the experimental design. TNF-α was injected 1 h before the tests. In the acute treatment, (*m*-CF_3-PhSe)$_2$ was administered to mice 30 min before the tests. In the subchronic treatment, (*m*-CF_3-PhSe)$_2$ was administered for 14 days. The 14th administration of (*m*-CF_3-PhSe)$_2$ was given to mice 30 min before the tests. CF3: (*m*-CF_3-PhSe)$_2$; LAM: locomotor activity monitor; FST: forced swimming test; TST: tail suspension test.

continuously tracks the animal's movement. General locomotor activity and the mouse's position in the chamber are detected by breaks of the photocell beams, which are recorded by a computer, using Monitor Activity® software (Insight). Subjects were placed in the center of the apparatus and allowed to freely explore the arena during 4 min. Number of crossings and rearings, total distance traveled (cm) and average velocity (mm/s) were recorded.

2.5.2. Forced swimming test

The FST was performed as described previously (Porsolt et al., 1979). Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1 °C. The total duration of immobility was recorded during 6 min period and a decrease in this parameter is indicative of an antidepressant-like effect. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water.

2.5.3. Tail suspension test

The TST was carried out based on Steru et al. (1985). Each mouse was suspended by its tail to a horizontal wooden bar approximately 30 cm above the floor. The mouse was secured to the bar by adhesive tape placed 1 cm from the tip of the tail. The total duration of immobility was recorded during 6 min. The mouse was considered immobile only when it hung passively and completely motionless. Mice that climbed their tails were discarded (less than 5%) once it could compromises the immobility time record.

2.6. Western-blot analysis

After the behavioral tests animals from acute and subchronic treatments with (*m*-CF_3-PhSe)$_2$ were killed by decapitation and the prefrontal cortex and hippocampus were excised from the brain and frozen (−80 °C) to perform the western-blot procedure. The doses of (*m*-CF_3-PhSe)$_2$ chosen to perform the western blot assays were 10 mg/kg (an intermediate dose) and 0.01 mg/kg (the lower dose) for acute and subchronic treatments, respectively. The concentration of TNF-α chosen to perform western-blot analysis was 0.1 ƒg/5 μL/site to both acute and subchronic (*m*-CF_3-PhSe)$_2$ treatments.

Samples of prefrontal cortex and hippocampus were homogenized in 10 mM Tris–HCl, 1 mM EDTA, pH 7.4, and centrifuged (9800 × g at 4 °C for 5 min) to concentrate proteins. The pellet was reconstituted in a buffer solution (10 mM Tris–HCl, pH 7.6, 5 mM $MgCl_2$, 1.5 mM KAc), 1% NP-40, and protease inhibitor cocktail (Sigma–Aldrich) and incubated for 30 min on ice followed by 10 min on ultrasonic bath, and then centrifuged (3900 × g for 10 min, at 4 °C). Tissue extracts were diluted to a final protein concentration 2 μg/mL in SDS-polyacrylamide gel electrophoresis buffer (SDS-PAGE buffer constituted by Tris·HCl 0.5 M, pH 6.8 (final concentration of 62.5 mM), glycine, SDS, 2-β-mercaptoethanol, the reducing agent, and bromophenol blue, used as a marker to monitor the process of electrophoresis). The samples (50 μg of protein) and pre stained molecular weight standards (Sigma–Aldrich) were separated on 12% resolving with 4% concentrating SDS-PAGE electrophoresis gels. Proteins were transferred to polyvinylidene fluoride (PVDF) membrane using Transfer-Blot® Turbo™ Transfer System (1.0 mA; 30 min) and/equal protein loading was confirmed by Ponceau S staining. After blocking with 5% bovine serum albumin solution, the blots were incubated overnight at 4 °C with rabbit anti-NF-κB p65 (1:200; Santa Cruz Biotechnology) or rabbit anti-p-p38 MAPK antibody (Thr180/Tyr182) (1:1000; Cell Signaling) and rabbit anti-p38 MAPK (1:1000; Cell Signaling). Mouse anti-α-tubulin (1:3000, abcam) was stained as additional control of the protein loading. After primary antibodies incubation, membranes were washed and incubated with secondary antibodies conjugated with horseradish peroxidase (goat anti-rabbit (1:10,000) to anti-NF-κB p65, anti-p-p38 MAPK and anti-p38 MAPK antibodies and goat anti-mouse (1:10,000) to anti-α-tubulin antibody; Bio-Rad Laboratories) for 1 h at room temperature and developed with chemiluminescence kit. Optical density (O.D.) of the western blotting bands was quantified using Image J (NIH, Bethesda, MD, USA) software for Windows. Each value was derived from the ratio between arbitrary units obtained by the protein band and the respective α-tubulin band. The results of NF-κB levels were shown by % of control of quantification of bands O.D. corrected by α-tubulin and the results of p-p38 MAPK and p38 MAPK were shown by % of control of quantification of the phosphorylated ratio: O.D. of the phosphorylated band/O.D. of the total band both corrected by α-tubulin.

2.7. Statistical analysis

All experimental results are given as the mean (s) ± S.E.M. Normality of data was evaluated by D'Agostino and Pearson omnibus normality test. Comparisons between experimental and control groups were performed by two-way ANOVA, including the factors TNF-α injection, (*m*-CF_3-PhSe)$_2$ treatment and interaction, followed by the Newman–Keuls test for post hoc comparison when appropriate. The main effects are presented only when interactions were not significant. In the acute treatment, each dose of (*m*-CF_3-PhSe)$_2$ was evaluated in a separate two-way analysis. A value of $p < 0.05$ was considered to be significant.

3. Results

3.1. Acute and subchronic treatments with (m-CF_3-PhSe)$_2$ attenuated TNF-α-induced depressive-like behavior in mice without changing spontaneous locomotor activity

Fig. 2 shows the immobility time of mice in the FST and TST after TNF-α and/or acute (*m*-CF_3-PhSe)$_2$ administration. In the FST data, two-way ANOVA demonstrated a TNF-α × (*m*-CF_3-PhSe)$_2$

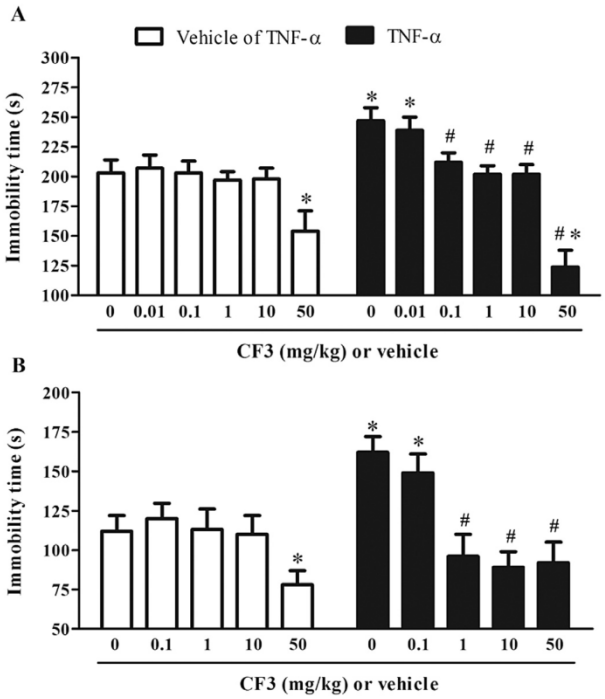

Fig. 2. Effect of TNF-α and/or acute treatment of mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.01–50 mg/kg) on immobility time in the (A) FST and (B) TST. TNF-α at concentrations of 0.1 ƒg/5 μL/site to FST and 0.001 ƒg/5 μL/site to TST was administered 1 h and $(m\text{-}CF_3\text{-}PhSe)_2$ 30 min before the tests. Data represent the mean (s) ± SEM. *n* to FST = 6–9/group (7 (control); 8 (CF3 0.01 mg/kg); 6 (CF3 0.1 mg/kg); 6 (CF3 1 mg/kg); 8 (CF3 10 mg/kg); 9 (CF3 50 mg/kg); 7 (TNF-α); 8 (CF3 0.01 mg/kg + TNF-α); 6 (CF3 0.1 mg/kg + TNF-α); 6 (CF3 1 mg/kg + TNF-α); 7 (CF3 10 mg/kg + TNF-α) and 9 (CF3 50 mg/kg + TNF-α)). *n* to TST = 8–10/group (8 (control); 8 (CF3 0.1 mg/kg); 8 (CF3 1 mg/kg); 8 (CF3 10 mg/kg); 10 (CF3 50 mg/kg); 8 (TNF-α); 8 (CF3 0.1 mg/kg + TNF-α); 8 (CF3 1 mg/kg + TNF-α); 10 (CF3 10 mg/kg + TNF-α) and 10 (CF3 50 mg/kg + TNF-α)). (*) $p < 0.05$ when compared to the control group. (#) $p < 0.05$ when compared to the TNF-α group (two-way ANOVA of each $(m\text{-}CF_3\text{-}PhSe)_2$ dose followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

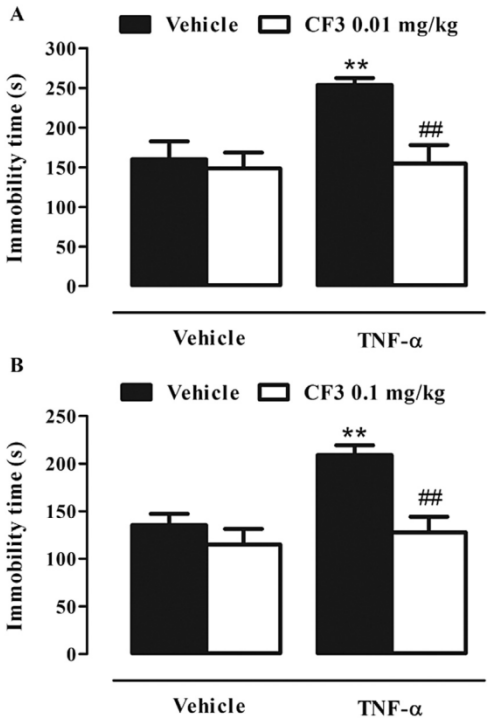

Fig. 3. Effect of TNF-α and/or subchronic treatment of mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.01 mg/kg to FST and 0.1 mg/kg to TST) on immobility time in the (A) FST and (B) TST. TNF-α at concentration of 0.1 ƒg/5 μL/site to FST and 0.001 ƒg/5 μL/site to TST was administered 1 h before the tests and $(m\text{-}CF_3\text{-}PhSe)_2$ was administered for 14 days. The last administration was given to mice 30 min before the tests. Data represent the mean (s) ± SEM. *n* to FST = 8–10/group (10 (control); 8 (CF3 0.01 mg/kg); 8 (TNF-α) and 10 (CF3 0.01 mg/kg + TNF-α)). *n* to TST = 9–11/group (9 (control); 11 (CF3 0.1 mg/kg); 9 (TNF-α) and 10 (CF3 0.1 mg/kg + TNF-α)). (**) $p < 0.01$ when compared to the control group. (##) $p < 0.01$ when compared to the TNF-α group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

interaction at doses of 0.1, 1, 10 and 50 mg/kg ($F_{(1,22)} = 4.85$, $p < 0.05$; $F_{(1,22)} = 7.20$, $p < 0.05$; $F_{(1,25)} = 4.86$, $p < 0.05$ and $F_{(1,28)} = 6.65$, $p < 0.05$, respectively). TNF-α at 0.1 ƒg/5 μL/site increased the mouse depressive-like behavior represented by an increase in immobility time and $(m\text{-}CF_3\text{-}PhSe)_2$ at doses of 0.1–50 mg/kg prevented this increase. The highest dose of $(m\text{-}CF_3\text{-}PhSe)_2$ reduced the immobility time of vehicle-treated mice (Fig. 2A). Two-way ANOVA of the TST data showed a TNF-α × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction at doses of 1 and 10 and 50 mg/kg ($F_{(1,28)} = 7.46$, $p < 0.05$; $F_{(1,30)} = 9.75$, $p < 0.01$ and $F_{(1,32)} = 4.86$, $p < 0.05$). TNF-α at 0.001 ƒg/5 μL/site increased the mouse immobility time in the TST and $(m\text{-}CF_3\text{-}PhSe)_2$ at doses of 1, 10 and 50 mg/kg was effective in blocking this increase. Similar to data obtained in the FST, the dose of 50 mg/kg $(m\text{-}CF_3\text{-}PhSe)_2$ decreased the immobility time of vehicle-treated mice (Fig. 2B).

The mice were submitted to a subchronic treatment with the doses of 0.01 and 0.1 mg/kg of $(m\text{-}CF_3\text{-}PhSe)_2$ because they were not effective in reducing TNF-α-induced immobility time in the FST and TST, respectively (Fig. 3). In the FST, $(m\text{-}CF_3\text{-}PhSe)_2$ treatment at a dose of 0.01 mg/kg for 14 days prevented the increase in mouse immobility time induced by TNF-α (0.1 ƒg/5 μL/site) (TNF-α × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,32)} = 4.50$, $p < 0.05$) (Fig. 3A). In the TST, subchronic $(m\text{-}CF_3\text{-}PhSe)_2$ treatment at a dose of 0.1 mg/kg also blocked the effect of TNF-α (0.001 ƒg/5 μL/site) (TNF-α × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,35)} = 4.34$, $p < 0.05$) (Fig. 3B).

The possible effects of treatments on locomotor activity of mice were evaluated in LAM. The i.c.v. injection of TNF-α at 0.1 ƒg/5 μL/site or 0.001 ƒg/5 μL/site and/or acute and subchronic $(m\text{-}CF_3\text{-}PhSe)_2$ treatments did not change any locomotor parameter evaluated, e.g., number of crossings and rearings, total distance traveled and average velocity of mice (Tables 1–4).

3.2. $(m\text{-}CF_3\text{-}PhSe)_2$ treatments prevented the increase in NF-κB levels induced by TNF-α

TNF-α injection increased NF-κB levels in the prefrontal cortex and hippocampus of mice and acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ at a dose of 10 mg/kg was effective in blocking this increase in both structures (TNF-α × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,8)} = 6.83$, $p < 0.05$ and $F_{(1,10)} = 8.66$, $p < 0.05$, respectively) (Fig. 4). In the subchronic treatment, two-way ANOVA of NF-κB data showed a TNF-α × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction in the prefrontal cortex ($F_{(1,12)} = 4.75$, $p < 0.05$) and hippocampus ($F_{(1,14)} = 5.75$, $p < 0.05$). *Post hoc* analysis demonstrated that treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ at a dose of 0.01 mg/kg for 14 days prevented the increase in NF-κB levels in the prefrontal cortex and attenuated this increase in hippocampus (not statistically significant) (Fig. 5).

Table 1
Locomotor parameters of mice treated with TNF-α 0.1 fg/5 μL/site and/or acute doses of (*m*-CF_3-PhSe)$_2$.

	Crossing	Rearing	Distance (cm)	Velocity (mm/s)
Control	252.5 ± 44.8	6.6 ± 1.7	414.4 ± 88.8	24.6 ± 4.0
CF3 0.01	290.0 ± 42.4	8.1 ± 1.9	388.3 ± 52.2	27.8 ± 2.8
CF3 0.1	250.1 ± 44.5	6.4 ± 1.5	394.0 ± 61.7	21.0 ± 2.8
CF3 1	240.9 ± 78.6	5.4 ± 1.5	427.2 ± 147.9	35.7 ± 2.7
CF3 10	240.2 ± 49.8	7.1 ± 1.6	438.3 ± 76.6	22.0 ± 2.5
CF3 50	232.8 ± 21.1	8.2 ± 1.6	514.4 ± 46.2	26.0 ± 2.5
TNF-α	295.8 ± 61.6	7.3 ± 2.0	456.6 ± 103.6	25.0 ± 3.5
CF3 0.01 + TNF-α	210.1 ± 60.3	4.4 ± 1.9	361.9 ± 86.9	26.6 ± 2.7
CF3 0.1 + TNF-α	321.0 ± 41.7	7.8 ± 1.8	433.0 ± 79.8	25.1 ± 3.2
CF3 1 + TNF-α	213.5 ± 41.7	6.1 ± 2.0	439.8 ± 89.5	31.1 ± 4.3
CF3 10 + TNF-α	367.0 ± 55.4	11.5 ± 0.9	540.4 ± 57.3	32.1 ± 2.2
CF3 50 + TNF-α	302.4 ± 83.0	9.8 ± 3.1	540.5 ± 167.9	32.0 ± 5.7

TNF-α at concentration of 0.1 fg/5 μL/site was administered 1 h and (*m*-CF_3-PhSe)$_2$ (0.01–50 mg/kg) 30 min before LAM. Data are reported as means ± SEM. $n = 6$–9/group (7 (control); 8 (CF3 0.01 mg/kg); 6 (CF3 0.1 mg/kg); 6 (CF3 1 mg/kg); 8 (CF3 10 mg/kg); 9 (CF3 50 mg/kg); 7 (TNF-α); 8 (CF3 0.01 mg/kg + TNF-α); 6 (CF3 0.1 mg/kg + TNF-α); 6 (CF3 1 mg/kg + TNF-α); 7 (CF3 10 mg/kg + TNF-α) and 9 (CF3 50 mg/kg + TNF-α)). CF3: (*m*-CF_3-PhSe)$_2$.

Table 2
Locomotor parameters of mice treated with TNF-α 0.001 fg/5 μL/site and/or acute doses of (*m*-CF_3-PhSe)$_2$.

