UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:
BIOQUÍMICA
TOXICOLÓGICA

MECANISMOS ENVOLVIDOS NOS EFEITOS DO SELENETO VINÍLICO BIS SUBSTITUÍDO (SVBS) EM MODELOS ANIMAIS DE DEPRESSÃO E DE DOR

Tese de doutorado

Cristiano Ricardo Jesse

Santa Maria, RS, Brasil
2011

MECANISMOS ENVOLVIDOS NOS EFEITOS DO SELENETO VINÍLICO BIS SUBSTITUÍDO (SVBS) EM MODELOS ANIMAIS DE DEPRESSÃO E DE DOR

por

Cristiano Ricardo Jesse

Tese apresentada ao 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 a obtenção do grau de Doutor em Bioquímica Toxicológica.

Orientadora: Prof[a]. Dr[a]. Cristina Wayne Nogueira
Co-orientador: Prof. Dr. Gilson Zeni

Santa Maria, RS, Brasil

Universidade Federal de Santa Maria

Centro de Ciências Naturais e Exatas

Programa de Pós-Graduação em Ciências Biológicas - Bioquímica Toxicológica

A Comissão Examinadora, abaixo assinada, aprova a Tese de doutorado

MECANISMOS ENVOLVIDOS NOS EFEITOS DO SELENETO VINÍLICO BIS SUBSTITUÍDO (SVBS) EM MODELOS ANIMAIS DE DEPRESSÃO E DE DOR

Elaborada por
Cristiano Ricardo Jesse
como requisito parcial para a obtenção do grau de **Doutor em Bioquímica Toxicológica**

COMISSÃO EXAMINADORA:

Profª. Drª. Cristina Wayne Nogueira (Orientadora)

Prof. Dr. Juliano Ferreira (UFSM)

Profª. Drª. Daniela Martí Barros (FURG)

Profª. Drª. Janine Inez Rossato (PUCRS)

Profª. Drª. Stela Maris Kuze Rates (UFRGS)

Santa Maria, março de 2011

“Mas para a grande maioria dos casos, a conclusão é simples: aqueles classificados de ‘gênios’ não têm um talento natural, mas uma paixão obsessiva pelo que fazem. A paixão sozinha não vai garantir o sucesso, mas é o primeiro passo. Sem esse amor incondicional por uma atividade, você jamais será classificado como genial”.

Alysson Muotri

AGRADECIMENTOS

A Deus, por reproduzir em cada um de nós sua manifestação criadora.
A minha família, pelo apoio incondicional, amor, paciência, força e otimismo.
Aos meus orientadores Cristina e Gilson, agradeço pela confiança em mim depositada e pelo exemplo de perseverança incansável ao ensino e a ciência. Cris e GZ, muito obrigado!

Ao professor João Batista por todos os ensinamentos que contribuíram para o meu crescimento pessoal e profissional. Obrigado!

Aos professores do Programa de Pós-Graduação em Bioquímica Toxicológica e Farmacologia, que contribuíram para a minha formação.

Aos colegas do Laboratório do Prof. Gilson, Félix e João pela amizade e companheirismo.

Aos colegas do laboratório da Profª Cristina, por aguentarem algumas brincadeiras "sem graça". Agradeço-os pela amizade, auxílio, compreensão, conhecimento e companheirismo compartilhados diariamente. Agradeço a todos por darem sentido a minha vida acadêmica. Agradeço em especial a Crisinha e a Ethel pela ajuda direta em muitos trabalhos realizados durante o mestrado.

Aos funcionários Angélica, Márcia, Rinaldo, Brondane, Elton, Silvandro e Maneco pela dedicação e competência com que realizam os seus trabalhos.

À Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Bioquímica Toxicológica pela possibilidade de realização deste curso.

A CAPES pela bolsa de estudos e pelos recursos financeiros concedidos.

Enfim, agradeço a todos que de alguma forma contribuíram para a realização deste trabalho.

RESUMO

Tese de doutorado
Programa de Pós-Graduação em Ciências Biológicas - Bioquímica Toxicológica
Universidade Federal de Santa Maria, RS, Brasil

MECANISMOS ENVOLVIDOS NOS EFEITOS DO SELENETO VINÍLICO BIS SUBSTITUÍDO (SVBS) EM MODELOS ANIMAIS DE DEPRESSÃO E DE DOR

AUTOR: Cristiano Ricardo Jesse
ORIENTADORA: Cristina Wayne Nogueira
CO-ORIENTADOR: Gilson Zeni
DATA E LOCAL DA DEFESA: Santa Maria, março de 2011.

A associação entre a depressão e a dor crônica é freqüente e bidirecional. O elevado número de pacientes com depressão e dores crônicas que são refratários aos medicamentos disponíveis atualmente torna importante a busca por novas drogas para o tratamento destas doenças. O seleneto vinílico bis substituído (SVBS) é um composto orgânico de selênio com atividades antioxidante, antinociceptiva e antiinflamatória em camundongos. Este estudo teve como objetivo investigar o efeito do SVBS em modelos de depressão e dor em camundongos, além dos mecanismos farmacológicos envolvidos. A administração oral do SVBS (5 mg/kg, per oral, p.o.) reduziu o tempo de imobilidade no teste da suspensão da cauda (TSC) com um efeito máximo em 1 hora e permaneceu significativo em relação ao controle até 6 horas. O efeito causado pelo SVBS em reduzir o tempo de imobilidade foi observado nas doses de 0,5, 1 e 5 mg/kg no TSC e no teste do nado forçado (TNF) (artigo 1). A atividade do tipo antidepressiva do SVBS foi revertida pelo tratamento prévio com p-clorofenilalanina etil éster (PCPA, inibidor da síntese de serotonina (5-HT)), cetanserina (antagonista dos receptores 5-$HT_{2A/2C}$), ondansetrona (antagonista dos receptores 5-HT_3) (artigo 1), L-arginina (aminoácido precursor de óxido nítrico (NO)), sildenafil (inibidor da fosfodiesterase tipo 5, PDE-V), S-nitroso-N-acetil-penicilamina (SNAP, um doador de NO) (artigo 2), cromacalina (abrem os canais de potássio - K^+), minoxidil (abrem os canais de K^+) e GW 9662 (antagonista dos receptores ativados da proliferação de peroxissomos-gama (PPARγ) (artigo 3). Entretanto, o efeito do tipo antidepressivo do SVBS (1 mg/kg, p.o.) não foi prevenido pelo tratamento prévio com prazosin (antagonista de receptores α_1), ioimbina (antagonista de receptores α_2), propranolol (antagonista de receptores β), SCH 23390 (antagonista de receptores D_1),

sulpirida (antagonista de receptores D_2) e WAY 100635 (antagonista seletivo de receptores 5-HT_{1A}) (artigo 1). Além disso, quando administrado em uma dose sub-ativa, o SVBS (0,1 mg/kg) apresentou