	Crossing	Rearing	Distance (cm)	Velocity (mm/s)
Control	297.1 ± 47.3	7.4 ± 1.7	535.0 ± 83.5	29.7 ± 6.0
CF3 0.1	273.2 ± 27.7	6.2 ± 1.7	480.2 ± 51.0	28.0 ± 3.6
CF3 1	268.5 ± 67.1	6.4 ± 2.8	439.9 ± 153.5	34.8 ± 5.1
CF3 10	387.2 ± 66.2	10.4 ± 2.8	582.3 ± 118.1	34.1 ± 5.5
CF3 50	258.4 ± 70.1	8.8 ± 2.5	506.5 ± 117.1	32.5 ± 7.8
TNF-α	308.6 ± 52.4	8.1 ± 2.1	540.5 ± 107.9	26.2 ± 3.9
CF3 0.1 + TNF-α	364.5 ± 94.8	7.3 ± 1.4	429.5 ± 97.4	31.6 ± 9.2
CF3 1 + TNF-α	486.2 ± 101.1	11.2 ± 3.1	688.7 ± 150.6	44.3 ± 9.5
CF3 10 + TNF-α	273.4 ± 51.3	8.0 ± 2.2	533.1 ± 113.4	20.9 ± 3.3
CF3 50 + TNF-α	245.3 ± 73.6	5.4 ± 1.8	467.6 ± 133.5	26.0 ± 3.2

TNF-α at concentration of 0.001 fg/5 μL/site was administered 1 h and (*m*-CF_3-PhSe)$_2$ (0.1–50 mg/kg) 30 min before LAM. Data are reported as means ± SEM. $n = 8$–10/group (8 (control); 8 (CF3 0.1 mg/kg); 8 (CF3 1 mg/kg); 8 (CF3 10 mg/kg); 10 (CF3 50 mg/kg); 8 (TNF-α); 8 (CF3 0.1 mg/kg + TNF-α); 8 (CF3 1 mg/kg + TNF-α); 10 (CF3 10 mg/kg + TNF-α) and 10 (CF3 50 mg/kg + TNF-α)). CF3: (*m*-CF_3-PhSe)$_2$.

3.3. (*m*-CF_3-PhSe)$_2$ treatments reduced p38 MAPK activation induced by TNF-α

In the acute treatment with (*m*-CF_3-PhSe)$_2$ at a dose of 10 mg/kg, the two-way ANOVA of p-p38/p38 MAPK ratio data showed a main effect of TNF-α in the prefrontal cortex ($F_{(1,12)} = 17.01$, $p < 0.01$) and a TNF-α × (*m*-CF_3-PhSe)$_2$ interaction in hippocampus

Table 3
Locomotor parameters of mice treated with TNF-α 0.1 fg/5 μL/site and/or subchronic doses of (*m*-CF_3-PhSe)$_2$ at 0.01 mg/kg.

	Crossing	Rearing	Distance (cm)	Velocity (mm/s)
Control	365.1 ± 41.0	10.3 ± 1.5	700.2 ± 79.3	34.3 ± 3.2
CF3 0.01	346.8 ± 44.9	09.5 ± 2.2	570.9 ± 80.7	28.5 ± 3.3
TNF-α	385.8 ± 60.0	12.6 ± 2.1	666.2 ± 90.0	30.8 ± 3.8
CF3 0.01 + TNF-α	436.4 ± 66.5	12.0 ± 2.2	726.5 ± 106.4	32.7 ± 4.3

TNF-α at concentration of 0.1 fg/5 μL/site was administered 1 h before the tests and (*m*-CF_3-PhSe)$_2$ (0.01 mg/kg) was administered for 14 days. The last administration was given to mice 30 min before the LAM. Data are reported as means ± SEM. $n = 8$–10/group (10 (control); 8 (CF3 0.01 mg/kg); 8 (TNF-α) and 10 (CF3 0.01 mg/kg + TNF-α)). CF3: (*m*-CF_3-PhSe)$_2$.

Table 4
Locomotor parameters of mice treated with TNF-α 0.001 fg/5 μL/site and/or subchronic doses of (*m*-CF_3-PhSe)$_2$ at 0.1 mg/kg.

	Crossing	Rearing	Distance (cm)	Velocity (mm/s)
Control	249.6 ± 57.9	6.0 ± 2.2	430.5 ± 114.8	21.6 ± 3.7
CF3 0.1	287.7 ± 63.7	6.3 ± 1.8	488.3 ± 111.0	25.7 ± 4.1
TNF-α	314.1 ± 71.6	8.2 ± 2.7	535.2 ± 139.6	27.9 ± 5.5
CF3 0.1 + TNF-α	296.3 ± 23.6	7.1 ± 1.4	498.3 ± 52.9	25.6 ± 2.9

TNF-α at concentration of 0.001 fg/5 μL/site was administered 1 h before the tests and (*m*-CF_3-PhSe)$_2$ (0.1 mg/kg) was administered for 14 days. The last administration was given to mice 30 min before the LAM. Data are reported as means ± SEM. $n = 9$–11/group (9 (control); 11 (CF3 0.1 mg/kg); 9 (TNF-α) and 10 (CF3 0.1 mg/kg + TNF-α)). CF3: (*m*-CF_3-PhSe)$_2$.

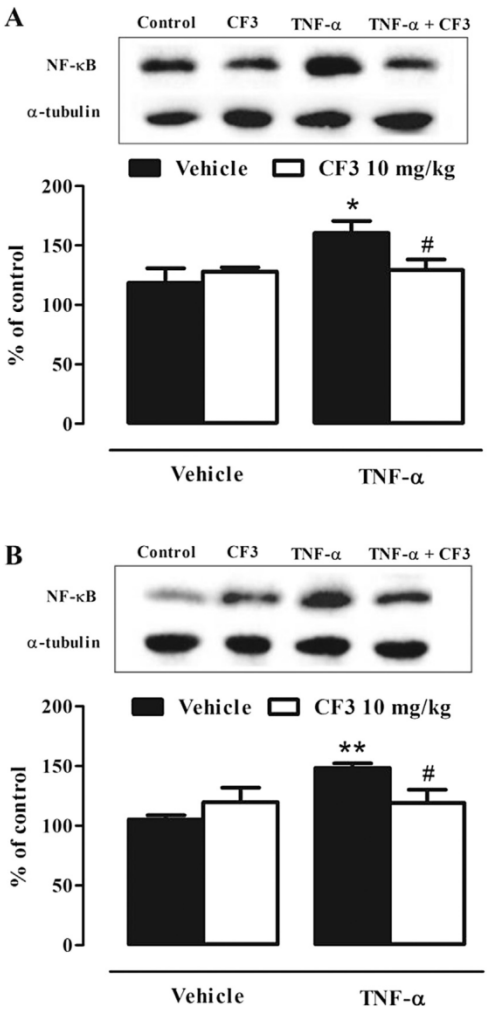

Fig. 4. Effect of TNF-α and/or acute treatment of mice with (*m*-CF_3-PhSe)$_2$ (10 mg/kg) on NF-κB levels in the (A) prefrontal cortex and (B) hippocampus. TNF-α (0.1 fg/5 μL/site) was administered 1 h and (*m*-CF_3-PhSe)$_2$ 30 min before the sample collection. The results of NF-κB levels were shown as % of control of quantification of bands O.D. Data represent the mean (s) ± SEM. *n* to prefrontal cortex = 3/group (3 (control); 3 (CF3 10 mg/kg); 3 (TNF-α) and 3 (CF3 10 mg/kg + TNF-α)). *n* to hippocampus = 3–4/group (3 (control); 3 (CF3 10 mg/kg); 4 (TNF-α) and 4 (CF3 10 mg/kg + TNF-α)). (*) $p < 0.05$ and (**) $p < 0.01$ when compared to the control group. (#) $p < 0.05$ when compared to the TNF-α group (two-way ANOVA followed by the Newman–Keuls test). CF3: (*m*-CF_3-PhSe)$_2$.

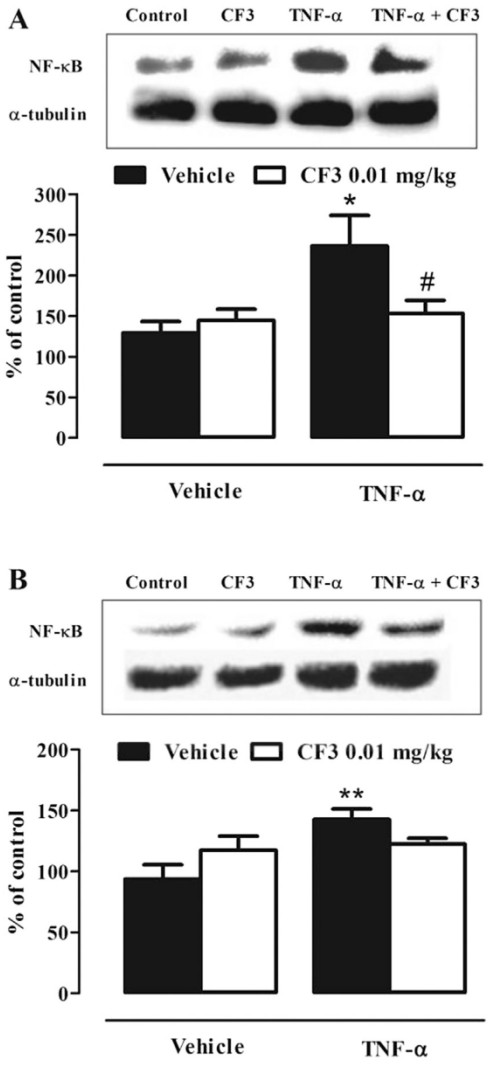

Fig. 5. Effect of TNF-α and/or subchronic treatment of mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.01 mg/kg) on NF-κB levels in the (A) prefrontal cortex and (B) hippocampus. TNF-α (0.1 ƒg/5 μL/site) was administered 1 h before the sample collection and $(m\text{-}CF_3\text{-}PhSe)_2$ was administered for 14 days. The last administration was given to mice 30 min before the sample collection. The results of NF-κB levels were shown as % of control of quantification of bands O.D. Data represent the mean (s) ± SEM. *n* to prefrontal cortex = 4/group (4 (control); 4 (CF3 0.01 mg/kg); 4 (TNF-α) and 4 (CF3 0.01 mg/kg + TNF-α)). *n* to hippocampus = 4–5/group (4 (control); 4 (CF3 0.01 mg/kg); 5 (TNF-α) and 5 (CF3 0.01 mg/kg + TNF-α)) (*) $p < 0.05$ and (**) $p < 0.01$ when compared to the control group. (#) $p < 0.05$ when compared to the TNF-α group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

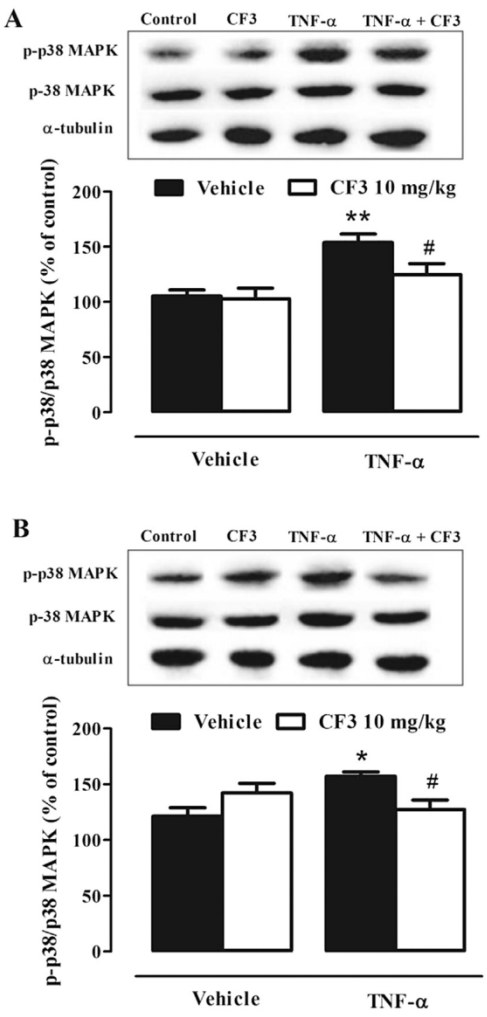

Fig. 6. Effect of TNF-α and/or acute treatment of mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (10 mg/kg) on p-p38/p38 MAPK ratio in the (A) prefrontal cortex and (B) hippocampus. TNF-α (0.1 ƒg/5 μL/site) was administered 1 h and $(m\text{-}CF_3\text{-}PhSe)_2$ 30 min before the sample collection. The results of p-p38 MAPK and p38 MAPK were shown as % of control of quantification of the phosphorylated ratio: O.D. of the phosphorylated band/O.D. of the total band, both corrected by α-tubulin. Data represent mean(s) ± SEM. *n* to prefrontal cortex = 4/group (4 (control); 4 (CF3 10 mg/kg); 4 (TNF-α) and 4 (CF3 10 mg/kg + TNF-α)). *n* to hippocampus = 4–5/group (4 (control); 4 (CF3 10 mg/kg); 5 (TNF-α) and 4 (CF3 10 mg/kg + TNF-α)). (*) $p < 0.05$ and (**) $p < 0.01$ when compared to the control group. (#) $p < 0.05$ when compared to the TNF-α group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

$(F_{(1,13)} = 11.50, p < 0.01)$. The i.c.v. TNF-α injection increased the activation of p38 MAPK represented by an increase in the ratio of p-p38/p38 MAPK in the prefrontal cortex and hippocampus and $(m\text{-}CF_3\text{-}PhSe)_2$ was effective in preventing this increase in both structures (Fig. 6). In the subchronic treatment, $(m\text{-}CF_3\text{-}PhSe)_2$ at a dose of 0.01 mg/kg for 14 days also prevented the increase in the ratio of p-p38/p38 MAPK in the prefrontal cortex as well as in hippocampus (a TNF-α × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction in the prefrontal cortex $(F_{(1,14)} = 5.85, p < 0.05)$ and a main effect of TNF-α in hippocampus $(F_{(1,12)} = 10.03, p < 0.01)$) (Fig. 7).

4. Discussion

The present study showed that acute and subchronic treatments of mice with the organoselenium compound $(m\text{-}CF_3\text{-}PhSe)_2$ prevented the depressive-like behavior induced by the i.c.v. injection of the pro-inflammatory cytokine TNF-α, which has been associated to etiology of major depressive disorders. $(m\text{-}CF_3\text{-}PhSe)_2$ was also effective in reducing the increase in NF-κB levels and p38 MAPK activation induced by TNF-α in the prefrontal cortex and hippocampus, two important brain areas involved in mood regulation (Phillips et al., 2003a,b).

The growing evidence of the relationship between the immune processes and neural activity is of particular relevance to patients

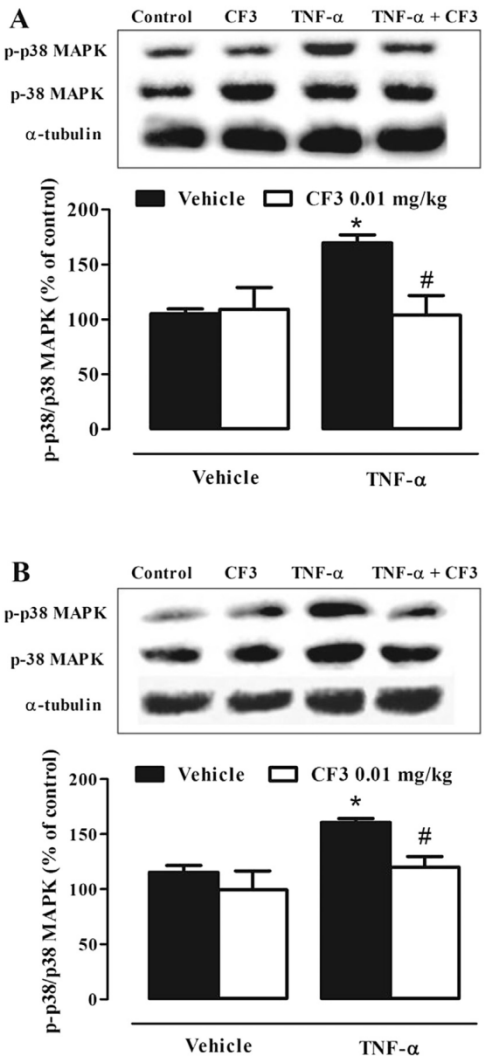

Fig. 7. Effect of TNF-α and/or subchronic treatment of mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.01 mg/kg) on p-p38/p38 MAPK ratio in the (A) prefrontal cortex and (B) hippocampus. TNF-α (0.1 fg/5 μL/site) was administered 1 h before the sample collection and $(m\text{-}CF_3\text{-}PhSe)_2$ was administered for 14 days. The last administration was given to mice 30 min before the sample collection. Data represent the mean (s) ± SEM. *n* to prefrontal cortex = 4–5/group (5(control); 5 (CF3 0.01 mg/kg); 4 (TNF-α) and 4 (CF3 0.01 mg/kg + TNF-α)). *n* to hippocampus = 4/group (4 (control); 4 (CF3 0.01 mg/kg); 4 (TNF-α) and 4 (CF3 0.01 mg/kg + TNF-α)). (*) $p < 0.05$ when compared to the control group. (#) $p < 0.05$ when compared to the TNF-α group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

suffering from psychiatric diseases such as major depression (Dantzer et al., 2008; Haroon et al., 2012) and has stimulated the search for animal models and treatments in this field. The depressogenic effect of TNF-α demonstrated, in this study, in the FST and TST is in accordance with the results of Kaster et al. (2012), which showed that low doses of TNF-α induce depressive-like behavior in mice and that classical antidepressants, such as fluoxetine and imipramine, block this effect. In addition, Krugel et al. (2013) reported that treatment of mice with the anti-TNF-α drug etanercept (Enbrel®; Amgen, USA) has antidepressant-like effect in a model of chronic mild stress. In humans, the administration of this TNF-α blocker attenuates the depressive symptoms that accompany immune system activation in psoriasis (Krishnan et al., 2007; Tyring et al., 2006).

In the present study, acute and subchronic treatments of mice with $(m\text{-}CF_3\text{-}PhSe)_2$, a blood–brain barrier (BBB)-permeant compound (Brüning et al., 2014), prevented the increase in immobility time induced by TNF-α in the FST and TST. The antidepressant-like effect of acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ was already demonstrated in the FST at doses higher than 10 mg/kg (Brüning et al., 2011). In the current study, low doses of $(m\text{-}CF_3\text{-}PhSe)_2$, that did not elicit an antidepressant-like effect in control animals, blocked the effect of TNF-α, showing that $(m\text{-}CF_3\text{-}PhSe)_2$ has an enhanced action when depressogenic stimulus is applied, given more predictive validity to the antidepressant effect of this compound.

In healthy individuals, there is a regulated balance between pro- and anti-inflammatory cytokines, such as interleukin-10 (IL-10), which exerts immuno-regulatory effect and maintains the homeostasis after an immunological challenge. By contrast, in a chronic inflammation condition this balance is disrupted and it can be shifted towards a pro-inflammatory environment (Dhabhar et al., 2009). The brain monitors peripheral innate immune responses by several means that could act in parallel through afferent nerves, humoral pathway or cytokine transporters at BBB (Banks, 2006; Bluthe et al., 1994; Quan et al., 1998). Under different conditions of peripheral inflammation diverse neuroimmune afferents can be activated. When localized inflammation is controlled by local immune activity the neuroimmune afferents are kept in a quiescent state. However, when local inflammagens exceed a threshold, sensory neurons are activated to relay immune signals to the brain and in a systemic inflammation situation both neural and BBB-dependent neuroimmune afferents can be activated. Finally, strong systemic inflammation stimulates microglia-mediated neuroinflammation in addition to all the neuroimmune afferents (Quan, 2014). Brain parenchymal macrophages, known as microglial cells, are more quiescent in comparison with other tissue macrophages but can respond to inflammatory stimuli by over producing pro-inflammatory cytokines. Particularly, TNF-α receptors, TNFR1 and TNFR2, are found in several regions of brain involved in mood and cognitive functioning. They are highly expressed in hippocampus and cortex (Simen et al., 2006) and constitutively expressed by microglia and neurons (Dopp et al., 1997; Khairova et al., 2009). In the model of i.c.v. injection of TNF-α, Kaster et al. (2012) propose that an initial inflammatory stimuli produced by low doses of TNF-α ultimately leads to the synthesis and release of endogenous TNF-α, given that the inhibition of TNF-α synthesis by thalidomide blocked the depressive-like effect of the exogenous TNF-α.

Considering the concept that acute activation of TNF-α during immune and inflammatory response leads to increase in brain levels of pro-inflammatory cytokines (Kaster et al., 2012; Qin et al., 2007), two important TNFR1 and TNFR2-associated signaling cascades, NF-κB and p38 MAPK (Aggarwal, 2003), were evaluated in this study. NF-κB regulates a wide variety of genes including those encoding pro-inflammatory cytokines (e.g., TNF-α, IL-1β and IL-6), chemokines, acute phase proteins and inducible effector enzymes. In the present study, TNF-α increased the NF-κB levels in the prefrontal cortex and hippocampus and acute and subchronic treatments with $(m\text{-}CF_3\text{-}PhSe)_2$ were effective in reducing these levels in mice. As a consequence, $(m\text{-}CF_3\text{-}PhSe)_2$ probably prevented the synthesis and release of endogenous pro-inflammatory cytokines induced by TNF-α, which could be associated to its antidepressant effect in this model. In accordance with earlier reports, NF-κB activation induces depressive behavior (Munhoz et al., 2006; Pace et al., 2006) and is a therapeutic target for depression (Koo et al., 2010). Accordingly, another diaryl diselenide compound, bis-(3-hydroxyphenyl) diselenide, has been shown to have anti-

inflammatory properties, which are related to the reduction in the expression of inducible nitric oxide synthase (iNOS), cyclo-oxygenase (COX)-2, TNF-α, IL-1β and IL-6 through the down-regulation of NF-κB binding activity (Shin et al., 2009).

Among the three mitogen-activated protein kinase (MAPK) pathways, extra-cellular-signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38, this last one is considered to be a central regulator of inflammation and also could underlies stress-induced depressive-like behavior in animal models (Bruchas et al., 2011; Kumar et al., 2003; Land et al., 2009; Saklatvala, 2004). Many inflammatory response proteins depend on p38 MAPK signaling for their production. TNF-α, IL-1 and COX-2 are among the most important inflammatory mediators that are regulated by p38 MAPK. In addition to mediating the production of inflammatory molecules, p38 MAPK is significantly activated by inflammatory cytokines, such as TNF-α, and has crucial functions in cellular responses to these cytokines (Dong et al., 2002). Inhibition of the p38 MAPK pathway leads to reduce production of inflammatory mediators and, consequently, blockade of the inflammatory process (Kumar et al., 2003; Saklatvala, 2004). In the present study, TNF-α induced a great increase in the activation of p38 MAPK, represented by an increase in the phosphorylated ratio of this protein in the prefrontal cortex and hippocampus, and acute and subchronic treatments with $(m\text{-}CF_3\text{-}PhSe)_2$ prevented this increase in both regions, which could be related to its antidepressant-like effect.

The depressogenic effects of pro-inflammatory cytokines have been related mainly to their action in the neurotransmitter serotonin (5-HT) homeostasis, which deficiency is considered the main pathophysiologic feature of depressive disorders and the target of most antidepressants (Nutt, 2008). Mice knockout to TNF-α receptors have increased levels of 5-HT in several brain areas (Yamada et al., 2000). By inference, elevations of TNF-α may reduce 5-HT availability providing an attractive molecular mechanism to explain the effects of TNF-α on mood. One of this effects of pro-inflammatory cytokines is the depletion of tryptophan, the precursor of 5-HT synthesis, through activation of the enzyme indoleamine-2,3-dioxygenase (IDO), which is significantly upregulated in response to inflammation (Dantzer et al., 2008). Activation of (IDO) is considered an inducible host defense mechanism against pathogen replication, once it reduces the essential amino acid tryptophan from local tissue microenvironment and for the same reason, this enzyme has been considered to play an important immunoregulatory effect in autoimmune disorders. However, the presence of chronic inflammation might provoke sustained IDO production, even though IDO fails to limit immune dysregulation under these pathological conditions (Mellor and Munn, 1999; Munn and Mellor, 2013). Pro-inflammatory cytokines could also reduce 5-HT availability through activation of neuronal serotonin transporters (SERT) (Malynn et al., 2013; Zhu et al., 2006). It has been demonstrated that IL-1β and TNF-α increase SERT activity via trafficking-dependent and independent processes through p38 MAPK activation (Bruchas et al., 2011; Zhu et al., 2005, 2010). In this way, the increase of p38 MAPK after TNF-α injection observed herein could increase the 5-HT uptake, decreasing the 5-HT availability in the synaptic cleft and inducing the depressive-like behavior observed in the FST and TST, which was prevented by acute and subchronic treatments with $(m\text{-}CF_3\text{-}PhSe)_2$. Of particular importance, $(m\text{-}CF_3\text{-}PhSe)_2$ inhibits 5-HT uptake *in vitro* (Borges et al., 2009) as well as *ex vivo* in mice (Brüning et al., 2014), and also inhibits the enzyme implicated in 5-HT degradation monoamine oxidase A (MAO-A) (Brüning et al., 2009), contributing to maintain 5-HT homeostasis.

Some of the limitations of this study include; first, the subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ was performed in mice without the depressogenic stimulus and could not represent a clinical situation. In addition, $(m\text{-}CF_3\text{-}PhSe)_2$ is a lipophilic compound and the effect of low doses in the subchronic treatment could be due an accumulation process. Lipophilic compounds are known to accumulate in adipose tissue and to be eliminated slowly (Nagahori et al., 2010). Lastly, it is not possible to affirm if the antidepressant-like effect of $(m\text{-}CF_3\text{-}PhSe)_2$ in this experimental model is due to an immunoregulatory action of this compound or the already demonstrated effects of $(m\text{-}CF_3\text{-}PhSe)_2$ in the serotonergic system. Despite these limitations the results reported herein support the notion that the mechanisms of antidepressant-like action of $(m\text{-}CF_3\text{-}PhSe)_2$ could involve effects on the serotonergic system and its anti-inflammatory property.

Multi-target profile has been considered a desirable property in comparison to drugs that are selective (Frantz, 2005). The antidepressant and anti-inflammatory actions of $(m\text{-}CF_3\text{-}PhSe)_2$ at low doses, preventing the increase in NF-κB levels and p38 MAPK activation, in association with direct effects of $(m\text{-}CF_3\text{-}PhSe)_2$ on 5-HT uptake and metabolism already demonstrated, may support this compound as an interesting molecule in the search for new drugs to treat depression disorders that have been largely linked to immune process and inflammation.

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Contributors

All authors contributed equally to the research and article preparation and have approved the final article form.

Conflict of interest

There is no conflict of interest in the conduct and reporting of research (e.g., financial interests in a test or procedure, funding by pharmaceutical companies for drug research).

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3.3 Artigo 3

_m_-Trifluoromethyl-diphenyl diselenide, a multi-target selenium compound, prevented mechanical allodynia and depressive-like behavior in a mouse comorbid pain and depression model

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ABSTRACT

Chronic pain and depression are two complex states that often coexist in the clinical setting and traditional antidepressants and analgesics have shown limited clinical efficacy. There is an intricate communication between the immune system and the central nervous system and inflammation has been considered a common mediator of pain–depression comorbidity. This study evaluated the effect of *m*-trifluoromethyl diphenyl diselenide [$(m\text{-}CF_3\text{-}PhSe)_2$], an organoselenium compound that has been reported to have both antinociceptive and antidepressant-like actions, in the comorbidity of chronic pain and depression induced by partial sciatic nerve ligation (PSNL) in an inflammatory approach. Mice were submitted to PSNL during 4 weeks and treated with $(m\text{-}CF_3\text{-}PhSe)_2$ acutely (0.1–10 mg/kg, i.g.) or subchronically (0.1 mg/kg, i.g., once a day during the 3rd and 4th weeks). Both treatments prevented PSNL-increased pain sensitivity and depressive-like behavior observed in Von-Frey hair (VFH) and forced swimming (FST) tests, respectively. These effects could be mainly associated with an anti-inflammatory action of $(m\text{-}CF_3\text{-}PhSe)_2$ which reduced the levels of pro-inflammatory cytokines, NF-κB and COX-2, and p38 MAPK activation that were increased by PSNL. $(m\text{-}CF_3\text{-}PhSe)_2$ also increased the BDNF levels and reduced glutamate release and 5-HT uptake altered by PSNL. Although acute and subchronic treatments with $(m\text{-}CF_3\text{-}PhSe)_2$ prevented these alterations induced by PSNL, the best results were found when $(m\text{-}CF_3\text{-}PhSe)_2$ was subchronically administered to mice. Considering the potential common mechanisms involved in the comorbidity of inflammation-induced depression and chronic pain, the results found in this study indicate that $(m\text{-}CF_3\text{-}PhSe)_2$ could become an interesting molecule to treat long-lasting pathological pain associated with depression.

1. Introduction

Chronic pain and depression are two complex states that often coexist in the clinical setting. The prevalence rate of depression is several times higher in patients with chronic pain than in the general population, whereas depression significantly increases the risk of developing chronic pain (Arnow et al., 2006; Von Korff and Simon, 1996), leading to a poorer prognosis. Although of great interest, the underlying causal mechanisms involved in pain and depression remain poorly understood.

A functional deficiency of key monoaminergic neurotransmitters, mainly serotonin (5-hydroxytryptamine — 5-HT), is thought to be the main pathophysiologic feature of depressive disorders and is the target of the most antidepressants (Nutt, 2008). Likewise, 5-HT has an important role in the control of nociception, through the descending pathway (Millan, 2002). In this way, current symptomatic management of chronic pain is often performed through combination of analgesics with antidepressants, but this clinical approach has achieved only limited success and significant side effects (Mao et al., 2011).

It has been reported an intricate communication between the immune system and the central nervous system and neuroinflammation as a common mediator of pain–depression comorbidity (Walker et al., 2014). The peripheral inflammation initiates a cascade of events with

Abbreviations: 5-HT, 5-hydroxytryptamine; TNF-α, tumor necrosis factor-α; IL, interleukin; BBB, blood-brain-barrier; HPA, hypothalamic-pituitary-adrenal axis; $(m\text{-}CF_3\text{-}PhSe)_2$, *m*-trifluoromethyl diphenyl diselenide; MAO-A, monoamine oxidase A; PSNL, partial sciatic nerve ligation; LAM, locomotor activity monitor; FST, forced swimming test; VFH, von-frey hair test; ACTH, adrenocorticotropic hormone; p38 MAPK, p38 mitogen-activated protein kinase; NF-κB, nuclear factor-κB; BDNF, brain-derived neurotrophic factor; COX-2, cyclooxygenase-2; SERT, selective serotonin transporter.

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the release of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), at the site of injury that can initiate a systemic immune response, as can be observed in the onset of a peripheral nerve injury (Walker et al., 2014). These cytokines overflowing in the systemic circulation can gain access to the brain through saturable transporters at the blood–brain-barrier (BBB) (Banks, 2006) or activate primary afferent nerves (Bluthe et al., 1994). In addition, both neuronal and non-neuronal brain cells express receptors for these mediators (Dantzer, 2007). Engagement of these immune-to-brain communication pathways ultimately leads to activation of microglial cells over-increasing pro-inflammatory cytokines, establishing a neuroinflammatory framework.

Inflammatory signals target downstream pathways, which can disturb neural function such as activation of hypothalamic–pituitary–adrenal (HPA) axis (Anisman et al., 2002), reduction of 5-HT availability (Malynn et al., 2013) and activation of glutamatergic system (Loftis et al., 2010). Chronic inflammation can lead to a permanent restructuring of these pathways and to the transition from acute pain and sickness responses into chronic pain and depression, even after the acute inflammatory response has dissipated. Indeed, neuroinflammation has been shown to play a pivotal role in the onset and maintenance of pain hypersensitivity after peripheral nerve injury (Backonja et al., 2008; Walker et al., 2014).

m-Trifluoromethyl diphenyl diselenide [$(m\text{-}CF_3\text{-}PhSe)_2$], an organoselenium compound BBB permeant, has been reported to have both antinociceptive and antidepressant-like effects in animal models (Brüning et al., 2010, 2011, 2014). Neurochemical data indicate that $(m\text{-}CF_3\text{-}PhSe)_2$ modulates the serotonergic system through mechanisms that involve a selective inhibition of monoamine oxidase A (MAO-A) activity (Brüning et al., 2009), an enzyme implicated in the 5-HT degradation, and the 5-HT uptake inhibition in synaptosomes of rat brains (Borges et al., 2009). These effects of $(m\text{-}CF_3\text{-}PhSe)_2$ could result in an overall increase of 5-HT availability in the synaptic cleft, contributing to its pharmacological effects. Besides, a structural parent compound of $(m\text{-}CF_3\text{-}PhSe)_2$ has been shown to have a remarkable anti-inflammatory effect (Rupil et al., 2012). Although $(m\text{-}CF_3\text{-}PhSe)_2$ has been reported to be a multi-target compound, detailed characterization of its effects remains largely unknown.

Considering the depression and pain comorbidity in the context of inflammation and that the efficacy of traditional antidepressants and analgesics yields success rates lower than 50%, it is important the development of novel effective pharmaceutical interventions to treat simultaneously both conditions (Kroenke et al., 2009). The aim of this study was to evaluate the effect of $(m\text{-}CF_3\text{-}PhSe)_2$ in the chronic pain and depression comorbidity under a neuroinflammatory perspective after peripheral nerve injury induced by partial sciatic nerve ligation (PSNL), a neuropathy model that mimics pathological neuropathic pain conditions in humans.

2. Experimental procedures

2.1. Animals

The experiments were carried out using male adult Swiss mice (25–30 g) from our own breeding colony. The animals were maintained at 22–25 °C with free access to water and food (Guabi, Brazil), under a 12:12 h light/dark cycle with lights on at 7:00 a.m. Mice were acclimated to the behavioral room at least 1 h before the test. Experimental procedures were conducted in compliance with the Ethical Research Committee of Federal University of Santa Maria (#042/2012), affiliated to the Council for Control of Animal Experiments (CONCEA) and in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used in the experiments and their suffering.

2.2. Drugs

$(m\text{-}CF_3\text{-}PhSe)_2$ was prepared and characterized in our laboratory based on a previous study carried out by Paulmier (1986). ^1H and ^{13}C nuclear magnetic resonance spectroscopy analysis showed analytical and spectroscopic agreement with the assigned structure. The chemical purity of $(m\text{-}CF_3\text{-}PhSe)_2$ (99.9%) was determined by gas chromatography–mass spectrometry (GC/MS combined). [^{3}H]5-HT creatinine sulfate (23 Ci/mmol) and L-[^{3}H]glutamate (50 Ci/mmol) were purchased from Labex Inc. (Hinsdale, Illinois, USA). Other chemicals were obtained from standard commercial suppliers. $(m\text{-}CF_3\text{-}PhSe)_2$ was dissolved in canola oil and administered to mice by intragastric (i.g.) route at volume of 10 ml/kg.

2.3. Peripheral nerve injury

PSNL was performed based on the method described by Seltzer et al. (1990) under intraperitoneal ketamine/xylazine (150 and 10 mg/kg, respectively) anesthesia. The right sciatic nerve (ipsilateral side) was exposed after the incision of skin and blunt separation of the muscle. The sciatic nerve was freed from surrounding connective tissues at a site near the trochanter just distal to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve, and one ligature (8/0 Ethicon GmbH, Norderstedt, Germany) was made around approximately one-third to one-half of the diameter of the sciatic nerve. In the sham-operated mice, the nerve was exposed without ligation.

2.4. Experimental design

The mice were acutely or subchronically treated with $(m\text{-}CF_3\text{-}PhSe)_2$ with the purpose of evaluating its effect on depressive-like behavior and mechanical allodynia induced by PSNL (Fig. 1). In the acute treatment, at the end of the 4th week after surgery, PSNL mice were treated with vehicle or $(m\text{-}CF_3\text{-}PhSe)_2$ (0.1 to 10 mg/kg) and after 30 min the animals were submitted to the locomotor activity monitor (LAM) and the forced swimming test (FST). In order to determine the effect of acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ on PSNL-induced mechanical allodynia, another set of animals was submitted to the Von-Frey hair test (VFH) 30 min after the administration of $(m\text{-}CF_3\text{-}PhSe)_2$ (0.1 to 10 mg/kg). In the subchronic treatment, sham and PSNL mice were treated with vehicle or $(m\text{-}CF_3\text{-}PhSe)_2$ at a dose of 0.1 mg/kg (a subeffective dose in the acute treatment), once a day for two weeks, during the 3rd and 4th weeks after surgery. At the end of the 4th week, 24 h after the last administration of $(m\text{-}CF_3\text{-}PhSe)_2$ the mice were submitted to the LAM, VFH and FST.

2.5. Behavioral testing

2.5.1. Spontaneous locomotor activity

The mouse spontaneous locomotor activity was performed in the LAM in order to discard non-specific effects of treatments. LAM is a clear acrylic plastic box (45 × 45 × 45 cm) with a removable plastic lid perforated with holes for ventilation. The monitor is surrounded by a frame consisting of 32 photocells mounted on opposite walls (16 L × 16 W, spaced 2 cm apart) that continuously tracks the animal's movement. The general locomotor activity and the mouse's position in the chamber are detected by breaks of the photocell beams, which are recorded by a computer, using Monitor Activity® software (Insight). The mice were placed in the center of the apparatus and allowed to freely explore the arena during 4 min. Number of crossings and rearings, total distance traveled (cm) and average velocity (mm/s) were recorded.

2.5.2. Forced swimming test

Depression-like behavior was assessed in the FST, a well-established preclinical animal model, as described previously (Porsolt et al., 1979).

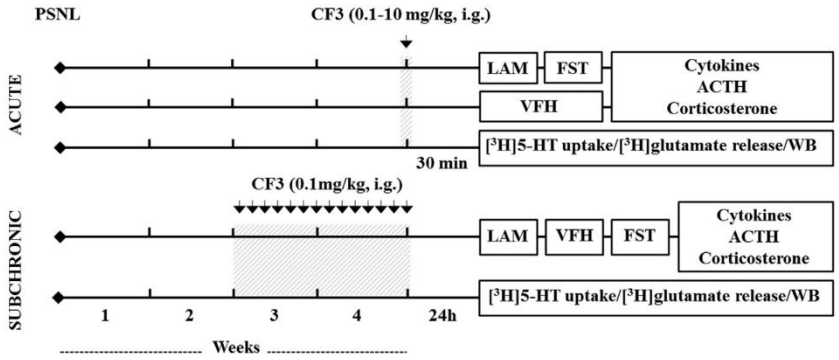

Fig. 1. Schematic view of the experimental study design. CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

The mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1 °C and their escape related mobility behavior (latency for the first immobility episode and total duration of immobility) was recorded during 6 min period by a blinded observer. Immobility was defined as floating passively in an upright position in the water, with only small movements made necessary to keep the head above the water surface.

2.5.3. Mechanical allodynia test

PSNL has been reported to induce persistent mechanical allodynia related to central sensitization (Kim and Moalem-Taylor, 2011). The mechanical allodynia of mice was evaluated with VFH (Stoelting, Chicago, IL). The mice were placed in individual plexiglas chambers (9 × 7 × 11 cm) with wire mesh bottoms and allowed to acclimatize to the environment. The response frequency was measured after ten applications (duration of 1–2 s each) of 0.6 g VFH in the plantar surface of each hind paw by a blinded observer. A previous study indicated that 0.6 g VFH produced a mean withdrawal frequency of ipsilateral hind paw of approximately 70% in PSNL mice, which was considered to be an adequate value for the measurement of mechanical allodynia. The contralateral hind paw was also tested in the VFH in order to evaluate the occurrence of mirror-image pain.

2.6. Ex vivo assays

At the end of behavioral tests in both acute and subchronic treatments with $(m\text{-}CF_3\text{-}PhSe)_2$, mice were anesthetized with isoflurane and blood samples were collected by cardiac puncture. In the acute treatment, a dose of 10 mg/kg was chosen to the ex vivo assays. Blood was centrifuged at 4000 ×*g* for 10 min, and serum was collected and stored at −80 °C for the determination of serum cytokines, adrenocorticotropic hormone (ACTH) and corticosterone levels. After this procedure, mice were killed by cervical dislocation and samples of the brain contralateral cortex and hippocampus, two important regions involved in both pain and depression (Robinson et al., 2009), were immediately excised and stored at −80 °C for the determination of cytokine levels. Other sets of animals from both acute and subchronic treatments with $(m\text{-}CF_3\text{-}PhSe)_2$, which were not submitted to the behavioral tests, were killed by cervical dislocation and the brains were removed to dissect the contralateral cortices and hippocampi. The [^{3}H]5-HT uptake and [^{3}H]glutamate release were determined in samples obtained from cerebral cortex and hippocampus. The tissues were flash-frozen and stored at −80 °C until the western-blot analysis (Fig. 1).

2.6.1. Determination of cytokine, serum ACTH and corticosterone levels

The cytokine levels in samples of serum, contralateral cortex and hippocampus of mice were measured by the enzyme linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN). Results were expressed in pg/ml (serum) or pg/mg of protein (contralateral cortex and hippocampus) for IL-1β, IL-6, TNF-α and IL-10 and μg/ml (serum) or μg/mg of protein (contralateral cortex and hippocampus) for INF-γ. The levels of ACTH and corticosterone in the mouse serum were measured through the radioimmunoassay test kit (Diagnostic Products Corporation, USA). ACTH and corticosterone levels were expressed in pg/ml and ng/ml, respectively.

2.6.2. Western-blot analysis

The samples of contralateral cortex and contralateral hippocampus were homogenized in 10 mM Tris (hydroxymethyl) aminomethane–hydrochloride (Tris–HCl), 1 mM ethylene diaminetetraacetic acid (EDTA), pH 7.4, and centrifuged (9800 ×*g* at 4 °C for 5 min) to concentrate the proteins. The pellet was reconstituted in a buffer solution (10 mM Tris–HCl, pH 7.6, 5 mM $MgCl_2$, 1.5 mM potassium acetate (KAc), 1% NP-40, and protein inhibitor cocktail (Sigma-Aldrich Co., St. Louis, Missouri, USA)) and incubated for 30 min on ice followed by 10 min on ultrasonic bath, and then centrifuged (3900 ×*g* for 10 min, at 4 °C). Tissue extracts were diluted to a final protein concentration of 2 μg/ml in sodium dodecyl sulfate–polyacrylamide gel electrophoresis buffer (SDS-PAGE buffer is essentially constituted by Tris·HCl 0.5 M, pH 6.8 (in a final concentration of 62.5 mM), glycine, SDS, 2-β-mercaptoethanol, the reducing agent, and bromophenol blue, used as a marker to monitor the process of electrophoresis). The samples (50 μg of protein) and pre stained molecular weight standards (Sigma-Aldrich Co., St. Louis, Missouri, USA) were separated on 12% resolving with 4% concentrating SDS-PAGE electrophoresis gels. Proteins were transferred to polyvinylidene fluoride (PVDF) membrane using Transfer-Blot® Turbo™ Transfer System (1.0 mA; 30 min) and/equal protein loading was confirmed by Ponceau S staining. After blocking with 5% bovine serum albumin solution, the blots were incubated overnight at 4 °C with rabbit anti-p-p38 MAPK antibody (phosphorylated p38 mitogen-activated protein kinase; Thr180/Tyr182) (1:1000; Cell Signaling) and rabbit anti-p38 MAPK (1:1000; Cell Signaling), rabbit anti-NF-κB p65 (nuclear factor-κB) (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-COX-2 (cyclooxygenase-2) (1:1000, Cell Signaling), and rabbit anti-BDNF (brain-derived neurotrophic factor,) (1:1000; Abcam). Mouse anti-β-actin (1:3000, Abcam) or anti-α-tubulin (1:3000, Abcam) was stained as additional control of the protein loading. After primary antibody incubation, membranes were washed and incubated with secondary antibodies conjugated with horseradish peroxidase (Bio-Rad Laboratories, Hercules, CA, USA) for 1 h at room temperature and developed with chemiluminescence kit (Amersham, São Paulo/Brazil). Optical density (O.D.) of the Western blotting bands was quantified using Image J (NIH, Bethesda, MD, USA) software for Windows. Each value was derived from the ratio between arbitrary units obtained by the protein band and the respective β-

actin or α-tubulin band. The results of p-p38 MAPK and p38 MAPK were shown by % of control of quantification of the phosphorylated ratio: O.D. of the phosphorylated band/O.D. of the total band. The results of NF-κB, COX-2 and BDNF levels were shown by % of control of quantification of bands O.D.

2.6.3. Synaptosomal [^{3}H]glutamate release

Synaptosomal suspensions from contralateral cortex and hippocampus (Gray and Whittaker, 1962) were washed three times in a non-depolarizing medium (low potassium), containing 27 mM HEPES, 133 mM NaCl, 2.4 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 12 mM glucose, and 1.0 mM $CaCl_2$, by centrifugation at 12,000 ×*g* for 15 min (4 °C). The determination of [^{3}H]glutamate release was carried out according to a method described previously (Migues et al., 1999). Results were expressed as percentage of [^{3}H]glutamate released.

2.6.4. Synaptosomal [^{3}H]5-HT uptake

Crude synaptosomes were obtained from contralateral cortex and hippocampus based on a previous method described by Gray and Whittaker (1962). [^{3}H]5-HT uptake into synaptosomes was carried out according to a previous study (Rocha et al., 2007), with some modifications. Results were expressed as fmol of [^{3}H]5-HT uptake·mg of protein^{-1}·min^{-1}.

2.7. Data presentation and statistical analysis

All experimental results are given as the mean ± SEM. Normality of data was evaluated by the D'Agostino and Pearson omnibus normality test. Statistical comparisons between experimental and control groups were performed using two-way ANOVA, including the factor surgery (sham and PSNL), treatment (vehicle or (m-CF_3-PhSe)$_2$), and interaction, followed by the Newman–Keuls test for post hoc comparison when appropriate. Main effects of first-order interactions are presented only when interaction was not significant. Value of $p < 0.05$ was considered to be significant. The two-way ANOVA analyses of the behavioral data for the acute treatment with (m-CF_3-PhSe)$_2$ were performed separately at each dose level.

3. Results

3.1. Acute and subchronic treatments with (m-CF_3-PhSe)$_2$ reduced PSNL-induced mechanical allodynia and depressive-like behavior

In the acute treatment of PSNL mice with (m-CF_3-PhSe)$_2$, the two-way ANOVA of VFH data in the ipsilateral paw showed a PSNL × (m-CF_3-PhSe)$_2$ interaction at doses of 1 and 10 mg/kg ($F_{(1,30)} = 33.82$, $p < 0.001$ and $F_{(1,26)} = 25.27$, $p < 0.001$, respectively). After 4 weeks of PSNL, there was an increase in mechanical allodynia of mice represented by an increase in the response frequency to the VFH stimulation and (m-CF_3-PhSe)$_2$ at doses of 1 and 10 mg/kg reduced this response, but not at a dose of 0.1 mg/kg (Fig. 2A). In the contralateral paw, there were no differences among groups in the response frequency to the VFH stimulation, i.e., PSNL did not induce mirror-image pain (data not shown). Likewise, the two-way ANOVA of the FST data demonstrated a PSNL × (m-CF_3-PhSe)$_2$ interaction at doses of 1 and 10 mg/kg ($F_{(1,28)} = 4.33$, $p < 0.05$ and $F_{(1,35)} = 4.24$, $p < 0.05$, respectively). PSNL increased the immobility time of mice and (m-CF_3-PhSe)$_2$ at doses of 1 and 10 mg/kg blocked this effect, attenuating the depressive-like behavior induced by nerve injury (Fig. 2B).

As acute administration of (m-CF_3-PhSe)$_2$ at a dose of 0.1 mg/kg was ineffective in reducing mechanical allodynia and the immobility time of PSNL mice, this dose was administered to mice in a subchronic treatment of two weeks (from the 3rd to 4th week after surgery), once a day. Two-way ANOVA showed a PSNL × (m-CF_3-PhSe)$_2$ interaction in both VFH and FST tests ($F_{(1,36)} = 29.50$, $p < 0.001$ and $F_{(1,32)} = 8.40$, $p < 0.01$, respectively). The subchronic treatment with the lowest dose

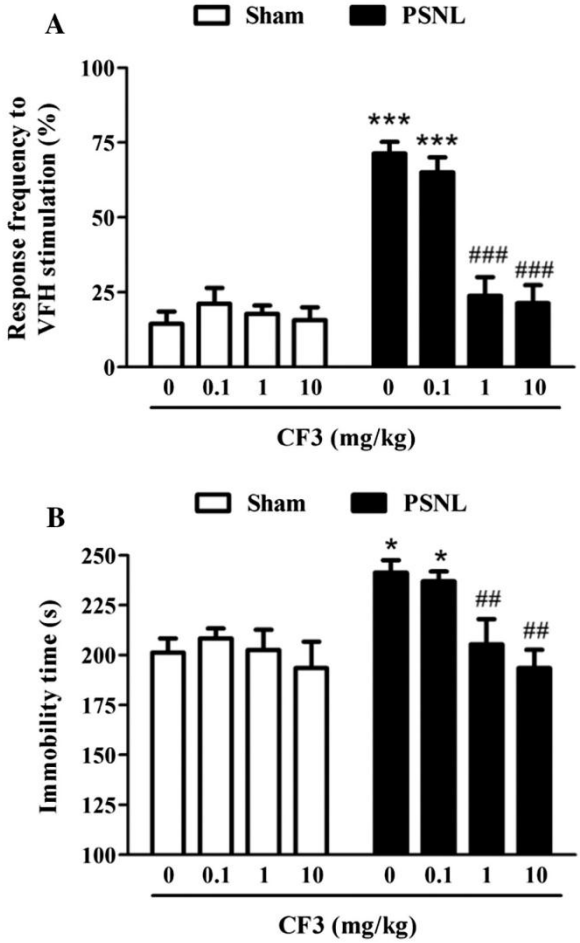

Fig. 2. Effect of acute treatment of PSNL mice with (m-CF_3-PhSe)$_2$ (0.1 mg/kg to 10 mg/kg) on (A) the response frequency to the VFH stimulation in the ipsilateral paw and (B) the immobility time in the FST. (m-CF_3-PhSe)$_2$ was administered 30 min before the test. Data represent means ± SEM, n = 7–9/group. (*) $p < 0.05$ and (***) $p < 0.001$ when compared to the sham-vehicle group. (##) $p < 0.01$ and (###) $p < 0.001$ when compared to the PSNL-vehicle group (two-way ANOVA of each (m-CF_3-PhSe)$_2$ dose followed by the Newman–Keuls test). CF3: (m-CF_3-PhSe)$_2$.

of (m-CF_3-PhSe)$_2$ blocked the increase in the response frequency to the VFH stimulation and the immobility time in the FST induced by PSNL (Fig. 3A and B). Similar to the acute treatment, there were no differences among groups in the response to the VFH stimulation in contralateral paws of mice after the subchronic treatment with (m-CF_3-PhSe)$_2$ (data not shown).

There were no significant differences among groups in all locomotor parameters, the number of crossings and rearings, the total distance traveled and average velocity, in both acute and subchronic treatments with (m-CF_3-PhSe)$_2$ (Tables 1 and 2, respectively).

3.2. (m-CF_3-PhSe)$_2$ treatment attenuated PSNL-induced alterations in cytokine levels

The cytokine levels in samples of serum, contralateral cortex and hippocampus of PSNL mice after acute treatment with (m-CF_3-PhSe)$_2$ (10 mg/kg) are shown in Table 3. In the serum, PSNL increased the levels of IL-1β, IL-6, TNF-α and INF-γ (main effects: $F_{(1,13)} = 455.57$, $p < 0.001$; $F_{(1,13)} = 406.16$, $p < 0.001$; $F_{(1,13)} = 359.17$, $p < 0.001$ and $F_{(1,13)} = 56.54$, $p < 0.001$, respectively) and the acute treatment with (m-CF_3-

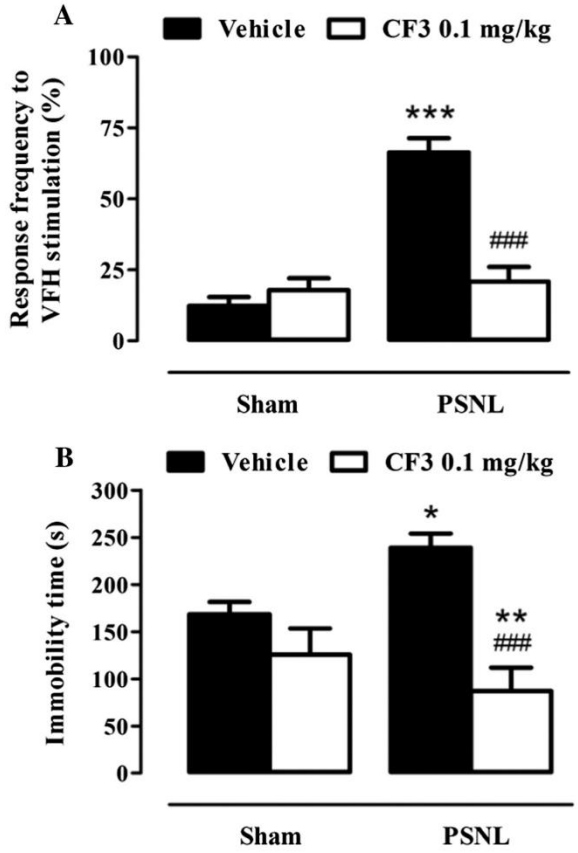

Fig. 3. Effect of subchronic treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.1 mg/kg) on (A) the response frequency to the VFH stimulation in the ipsilateral paw and (B) the immobility time in the FST. $(m\text{-}CF_3\text{-}PhSe)_2$ was administered once a day during the 3rd and 4th weeks after PSNL surgical procedure. Data represent means ± SEM, n = 9–11/group. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ when compared to the sham-vehicle group. (##) $p < 0.01$ and (###) $p < 0.001$ when compared to the PSNL-vehicle group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

$PhSe)_2$ was ineffective against this increase on pro-inflammatory cytokines. Similar results were found in the contralateral hippocampus, in which PSNL also increased the levels of IL-1β, IL-6, TNF-α and INF-γ (main effects: $F_{(1,13)} = 79.06$, $p < 0.001$; $F_{(1,13)} = 20.97$, $p < 0.001$; $F_{(1,13)} = 138.20$, $p < 0.001$ and $F_{(1,11)} = 22.64$, $p < 0.001$, respectively) and the acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ (10 mg/kg) did not reduce the levels of cytokines. In the contralateral cortex, two-way ANOVA showed a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction in the levels of IL-1β, IL-6 and TNF-α ($F_{(1,13)} = 14.52$, $p < 0.01$; $F_{(1,13)} = 4.31$, $p < 0.05$

Table 1
Locomotor parameters of mice evaluated in the activity monitor (LAM) after PSNL and/or acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.1 mg/kg to 10 mg/kg).

	Crossing	Rearing	Distance (cm)	Velocity (mm/s)
Sham	299.5 ± 43.6	9.3 ± 1.9	456.8 ± 74.8	21.6 ± 3.3
CF3 0.1	422.7 ± 52.6	9.7 ± 1.0	573.6 ± 84.8	31.8 ± 6.7
CF3 1	358.1 ± 76.6	12.1 ± 0.9	544.9 ± 87.7	29.8 ± 3.0
CF3 10	202.5 ± 20.2	5.6 ± 1.0	313.3 ± 48.7	19.3 ± 4.0
PSNL	231.6 ± 50.2	7.3 ± 2.1	354.7 ± 89.0	18.8 ± 3.5
CF3 0.1 + PSNL	376.3 ± 70.2	8.5 ± 0.9	539.9 ± 128.5	23.4 ± 4.4
CF3 1 + PSNL	481.9 ± 87.5	8.3 ± 0.8	464.4 ± 63.9	23.4 ± 3.5
CF3 10 + PSNL	180.4 ± 13.4	5.6 ± 1.0	313.3 ± 48.7	19.3 ± 4.0

$(m\text{-}CF_3\text{-}PhSe)_2$ was administered 30 min before the test. Data represent means ± SEM, n = 7–9/group. CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

Table 2
Locomotor parameters of mice evaluated in the activity monitor (LAM) after PSNL and/or subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.1 mg/kg).

	Crossing	Rearing	Distance (cm)	Velocity (mm/s)
Sham	411.7 ± 98.1	11.1 ± 3.3	617.8 ± 149.1	29.3 ± 5.1
CF3	464.0 ± 47.3	18.0 ± 2.9	696.9 ± 75.1	29.8 ± 2.8
PSNL	343.7 ± 19.9	11.7 ± 1.5	567.2 ± 36.6	26.3 ± 2.5
CF3 + PSNL	512.3 ± 47.3	17.9 ± 1.7	759.9 ± 71.1	33.0 ± 2.9

$(m\text{-}CF_3\text{-}PhSe)_2$ was administered once a day during the 3rd and 4th weeks after PSNL surgical procedure. Data represent means ± SEM, n = 9–11/group. CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

and $F_{(1,13)} = 5.84$, $p < 0.05$, respectively) and a main effect of PSNL in INF-γ levels ($F_{(1,13)} = 121.61$, $p < 0.001$). PSNL increased the levels of IL-1β, IL-6, TNF-α and INF-γ in the contralateral cortex and the acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ partially normalized these levels. The levels of anti-inflammatory cytokine, IL-10, were reduced by PSNL in serum and contralateral cortex and the acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ greatly increased the levels of IL-10, over those of the sham group (a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,13)} = 84.69$, $p < 0.001$ and $F_{(1,13)} = 39.79$, $p < 0.001$, respectively). In the contralateral hippocampus, PSNL did not alter IL-10 levels, although PSNL mice treated acutely with $(m\text{-}CF_3\text{-}PhSe)_2$ had an increase in the levels of this cytokine (PSNL×$(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,13)} = 20.72$, $p < 0.001$).

In the subchronic treatment of PSNL mice with a dose of 0.1 mg/kg of $(m\text{-}CF_3\text{-}PhSe)_2$ (Table 4), the two-way ANOVA of the serum pro-inflammatory cytokine levels demonstrated a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction for IL-1β ($F_{(1,12)} = 32.63$, $p < 0.001$) and a PSNL main effect for IL-6, TNF-α and INF-γ ($F_{(1,12)} = 332.02$, $p < 0.001$; $F_{(1,12)} = 389.30$, $p < 0.001$ and $F_{(1,12)} = 367.98$, $p < 0.001$, respectively). The levels of these pro-inflammatory cytokines were greatly increased by PSNL in the serum and $(m\text{-}CF_3\text{-}PhSe)_2$ partially normalized them, except for the levels of INF-γ. The effect of the subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ in the cytokine levels on both brain regions was similar. There was a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction for IL-1β, IL-6, TNF-α and INF-γ levels in the contralateral cortex ($F_{(1,11)} = 24.91$, $p < 0.001$; $F_{(1,11)} = 45.47$, $p < 0.001$; $F_{(1,11)} = 71.32$, $p < 0.001$ and $F_{(1,11)} = 80.78$, $p < 0.001$, respectively) and hippocampus ($F_{(1,12)} = 10.85$, $p < 0.01$; $F_{(1,12)} = 11.19$, $p < 0.01$; $F_{(1,12)} = 39.60$, $p < 0.001$ and $F_{(1,12)} = 25.39$, $p < 0.001$, respectively). PSNL increased the levels of all pro-inflammatory cytokines measured and $(m\text{-}CF_3\text{-}PhSe)_2$ was effective in preventing this increase in the contralateral cortex as well as in the contralateral hippocampus. Regarding the IL-10, PSNL diminished the levels of this anti-inflammatory cytokine in serum and contralateral cortex and the subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ partially prevented this decrease in serum (a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,12)} = 41.37$, $p < 0.001$) and normalized the levels of this cytokine in the contralateral cortex (a PSNL main effect: $F_{(1,11)} = 11.77$, $p < 0.01$). IL-10 levels were not altered in the contralateral hippocampus either by PSNL or by $(m\text{-}CF_3\text{-}PhSe)_2$ treatment.

3.3. $(m\text{-}CF_3\text{-}PhSe)_2$ treatment attenuated the hypothalamic–pituitary–adrenal activation induced by PSNL

Table 5 shows the ACTH and corticosterone levels after acute and subchronic treatments of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$. In the acute treatment, two-way ANOVA demonstrated a main effect of PSNL in ACTH and corticosterone levels ($F_{(1,13)} = 100.32$, $p < 0.001$ and $F_{(1,13)} = 19.16$, $p < 0.001$, respectively). PSNL increased the levels of both ACTH and corticosterone and the acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ did not prevent this increase. By contrast, when PSNL mice were subchronically treated with $(m\text{-}CF_3\text{-}PhSe)_2$ both ACTH and corticosterone levels were greatly reduced, although not to the levels of sham (a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,12)} = 33.58$, $p < 0.001$ and $F_{(1,12)} = 86.85$, $p < 0.001$, respectively).

Table 3
Effect of acute treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (10 mg/kg) in cytokine levels in serum, contralateral cortex and contralateral hippocampus.

		IL-1β	IL-6	TNF-α	INF-γ	IL-10
Serum	Sham	48.6 ± 2.6	55.8 ± 2.1	65.8 ± 3.4	79.8 ± 1.7	127.6 ± 3.0
	CF3	41.6 ± 0.8	51.0 ± 0.6	54.6 ± 2.8	78.6 ± 2.0	129.3 ± 1.4
	PSNL	140.0 ± 6.3***	152.2 ± 7.4***	174.6 ± 9.3***	211.6 ± 26.5***	81.8 ± 3.2***
	CF3 + PSNL	137.0 ± 2.4***	150.7 ± 2.2***	185.5 ± 2.8***	241.0 ± 22.4***	144.2 ± 3.8**###
Contralateral Cortex	Sham	2.5 ± 0.1	3.9 ± 0.1	5.5 ± 0.9	5.8 ± 0.1	7.5 ± 0.2
	CF3	1.8 ± 0.1	3.2 ± 0.1	5.0 ± 0.2	3.9 ± 0.2*	6.8 ± 0.4
	PSNL	7.1 ± 0.5***	9.1 ± 0.2***	14.6 ± 0.7***	11.3 ± 0.4***	5. 1 ± 0.3*
	CF3 + PSNL	3.9 ± 0.2*###	7.2 ± 0.4***###	10.1 ± 0.9**##	8.4 ± 0.7**###	12.0 ± 1.1***###
Contralateral hippocampus	Sham	7.5 ± 0.5	12.3 ± 0.9	14.2 ± 0.7	48.5 ± 0.6	22.6 ± 1.5
	CF3	7.0 ± 0.3	10.8 ± 0.3	12.9 ± 0.6	45.8 ± 1.3	29.1 ± 1.5
	PSNL	23.8 ± 2.8***	29.1 ± 3.0*	51.0 ± 4.3***	61.4 ± 3.6*	17.9 ± 2.0
	CF3 + PSNL	26.3 ± 1.9***	25.2 ± 5.8*	61.7 ± 5.2***	70.3 ± 6.15**	55.3 ± 6.2***###

$(m\text{-}CF_3\text{-}PhSe)_2$ was administered to mice 30 min before the sample collection. Data represent means ± SEM, n = 3–4/group. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ when compared to the sham group. (##) $p < 0.01$ and (###) $p < 0.001$ when compared to the PSNL group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

3.4. $(m\text{-}CF_3\text{-}PhSe)_2$ prevented activation of p38 MAPK and alterations in NF-κB, COX-2 and BDNF levels induced by PSNL

The western-blot data obtained from the acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ are depicted in Fig. 4. Regarding the p-p38/p38 MAPK ratio, two-way ANOVA showed a main effect of PSNL in the contralateral cortex ($F_{(1,15)} = 11.16$, $p < 0.01$) and a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction in the contralateral hippocampus ($F_{(1,14)} = 15.99$, $p < 0.01$). PSNL increased the p-p38/p38 MAPK ratio in the contralateral cortex and hippocampus and $(m\text{-}CF_3\text{-}PhSe)_2$ treatment prevented the activation of p38 MAPK in both regions (Fig. 4A and E). The total p38 and p-p38 MAPK levels were also significantly increased by PSNL in the contralateral cortex and $(m\text{-}CF_3\text{-}PhSe)_2$ treatment prevented this increase (PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,15)} = 12.53$, $p < 0.001$ for p38 and $F_{(1,15)} = 10.25$, $p < 0.01$ for p-p38 MAPK). In the contralateral hippocampus, there were no significant changes in the levels of total p38 MAPK and p-p38 MAPK. Although NF-κB levels were not altered in the contralateral hippocampus, PSNL induced a great increase in this factor in the contralateral cortex and $(m\text{-}CF_3\text{-}PhSe)_2$ normalized its levels (PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,12)} = 5.04$, $p < 0.05$) (Fig. 4B and F). Two-way ANOVA also demonstrated a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction in COX-2 and BDNF levels in the contralateral cortex ($F_{(1,10)} = 11.95$, $p < 0.01$ and $F_{(1,14)} = 7.31$, $p < 0.05$, respectively) and hippocampus ($F_{(1,10)} = 12.09$, $p < 0.01$ and $F_{(1,12)} = 7.19$, $p < 0.01$, respectively). After 4 weeks of PSNL, there were an increase in COX-2 levels and a decrease in BDNF levels in both brain regions and the acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ reduced the levels of COX-2 in the contralateral cortex and hippocampus. $(m\text{-}CF_3\text{-}PhSe)_2$ was also effective in normalizing BDNF levels in both regions (Fig. 4C, D, G and H).

Similar results were found in the western-blot analyses from the subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ (Fig. 5). $(m\text{-}CF_3\text{-}PhSe)_2$ was effective in reducing to the levels of sham group the ratio of p-p38/p38 MAPK increased by PSNL in both contralateral cortex and hippocampus (PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,14)} = 9.72$, $p < 0.01$ and $F_{(1,16)} = 4.76$, $p < 0.05$) (Fig. 5A and E). In the contralateral cortex, both total p38 MAPK and p-p38 MAPK were increased by PSNL and $(m\text{-}CF_3\text{-}PhSe)_2$ prevented this increase (PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,14)} = 9.47$, $p < 0.01$ for p38 and $F_{(1,14)} = 5.23$, $p < 0.05$ for p-p38 MAPK). In the contralateral hippocampus, although the ratio of p-p38/p38 MAPK remained at the control levels in the group PSNL + $(m\text{-}CF_3\text{-}PhSe)_2$, there has been a statistically significant increase in the levels of total p38 and p-p38 MAPK in this group. NF-κB levels in the contralateral hippocampus were not altered by any treatment (Fig. 5F) whereas the subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ prevented the increase in NF-κB levels induced by PSNL in the contralateral cortex (PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,19)} = 13.65$, $p < 0.01$) (Fig. 5B). The increase in COX-2 levels induced by PSNL was only normalized by the subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ in the contralateral cortex (PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,20)} = 8.80$, $p < 0.01$) (Fig. 5C and G). The PSNL-induced a decrease in BDNF levels was prevented by the subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ in both the contralateral cortex (PSNL main effect: $F_{(1,14)} = 6.63$, $p < 0.05$) and hippocampus (PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,15)} = 16.37$, $p < 0.01$) of mice (Fig. 5D and H).

3.5. $(m\text{-}CF_3\text{-}PhSe)_2$ prevented PSNL-induced the increase in the [^{3}H]glutamate release from synaptosomes

The two-way ANOVA of synaptosomal [^{3}H]glutamate release data from acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ of PSNL mice demonstrated a main effect of PSNL in the contralateral cortex ($F_{(1,18)} = 14.06$, $p < 0.01$) and a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction ($F_{(1,11)} = 9.17$,

Table 4
Effect of subchronic treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.1 mg/kg) in cytokine levels in serum, contralateral cortex and contralateral hippocampus.

		IL-1β	IL-6	TNF-α	INF-γ	IL-10
Serum	Sham	50.7 ± 1.9	57.5 ± 1.5	68.7 ± 2.3	78.5 ± 1.5	127.7 ± 3.9
	CF3	44.5 ± 2.3	52.7 ± 1.7	61.0 ± 1.7	69.5 ± 1.0	103.0 ± 3.2**
	PSNL	137.5 ± 7.5***	149.0 ± 8.7***	168.2 ± 8.8***	186.2 ± 10.0***	81.0 ± 4.0***
	CF3 + PSNL	84.5 ± 1.3***###	132.0 ± 2.7***#	152.5 ± 2.9***#	174.7 ± 4.3***	107.5 ± 4.6**##
Contralateral cortex	Sham	2.5 ± 0.2	3.9 ± 0.2	4.6 ± 0.1	5.7 ± 0.1	7.4 ± 0.3
	CF3	3.2 ± 0.2	3.8 ± 0.3	4.3 ± 0.1	5.4 ± 0.1	8.2 ± 0.2
	PSNL	7.3 ± 0.6***	9.0 ± 0.3***	14.7 ± 0.8***	15.8 ± 0.8***	5. 0 ± 0.2*
	CF3 + PSNL	3.5 ± 0.5###	3.8 ± 0.5###	4.4 ± 0.6###	5.2 ± 0.6###	6.9 ± 0.8#
Contralateral hippocampus	Sham	7.5 ± 0.7	12.2 ± 1.2	14.2 ± 0.9	17.6 ± 1.4	22.1 ± 1.9
	CF3	7.3 ± 0.5	11.1 ± 1.0	15.3 ± 1.6	18.3 ± 2.0	21.7 ± 1.2
	PSNL	23.9 ± 3.6***	29.0 ± 3.9***	51.1 ± 5.6***	57.0 ± 7.0***	18.1 ± 2.6
	CF3 + PSNL	11.13 ± 0.9###	13.2 ± 1.2###	14.2 ± 1.2###	19.2 ± 1.6###	24.9 ± 2.0

$(m\text{-}CF_3\text{-}PhSe)_2$ was administered once a day to mice from the 3rd to 4th weeks after PSNL surgical procedure. Data represent means ± SEM, n = 3–4/group. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ when compared to the sham-vehicle group. (#) $p < 0.05$, (##) $p < 0.01$ and (###) $p < 0.001$ when compared to the PSNL-vehicle group (two-way ANOVA followed by the

Table 5
Effect of acute or subchronic treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$ in serum ACTH and corticosterone levels.

	Acute treatment		Subchronic treatment	
	ACTH	Corticosterone	ACTH	Corticosterone
Sham	65.0 ± 1.4	132.6 ± 16.5	65.2 ± 1.9	116.2 ± 2.5
CF3	63.3 ± 2.0	180.3 ± 4.1	60.7 ± 4.1	101.0 ± 5.9
PSNL	149.6 ± 11.4***	325.0 ± 54.9**	139.5 ± 6.8***	270.7 ± 10.7***
CF3 + PSNL	150.2 ± 10.0***	297.7 ± 6.2*	86.7 ± 2.0**###	136.7 ± 2.7*###

Data represent means ± SEM, n = 3–4/group. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ when compared to the sham group. (###) $p < 0.001$ when compared to the PSNL group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

$p < 0.05$) in the contralateral hippocampus. PSNL increased the [^{3}H]glutamate release in both brain regions and the acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ was effective in reducing this release only in the contralateral hippocampus (Fig. 6A and B). By contrast, when PSNL mice were subchronically treated with $(m\text{-}CF_3\text{-}PhSe)_2$, the [^{3}H]glutamate release from synaptosomes was reduced to the levels of sham group in both the contralateral cortex and hippocampus (a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction: $F_{(1,15)} = 5.29$, $p < 0.05$ and $F_{(1,14)} = 6.44$, $p < 0.05$, respectively) (Fig. 7A and B).

3.6. Synaptosomal [^{3}H]5-HT uptake was altered by PSNL and $(m\text{-}CF_3\text{-}PhSe)_2$ treatment

In the acute treatment with $(m\text{-}CF_3\text{-}PhSe)_2$, there was a main effect of $(m\text{-}CF_3\text{-}PhSe)_2$ and PSNL on synaptosomal [^{3}H]5-HT uptake in the contralateral cortex ($F_{(1,18)} = 13.20$, $p < 0.01$ and $F_{(1,18)} = 16.63$, $p < 0.001$, respectively) and in the contralateral hippocampus ($F_{(1,19)} = 9.29$, $p < 0.01$ and $F_{(1,19)} = 32.78$, $p < 0.001$). PSNL increased synaptosomal [^{3}H]5-HT uptake in both brain regions. $(m\text{-}CF_3\text{-}PhSe)_2$ at a dose of 10 mg/kg reduced synaptosomal [^{3}H]5-HT uptake in the contralateral cortex and hippocampus and when administered to PSNL mice, $(m\text{-}CF_3\text{-}PhSe)_2$ prevented the increase in synaptosomal [^{3}H]5-HT uptake in the contralateral cortex, but not in the contralateral hippocampus (Fig. 8A and B). In the subchronic treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$, two-way ANOVA revealed a PSNL × $(m\text{-}CF_3\text{-}PhSe)_2$ interaction in the contralateral cortex ($F_{(1,12)} = 5.15$, $p < 0.05$) and a main effect of PSNL in the contralateral hippocampus ($F_{(1,16)} = 14.96$, $p < 0.001$). The subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ was effective in reducing the increase of synaptosomal [^{3}H]5-HT uptake induced by PSNL in the contralateral cortex, but not in the contralateral hippocampus (Fig. 9A and B).

4. Discussion

The present study demonstrates that the organoselenium compound $(m\text{-}CF_3\text{-}PhSe)_2$ was effective in reducing mechanical allodynia and the depressive-like behavior of mice submitted to PSNL, a pain and depression comorbidity model. This effect could be mainly associated to an anti-inflammatory action of $(m\text{-}CF_3\text{-}PhSe)_2$, which was demonstrated by the reduction in the levels of pro-inflammatory cytokines and NF-κB as well as by the activation of p38 MAPK increased by PSNL and by regulating glutamatergic and serotonergic systems functioning.

The clustering of chronic pain and depression occurs with high prevalence in general population (Arnow et al., 2006) suggesting a common underlying mechanism that may be responsible for such

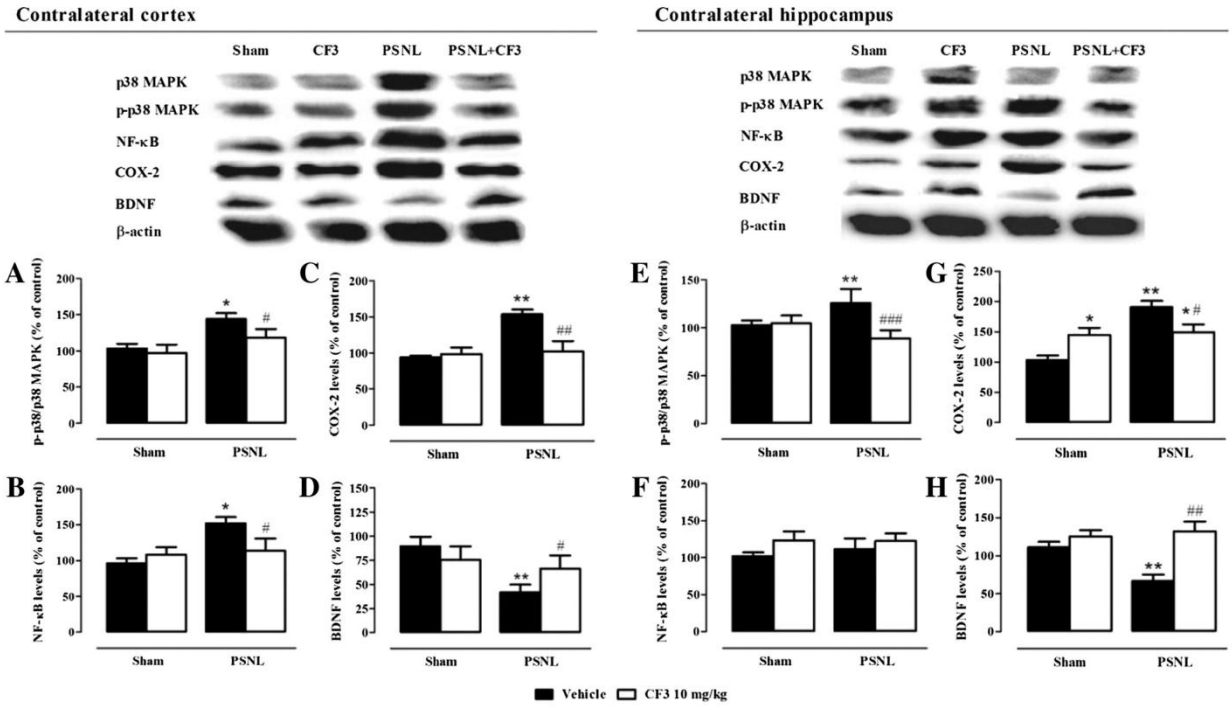

Fig. 4. Effect of acute treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (10 mg/kg) on (A) the p-p38/p38 MAPK ratio and (B) NF-κB, (C) COX-2 and (D) BDNF levels in the contralateral cortex (left panels) and on (E) the p-p38/p38 MAPK ratio and (F) NF-κB, (G) COX-2 and (H) BDNF levels in the contralateral hippocampus (right panels). $(m\text{-}CF_3\text{-}PhSe)_2$ was administered 30 min before the sample collection. The results of p-p38 MAPK and p38 MAPK were shown by % of control of quantification of the phosphorylated ratio: O.D. of the phosphorylated band/O.D. of the total band. The results of NF-κB, COX-2 and BDNF levels were shown by % of control of quantification of bands O.D. Data represent means ± SEM, n = 3–4/group. (*) $p < 0.05$ and (**) $p < 0.01$ when compared to the sham-vehicle group. (#) $p < 0.05$, (##) $p < 0.01$ and (###) $p < 0.001$ when compared to the PSNL-vehicle group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

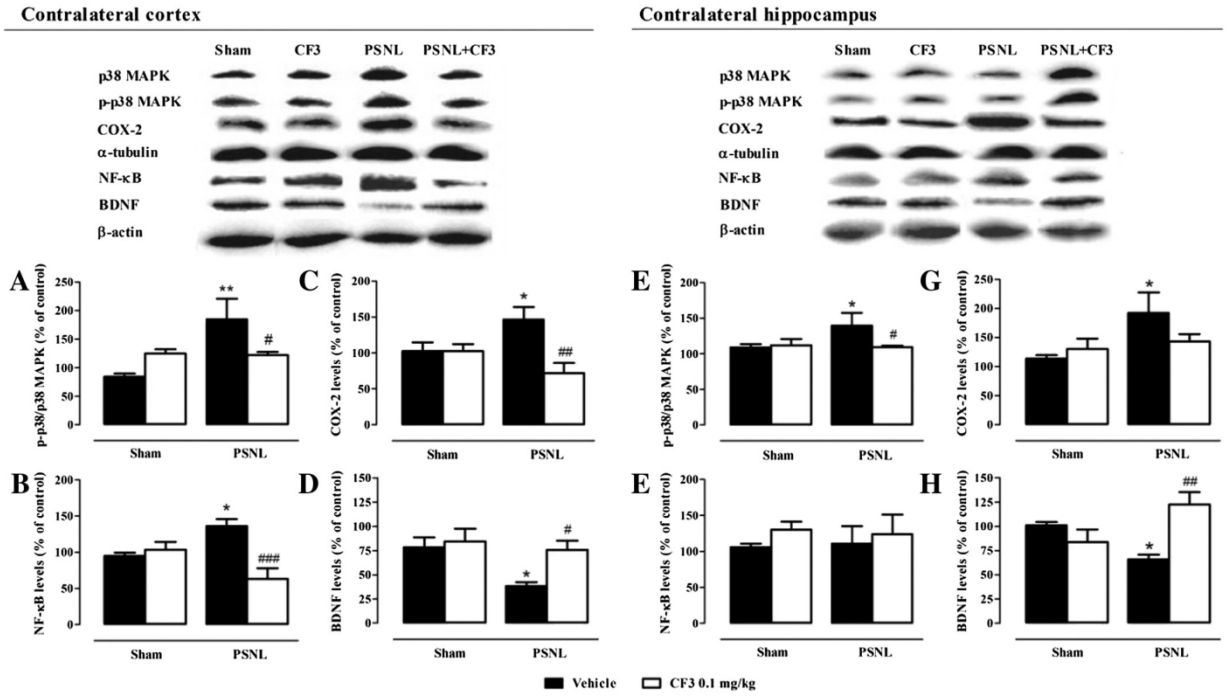

Fig. 5. Effect of subchronic treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.1 mg/kg) on (A) the p-p38/p38 MAPK ratio and (B) NF-κB, (C) COX-2 and (D) BDNF levels in the contralateral cortex (left panels) and on (E) the p-p38/p38 MAPK ratio and (F) NF-κB, (G) COX-2 and (H) BDNF levels in the contralateral hippocampus (right panels). $(m\text{-}CF_3\text{-}PhSe)_2$ was administered once a day during the 3rd and 4th weeks after PSNL surgical procedure. The results of p-p38 MAPK and p38 MAPK were shown by % of control of quantification of the phosphorylated ratio: O.D. of the phosphorylated band/O.D. of the total band. The results of NF-κB, COX-2 and BDNF levels were shown by % of control of quantification of bands O.D. Data represent means ± SEM, n = 4–5/group. (*) $p < 0.05$ and (**) $p < 0.01$ when compared to sham-vehicle group. (#) $p < 0.05$, (##) $p < 0.01$ and (###) $p < 0.001$ when compared to PSNL-vehicle group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

clustering. A recent research field has appointed inflammation as the common denominator to both pain and depression, which could activate some pathways that can trigger the transition from "sickness behavior" and acute pain induced by inflammation to depression and chronic pain (Walker et al., 2014). In fact, increased plasma concentrations of pro-inflammatory cytokines, such as IL-6, have been observed in depressed patients and increased levels of IL-1β and IL-6 have been found in the cerebrospinal fluid of chronic pain patients (Alexander et al., 2005; Sluzewska et al., 1996). Further, in rodents both centrally and peripherally administered recombinant pro-inflammatory cytokines induce a spectrum of symptoms of major depressive disorders (Bluthe et al., 2000; Dantzer, 2004) and peripheral nerve injury could lead to chronic inflammation and comorbid chronic pain and depression (Bravo et al., 2012). The present study accounts to this hypothesis, in view of the fact that PSNL greatly increased the pro-inflammatory cytokines in the serum and centrally in the cerebral contralateral cortex and hippocampus of mice and also reduced the anti-inflammatory cytokine IL-10, which maintains an up-regulated inflamed response. Although $(m\text{-}CF_3\text{-}PhSe)_2$ has been reported to have antidepressant-like and antinociceptive actions in animal models, its effect on chronic pain and depression comorbidity was never evaluated. In this study, we demonstrated that $(m\text{-}CF_3\text{-}PhSe)_2$ in both acute and subchronic treatments at a low dose prevented PSNL-induced depressive-like behavior and mechanical allodynia, one of the typical symptoms of neuropathic pain, characterized by hypersensitivity to innocuous mechanical stimuli. It is important to note that $(m\text{-}CF_3\text{-}PhSe)_2$ was effective to attenuate the increase in the levels of pro-inflammatory cytokines induced by nerve injury and that the best results were found when $(m\text{-}CF_3\text{-}PhSe)_2$ was subchronically administered to mice. $(m\text{-}CF_3\text{-}PhSe)_2$ also increased the levels of IL-10, which could downregulate nociception and sickness (Walker et al., 2014). In this way, the normalization of pro-inflammatory cytokine levels could account to the antiallodynic and antidepressant-like effects of $(m\text{-}CF_3\text{-}PhSe)_2$ after peripheral nerve injury.

Of particular importance, the present findings also demonstrated that PSNL accompanied by mechanical allodynia and depressive-like behavior increased the nuclear factor kappa B (NF-κB) levels in the contralateral cortex of PSNL mice and both acute and subchronic treatments with $(m\text{-}CF_3\text{-}PhSe)_2$ normalized these levels. NF-κB is a critical transcription factor and when activated translocates into the nucleus and leads to transcription of a wide variety of relevant effected genes, including pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, and inducible effector enzymes, such as COX-2. Accordingly, it has been shown that NF-kB activation plays a critical role in the procession of neuropathic pain and targeting of the NF-kB pathway is considered a potential novel approach in treatment of chronic pain mainly because of the stimulus-evoked pro-inflammatory role of NF-kB in immune cells (Niederberger and Geisslinger, 2008). In this way, a decrease of NF-κB activation could contribute to the pharmacological effects of $(m\text{-}CF_3\text{-}PhSe)_2$ after peripheral nerve injury.

Several mechanisms can be responsible for high comorbidity of pain and depression that stem from precipitating inflammation after peripheral nerve injury, which mainly include direct or indirect effects of cytokines on neuronal environment. The activation of HPA axis is one of these effects of cytokines. It has been demonstrated that in conditions of chronic inflammation, pro-inflammatory cytokines can cause glucocorticoid receptor resistance that reduces the ability of glucocorticoids to down-regulate HPA axis by negative feedback and at the level of peripheral and central innate immune cells, the normal inhibitory effect of glucocorticoids on cytokine production and action would no longer be operative, setting the condition for a feed-forward cascade that would result in an over-increasing production of pro-

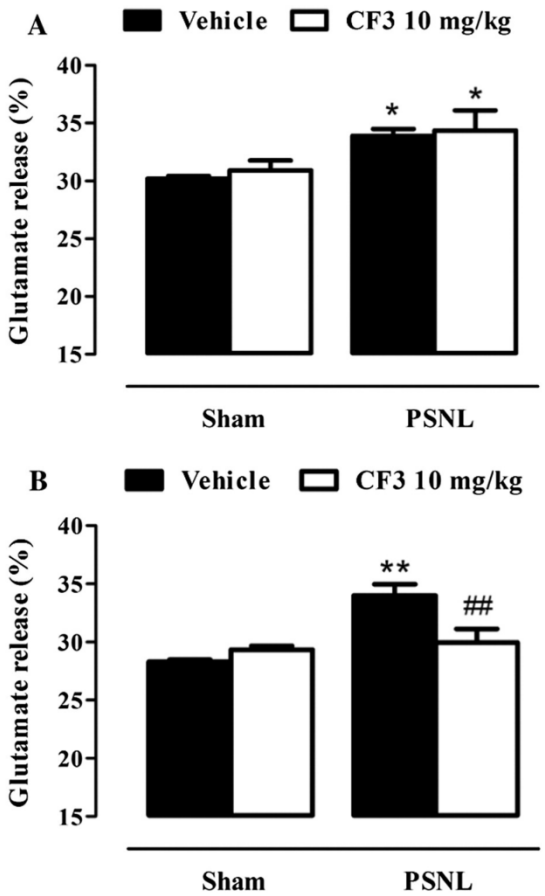

Fig. 6. Effect of acute treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (10 mg/kg) on glutamate release in (A) the contralateral cortex and (B) the contralateral hippocampus. $(m\text{-}CF_3\text{-}PhSe)_2$ was administered to mice 30 min before the sample collection. Data represent means ± SEM, n = 4–5/group. (*) $p < 0.05$ and (**) $p < 0.01$ when compared to the sham-vehicle group. (##) $p < 0.01$ when compared to the PSNL-vehicle group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

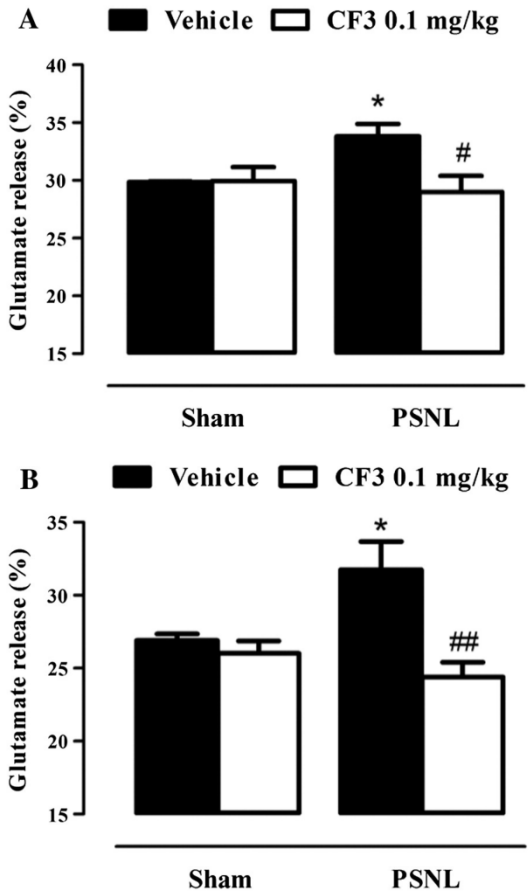

Fig. 7. Effect of subchronic treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.1 mg/kg) on glutamate release in (A) the contralateral cortex and (B) the contralateral hippocampus. $(m\text{-}CF_3\text{-}PhSe)_2$ was administered once a day to mice from the 3rd to 4th week after PSNL surgical procedure. Data represent means ± SEM, n = 4–5/group. (*) $p < 0.05$ when compared to the sham-vehicle group. (#) $p < 0.05$ and (#) $p < 0.01$ when compared to the PSNL-vehicle group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

inflammatory cytokines and stress-related hormones (Irwin and Miller, 2007; Raison and Miller, 2003) that may lead to behavioral alterations including depression-like symptoms (Busquet et al., 2010). The present study found an increase in serum ACTH and corticosterone levels in PSNL mice that could be due to an increase in cytokine levels. $(m\text{-}CF_3\text{-}PhSe)_2$ was effective in reducing the levels of these hormones only in the subchronic treatment, probably because this treatment was more effective in normalizing the levels of pro-inflammatory cytokines. We also found a reduction of brain-derived neurotrophic factor (BDNF) levels in the contralateral cortices and hippocampi of mice after 4 weeks of PSNL that may be an effect of increased levels of cytokines and corticosterone (Koo and Duman, 2008; Liu et al., 2003) and acute and subchronic treatments with $(m\text{-}CF_3\text{-}PhSe)_2$ were effective in restoring the BDNF levels in both regions. The lack of BDNF in the limbic system has been related to pathophysiology of depression and also to neuropathic pain concurrent with mood disorders (Duman and Monteggia, 2006; Fukuhara et al., 2012).

Activation of microglia and more specifically subsequent neuroinflammation have been shown to play a pivotal role in development and maintenance of comorbid neuropathic pain and depression after peripheral nerve injury (Clark et al., 2007; Walker et al., 2014). Phosphorylated p38 (p-p38) MAPK, the active form of p38 MAPK, is highly expressed in hyperactive microglia in the central nervous system (CNS) after peripheral nerve injury and is essential for activation of microglia (Tsuda et al., 2004). In addition, it has been reported that p38 MAPK activation is strongly implicated in neuropathic pain state (Chiang et al., 2013). Possible upstream mechanisms causing p38 MAPK activation in the CNS after peripheral nerve injury could involve direct activation by pro-inflammatory cytokines or increase of glutamate release from primary afferents as a result of an immediate injury discharge or altered ectopic activity (Ji and Suter, 2007). Glutamate acting in N-methyl-D-aspartate (NMDA) receptors of microglia depolarizes cell membrane and thereby opens voltage-gated Ca^{2+} channels causing a large influx of Ca^{2+} ions that could activate p38 MAPK (Farber and Kettenmann, 2006). Once active, microglia releases a wide range of inflammatory mediators, such as IL-6, IL-1β, TNF-α and COX-2, and it self-maintains active given that these mediators can also feedback to microglia via an autocrine fashion contributing to maintenance of neuroinflammation after peripheral nerve injury (Hanisch, 2002). Although we did not evaluate microglia activation in this study, PSNL increased p38 MAPK activation, represented by increased p-p38/p38 MAPK ratio, in the contralateral cortices and hippocampi of mice. Both acute and subchronic treatments with $(m\text{-}CF_3\text{-}PhSe)_2$ significantly reduced the p38 MAPK phosphorylation ratio suggesting that $(m\text{-}CF_3\text{-}PhSe)_2$ could block microglia activation. As a result, $(m\text{-}CF_3\text{-}PhSe)_2$ also

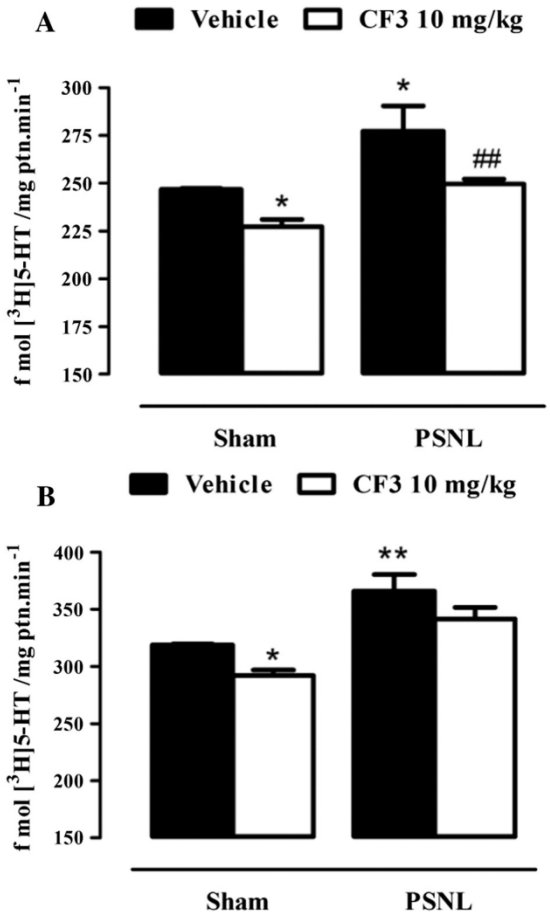

Fig. 8. Effect of acute treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (10 mg/kg) on [^{3}H]5-HT uptake in (A) the contralateral cortex and (B) the contralateral hippocampus. $(m\text{-}CF_3\text{-}PhSe)_2$ was administered to mice 30 min before the sample collection. Data represent means ± SEM, n = 5–6/group. (*) $p < 0.05$ and (**) $p < 0.01$ when compared to the sham-vehicle group. (##) $p < 0.01$ when compared to the PSNL-vehicle group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

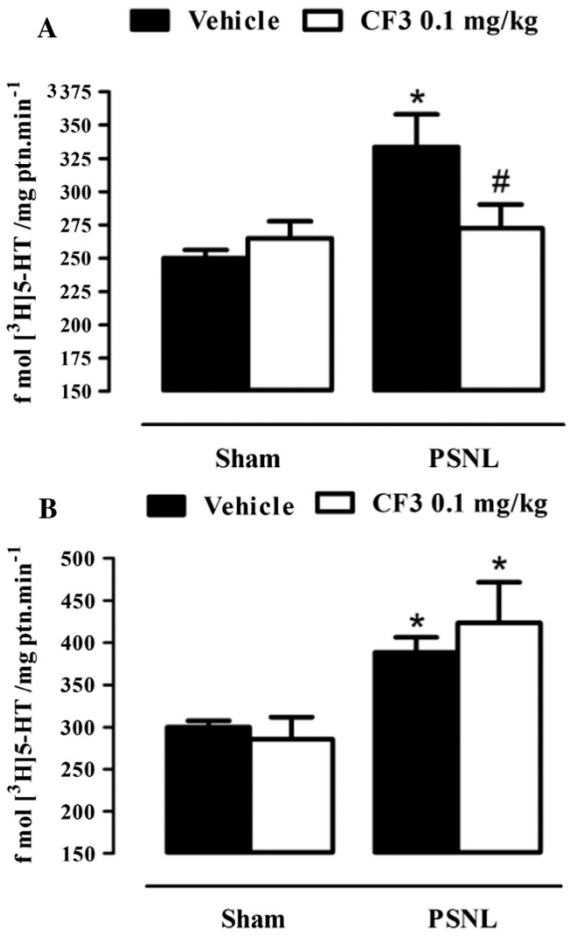

Fig. 9. Effect of subchronic treatment of PSNL mice with $(m\text{-}CF_3\text{-}PhSe)_2$ (0.1 mg/kg) on [^{3}H]5-HT uptake in (A) the contralateral cortex and (B) the contralateral hippocampus. $(m\text{-}CF_3\text{-}PhSe)_2$ was administered once a day to mice from the 3rd to 4th week after PSNL surgical procedure. Data represent means ± SEM, n = 4–5/group. (*) $p < 0.05$ when compared to the sham-vehicle group. (#) $p < 0.05$ when compared to the PSNL-vehicle group (two-way ANOVA followed by the Newman–Keuls test). CF3: $(m\text{-}CF_3\text{-}PhSe)_2$.

normalized COX-2 and pro-inflammatory cytokine levels increased by PSNL in those brain regions. Although PSNL increased the p-p38/p38 MAPK ratio in the contralateral cortex and hippocampus, the phosphorylated form and total p38 MAPK were significantly increased only in the contralateral cortex. Considering that protein levels of p38 MAPK were also increased, further studies should be performed to investigate if there is an increase in the relative amount of p38 MAPK per cell or/and an increase in the number of cells expressing p38 MAPK and also the cell type-specific activation of p38 MAPK.

In line with the glutamate release-induced p38 MAPK activation hypothesis, this study found an increase in the glutamate release from synaptosomes of contralateral cortex and hippocampus of PSNL mice. The subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ reduced significantly PSNL-induced release of this excitatory neurotransmitter in both regions, while acute treatment was effective only in the contralateral hippocampus. In fact, changes in glutamate signaling contribute critically to central sensitization and development of neuropathic pain (Walker et al., 2014). Regarding depression, it has been demonstrated that excessive glutamatergic neurotransmission can lead to depressive symptoms (Mitani et al., 2006). In this way, the decrease of glutamate release could be another target of $(m\text{-}CF_3\text{-}PhSe)_2$ to reduce PSNL-induced mechanical allodynia and depressive-like behavior.

The 5-HT transmitter system in mammalian brain is an essential modulator of homeostatic responses that control emotional behavior and also nociceptive response. Given that, most of antidepressants focus on increase 5-HT availability in the synaptic cleft, mainly through selective serotonin transporter (SERT) inhibition. Although alterations in SERT expression and activity have been associated with mental illness (Murphy et al., 2003), few studies have identified critical upstream signals that modulates SERT function. Of importance, some recent in vitro and in vivo studies have shown that pro-inflammatory cytokines increase SERT activity through activation of p38 MAPK (Malynn et al., 2013; Zhu et al., 2010). It is suggested that in serotonin neurons p38 MAPK acts to directly influence SERT trafficking to the plasma membrane and ultimately to increase the rate of serotonin reuptake (Bruchas et al., 2011). Our findings contribute to this hypothesis, given that PSNL increased the [^{3}H]5-HT uptake in both the contralateral cortices and hippocampi of mice, probably due to the activation of p38 MAPK in these regions, decreasing 5-HT availability. In the acute treatment, $(m\text{-}CF_3\text{-}PhSe)_2$ inhibited the [^{3}H]5-HT uptake in both brain regions, an effect already shown in vitro (Borges et al., 2009) and ex vivo (Brüning et al., 2014), but when administered to PSNL mice $(m\text{-}CF_3\text{-}PhSe)_2$ prevented the increase in the [^{3}H]5-HT uptake only in the contralateral cortex. Similar results were found in the subchronic treatment,

although $(m\text{-}CF_3\text{-}PhSe)_2$ did not inhibit [^{3}H]5-HT uptake. These results suggest that $(m\text{-}CF3\text{-}PhSe)_2$ could increase the 5-HT availability in the synaptic cleft by directly inhibiting SERT activity or reducing the SERT trafficking to membrane, by decreasing the p38 MAPK activation, besides to its inhibitory effect on the MAO-A activity (Brüning et al., 2009). As an overall result, $(m\text{-}CF_3\text{-}PhSe)_2$ treatment prevented the PSNL-induced depressive-like behavior and mechanical allodynia.

According to the fact that antidepressants require long-lasting treatments to achieve a therapeutic effect (Joffe et al., 1996), the results found in the present study indicate that the subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ was more effective than the acute treatment in preventing behavioral and neurochemical alterations induced by PSNL in mice. In this way, studies dealing with possible side effects of the subchronic treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ should be performed, given that other organoselenium-based compounds have shown some toxicity at acute high doses (Nogueira and Rocha, 2011; Nogueira et al., 2004).

Considering the potential common mechanisms of comorbid relationship between inflammation-induced depression and chronic pain, the results found in this study indicate that $(m\text{-}CF_3\text{-}PhSe)_2$ could become an interesting approach to treat long-lasting pathological pain associated with depression, given that this compound was effective in reducing inflammatory mediators and normalizing glutamatergic and serotonergic functioning after peripheral nerve injury. Polypharmacology has been considered a desirable property (Frantz, 2005) and could be the better way of treating complex states, such as chronic pain and depression comorbidity.

Author disclosure

None.

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4 DISCUSSÃO

O (*m*-CF_3-PhSe)$_2$ pertence a uma classe de compostos orgânicos de selênio com diversas propriedades farmacológicas (Nogueira et al., 2004), e apresenta tanto efeito antinociceptivo como antidepressivo demonstrados em estudos anteriores (Brüning et al., 2010; 2011). O seu efeito antinociceptivo foi demonstrado em modelos químicos e térmicos de nocicepção em camundongos, como o teste da injeção de capsaicina na pata, contorções abdominais induzidas por ácido acético, teste da imersão da cauda em água quente e teste da chapa quente. No teste de imersão da cauda em água quente, a pré-administração de antagonistas opióides naloxona (antagonista opióide não seletivo), naloxonazina (antagonista seletivo de receptores μ) e naltrindol (antagonista seletivo de receptores δ) bloqueou o efeito antinociceptivo do (*m*-CF_3-PhSe)$_2$. Em contrapartida, a pré-administração do antagonista seletivo de receptores opióides κ nor-binaltorfimina e do antagonista seletivo de receptores opióides periféricos naloxona metiodide não alterou seu efeito. Esses dados demonstraram o envolvimento dos receptores opióides centrais μ e δ, mas não κ, no efeito antinociceptivo deste composto (Brüning et al., 2010). O efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ foi demonstrado no TNF em camundongos e a pré-administração dos antagonistas de receptores serotoninérgicos WAY100635 (antagonista de receptores 5-HT_{1A}), ritanserina (antagonista de receptores 5-$HT_{2A/2C}$) e ondansetrona (antagonista de receptores 5-HT_3) bloqueou a redução do tempo de imobilidade dos animais induzida pelo (*m*-CF_3-PhSe)$_2$. Interessantemente, a administração de naloxona também bloqueou a redução no tempo de imobilidade dos animais no TNF, demonstrando que além do sistema serotoninérgico, o sistema opióide também está envolvido no efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ (Brüning et al., 2011).

O envolvimento do (*m*-CF_3-PhSe)$_2$ com o sistema serotoninérgico também já foi evidenciado em outros estudos. Borges e cols. (2009) demonstraram que o (*m*-CF_3-PhSe)$_2$ inibe a recaptação de 5-HT em sinaptossomas de ratos *in vitro*. Somado a isso, este composto apresenta efeito ansiolítico associado à interação com receptores serotoninérgicos 5-HT_{1A}, 5-$HT_{2A/2C}$ e 5-HT_3, e inibe a atividade da MAO-A *ex vivo* em camundongos (Brüning et al., 2009). Considerando esses aspectos, o presente estudo procurou melhor avaliar os efeitos do (*m*-CF_3-PhSe)$_2$ no sistema serotoninérgico, investigando se o mesmo também poderia estar envolvido no efeito antinociceptivo deste composto. Também tendo em vista que a inflamação pode ter um importante papel na patofisiologia da depressão (Dantzer et al., 2008) e ser um mecanismo central da díade dor-depressão (Walker et al., 2014) e análogos do (*m*-

CF_3-PhSe)$_2$ demonstram efeito anti-inflamatório (Shin et al., 2009; Rupil et al., 2012), outro importante foco deste trabalho foi avaliar se o (*m*-CF_3-PhSe)$_2$ apresenta efeito no comportamento do tipo depressivo induzido por fatores inflamatórios e na comorbidade entre dor e depressão associada à inflamação.

Os resultados apresentados no **artigo 1** demonstram que o (*m*-CF_3-PhSe)$_2$ também apresenta efeito antinociceptivo no teste da injeção de glutamato na pata e de fato também relacionado ao sistema serotoninérgico, uma vez que antagonistas de receptores 5-HT_{1A} e 5-HT_{2A} bloquearam seu efeito. Além disso, foi evidenciado pela primeira vez que o (*m*-CF_3-PhSe)$_2$ inibe a recaptação de 5-HT *ex vivo*, corroborando com os resultados *in vitro* (Borges et al., 2009). Por outro lado, o resultado do teste de ligação específica *in vitro* demonstrou que o (*m*-CF_3-PhSe)$_2$ desloca a 5-HT apenas em concentrações mais elevadas, e conclui-se que a modulação do sistema serotoninérgico pelo (*m*-CF_3-PhSe)$_2$ deva-se principalmente à inibição da MAO-A e da recaptação de 5-HT, e não à ligação à receptores serotoninérgicos. Esses efeitos somados possivelmente podem aumentar a disponibilidade de 5-HT na fenda sináptica o que poderia contribuir para o efeito antinociceptivo deste composto. Como mencionado anteriormente, a 5-HT é o principal neurotransmissor da via descendente, responsável pela modulação negativa do impulso nociceptivo na medula espinhal (Millan, 2002). Interessantemente, os opióides também contribuem para a facilitação da via descendente e o envolvimento do (*m*-CF_3-PhSe)$_2$ com este sistema, ou por ligar-se diretamente à receptores opióides ou induzir a liberação de opióides endógenos, pode somar-se ao seus efeitos serotoninérgicos na modulação da nocicepção.

Inúmeras evidências têm apontado que o sistema imune pode induzir diversos efeitos no SNC e eventos inflamatórios podem ter um papel importante da patogênese da depressão (Dantzer et al., 2008; 2009; Quan, 2014). Por muito tempo o cérebro foi considerado um órgão imunologicamente privilegiado (Galea et al., 2007), porém sabe-se que citocinas pró-inflamatórias circulantes podem atravessar a barreira hematoencefálica e/ou ativar as células microgliais a produzirem mais citocinas via ativação de nervos aferentes, e dessa forma iniciar um processo de neuroinflamação (Bluthe et al., 1994; Watkins et al., 1994; Vitkovic et al., 2000; Romeo et al., 2001; Banks, 2006). Além disso, recentemente Louveau and cols. (2015) identificaram, pela primeira vez, a presença de um sistema linfático no SNC em camundongos, o que permitiria a comunicação direta entre o sistema imune e o cérebro. Os resultados apresentados no **artigo 2** acerca do efeito do tipo depressivo induzido pela injeção i.c.v. da citocina pró-inflamatória TNF-α em camundongos estão de acordo com trabalhos

anteriores (Kaster et al., 2012; Manosso et al., 2013) e demonstram que o (*m*-CF_3-PhSe)$_2$, em doses baixas, é eficaz em bloquear este efeito no TNF e no TSC, dois testes consolidados para a identificação do comportamento do tipo depressivo. Dados anteriores apontam que o (*m*-CF_3-PhSe)$_2$ apresenta efeito do tipo antidepressivo apenas em doses mais elevadas (Brüning et al., 2011), no entanto quando o comportamento do tipo depressivo foi induzido pelo TNF-α, doses significantemente menores (500× no TNF e 50× no TSC) foram eficazes em bloquear este efeito. Além disso, doses de (*m*-CF_3-PhSe)$_2$ que não foram eficazes no tratamento agudo, quando administradas subcronicamente por 2 semanas foram igualmente efetivas em bloquear o efeito do TNF-α. É importante salientar também que nenhum dos tratamentos alterou a atividade locomotora dos animais observada no monitor de atividades, o que descarta que o comportamento dos camundongos no TNF e no TSC possa ter sido influenciado por alterações na atividade locomotora.

A injeção i.c.v. de TNF-α também induziu um aumento dos níveis de NF-κB e ativação da p38 MAPK no córtex pré-frontal e no hipocampo dos camundongos, duas importantes regiões envolvidas na regulação do humor (Phillips et al., 2003). Essas duas proteínas estão associadas à cascata de sinalização dos receptores TNFR1 e TNFR2 e tanto o NF-κB quando a p38 MAPK estão envolvidos na indução de diversos fatores inflamatórios (Aggarwal, 2003; Kumar et al., 2003; Saklatvala, 2004). Além disso, a p38 MAPK pode ter efeitos diretos no sistema serotoninérgico, aumentando a recaptação de 5-HT e diminuindo a disponibilidade desta na fenda sináptica (Zhu et al., 2005; 2006; Malynn et al., 2013). Notavelmente, o (*m*-CF_3-PhSe)$_2$, em baixas doses, bloqueou o aumento dos níveis de NF-κB e a ativação da p38 MAPK induzidas pelo TNF-α em ambos os tratamentos, agudo e subcrônico, demonstrando que este composto também apresenta efeito anti-inflamatório, o que poderia contribuir para seu efeito antidepressivo, em associação aos seus efeitos no sistema serotoninérgico e opióide.

Tendo em vista as propriedades farmacológicas do (*m*-CF_3-PhSe)$_2$ observadas e o fato da comorbidade entre dor e depressão ser uma condição multipatogênica podendo ter a neuroinflamação como mecanismo central (Walker et al., 2014), procurou-se avaliar se este composto orgânico de selênio poderia ser efetivo no tratamento desta comorbidade. Os resultados apresentados no **artigo 3** demonstraram que a ligação parcial do nervo ciático (LPNC) em camundongos induziu alodínia mecânica, após 4 semanas, observada no teste dos filamentos de Von-frey, e o comportamento do tipo depressivo no TNF, sem alterações da atividade locomotora. O tratamento dos animais com baixas doses de (*m*-CF_3-PhSe)$_2$ preveniu

tanto a alodínia quanto o comportamento do tipo depressivo e a dose que não apresentou efeito no tratamento agudo foi efetiva no tratamento subcrônico. De um modo geral, a LPNC induziu um significativo aumento dos níveis de citocinas pró-inflamatórias e redução da IL-10, tanto no soro como no córtex cerebral e hipocampo contralaterais e o tratamento com (*m*-CF_3-PhSe)$_2$ normalizou ou parcialmente normalizou essas alterações, sendo que os melhores resultados foram observados no tratamento subcrônico. Além disso, a ativação do eixo HPA com o aumento de ACTH e corticosterona no soro dos animais induzida pela LPNC foi prevenida pelo tratamento subcrônico com (*m*-CF_3-PhSe)$_2$. A nível central, a LPNC induziu a ativação da p38 MAPK, aumento dos níveis de NF-κB e COX-2 e diminuição do BDNF, alterações que foram normalizadas pelo tratamento com (*m*-CF_3-PhSe)$_2$. A LPNC também induziu alterações neuroquímicas como o aumento da recaptação de 5-HT e a liberação de glutamato no córtex e hipocampo contralaterais, sendo que os tratamentos agudo e subcrônico com (*m*-CF_3-PhSe)$_2$, de maneira geral, preveniram esses efeitos. Como amplamente discutido anteriormente, todas essas alterações induzidas pela LPNC podem estar envolvidas na indução e manutenção da dor crônica e do estado depressivo e as citocinas pró-inflamatórias podem ser o fator chave no desencadeamento das mesmas. O (*m*-CF_3-PhSe)$_2$ ao prevenir grande parte dessas alterações, demonstrou amplo espectro de ação.

A propriedade multialvo de uma molécula tem sido considerada a melhor alternativa terapêutica de doenças multipatogênicas e complexas, como é caso da depressão, da dor crônica e da díade dor-depressão (Millan, 2014). O principal desafio no desenvolvimento de fármacos multialvo é a integração de dois ou mais farmacóforos em pequenas estruturas com peso molecular idealmente não ultrapassando 500, a fim de conciliar os efeitos farmacológicos com adequadas propriedades farmacocinéticas, como acesso ao SNC e bioestabilidade (Hopkins et al., 2006; Millan, 2006; Zimmermann et al., 2007; Wong et al., 2010). Os resultados do presente estudo e de anteriores, resumidos na figura 6, demonstram que o (*m*-CF_3-PhSe)$_2$ é uma droga multialvo concentrando propriedades antidepressiva e antinociceptiva, provavelmente por interagir com o sistema serotoninérgico (através da inibição da recaptação de 5-HT e da MAO-A), sistema opióide e possuir efeito anti-inflamatório. Além disso, dados de distribuição de selênio apresentados no **artigo 1** demonstram que este composto atravessa a BHE, embora não seja possível afirmar se é a molécula integra ou um metabólito que tem acesso ao SNC. No entanto considerando o efeito do (*m*-CF_3-PhSe)$_2$ na inibição da recaptação de 5-HT tanto *in vitro* como *ex vivo*, é provável que a molécula atinja o SNC sem sofrer biotransformação.

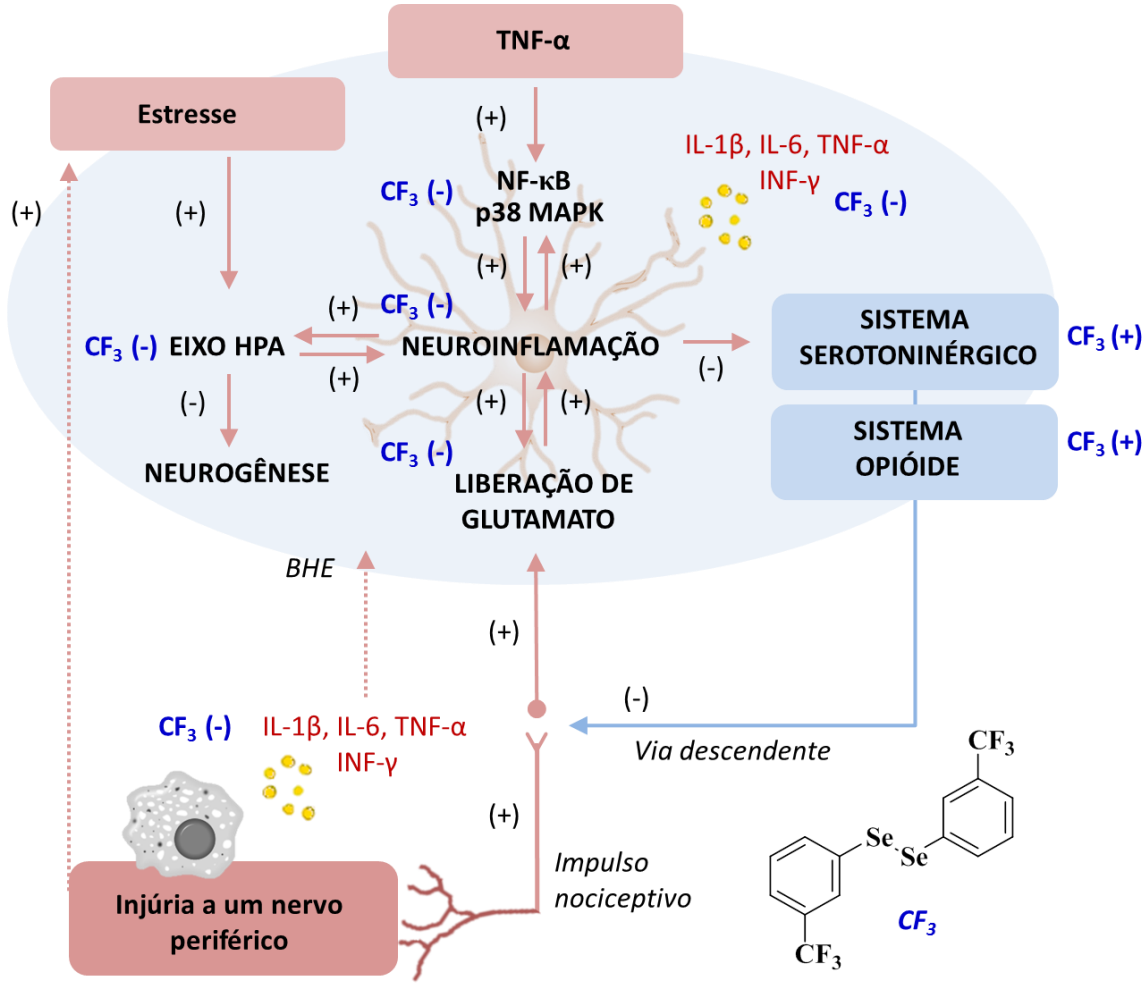

Figura 6. Esquema geral dos mecanismos envolvidos nos efeitos farmacológicos do (*m*-CF_3-PhSe)$_2$. (+) representa modulação positiva enquanto (-) representa modulação negativa. CF3: (*m*-CF_3-PhSe)$_2$.

Diversos estudos têm demonstrado que a ativação da p38 MAPK está de fato envolvida tanto na depressão quanto na dor neuropática (Miller e Raison, 2006; Ji e Suter, 2007; Bruchas et al., 2011; Chiang et al., 2013). Além disso, um estudo recente demonstrou que em neurônios a p38 MAPK pode ser ativada pela cascata de sinalização do receptor opióide κ, o que leva à translocação dos transportadores de 5-HT para a membrana sináptica e consequente aumento da recaptação deste neurotransmissor, e este mecanismo poderia estar envolvido no desenvolvimento de dependência a drogas de abuso, como a cocaína e a morfina (Bruchas et al., 2011). Os receptores opióides κ e seus ligantes endógenos, as dinorfinas, são amplamente distribuídos em regiões cerebrais relacionadas ao humor e a recompensa (Simonin et al., 1995; Yuferov et al., 2004) e sabe-se que ocorre um aumento dos níveis de

mRNA das dinorfinas após a exposição ao estresse ou drogas de abuso (Daunais et al., 1993; Tjon et al., 1997; Wang et al., 1999; Butelman et al., 2012). A administração de agonistas de receptores κ induz efeitos do tipo depressivo (Carlezon et al., 2006) e causa recaída à cocaína (Zhang et al., 2004). Por outro lado, antagonistas de receptores opióides κ bloqueiam o efeito do tipo depressivo, observado após estresse ou retirada de cocaína (Shirayama et al., 2004; Chartoff et al., 2012). Conforme discutido, o (*m*-CF_3-PhSe)$_2$ possivelmente não interage com receptores opióides κ e além disso inibe a ativação da p38 MAPK. Embora os mecanismos do desenvolvimento de dependência possam ser bastante complexos (Butelman et al., 2012), especulativamente o (*m*-CF_3-PhSe)$_2$ poderia não apresentar os efeitos de dependência usualmente demonstrados pelas moléculas opióides.

Em relação às propriedades toxicológicas do (*m*-CF_3-PhSe)$_2$, dados anteriores demonstram que este composto apresenta baixa toxicidade em camundongos em doses agudas, com DL50 (dose letal em 50% dos animais) de 278 mg/kg, sem alteração de parâmetros bioquímicos como a alanina aminotransferase (ALT), a aspartato aminotransferase (AST), a ureia e a creatinina (Savegnago et al., 2009). Considerando os efeitos farmacológicos do (*m*-CF_3-PhSe)$_2$ observados no presente estudo, no qual a dose de 1mg/kg foi efetiva em todos os testes, pode-se considerar que este composto apresenta uma janela terapêutica segura. Além disso, doses menores de 1 mg/kg como 0.1 mg/kg e 0.01 mg/kg que em geral não apresentaram efeito nos tratamentos agudos foram efetivas nos tratamentos subcrônicos, o que poderia reduzir ainda mais a possibilidade de efeitos indesejáveis.

5 CONCLUSÃO

Os resultados apresentados nesse estudo indicam que o (*m*-CF_3-PhSe)$_2$ (i) apresentou efeito antinociceptivo por modular o sistema serotoninérgico, através da inibição da recaptação de 5-HT, (ii) preveniu o comportamento do tipo depressivo em um modelo inflamatório, inibindo a ativação da p38 MAPK e o aumento dos níveis de NF-κB e (iii) bloqueou tanto a alodínia mecânica quanto o comportamento do tipo depressivo induzido pela lesão à um nervo periférico, normalizando os níveis de citocinas pró-inflamatórias, NF-κB, COX-2 e BDNF e inibindo a ativação da p38 MAPK e do eixo HPA, bem como o aumento da liberação de glutamato e da recaptação de 5-HT.

A díade dor-depressão apresenta múltiplos mecanismos patofisiológicos e moléculas multialvo seriam possivelmente a melhor maneira de tratar essa comorbidade. Considerando os efeitos do (*m*-CF_3-PhSe)$_2$ demonstrados no presente estudo bem como suas propriedades já conhecidas, este composto orgânico de selênio poderia ser uma interessante alternativa terapêutica para o tratamento da dor crônica associada à depressão.

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ANEXOS

ANEXO A – Carta de aprovação do projeto de pesquisa pela Comissão de Ética no Uso de Animais (CEUA) da Universidade federal de Santa Maria

UNIVERSIDADE FEDERAL DE SANTA MARIA
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS-UFSM

CARTA DE APROVAÇÃO

A Comissão de Ética no Uso de Animais-UFSM, analisou o protocolo de pesquisa:

Título do Projeto: "Avaliação da ação do tipo antidepressiva e das propriedades farmacocinéticas do composto de (M-trifluormetil) difenila em camundongos."

Numero do Parecer: 042/2012

Pesquisador Responsável: Cristina Wayne Nogueira

Este projeto foi **APROVADO** em seus aspectos éticos e metodológicos. Toda e qualquer alteração do Projeto, assim como os eventos adversos graves, deverão ser comunicados imediatamente a este Comitê.

Os membros da CEUA-UFSM não participaram do processo de avaliação dos projetos onde constam como pesquisadores.

DATA DA REUNIÃO DE APROVAÇÃO:

Santa Maria, 18 de Junho de 2012.

Roselei Fachinetto
Vice-Presidente da Comissão de Ética no Uso de Animais-UFSM

Comissão de Ética no Uso de Animais - UFSM - Av. Roraima, 1000 – Prédio da Reitoria - 2º andar - Campus Universitário 97105-900 – Santa Maria – RS - - Tel: 0 xx 55 3220 9362

ANEXO B – Autorização para reprodução do artigo científico "Serotonergic systems are implicated in antinociceptive effect of *m*-trifluoromethyl diphenyl diselenide in the mouse glutamate test" publicado na *Pharmacology, Biochemistry and Behavior 125 (2014) 15–20.*

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ANEXO C – Autorização para reprodução do artigo científico "Depressive-like behavior induced by tumor necrosis factor-α is attenuated by *m*-trifluoromethyl-diphenyl diselenide in mice" publicado na *Journal of Psychiatric Research 66-67 (2015) 75-83.*

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ANEXO D – Autorização para reprodução do artigo científico "*m*-Trifluoromethyl-diphenyl diselenide, a multi-target selenium compound, prevented mechanical allodynia and depressive-like behavior in a mouse comorbid pain and depression model" publicado na *Progress in Neuro-Psychopharmacology & Biological Psychiatry 63 (2015) 35- 46.*

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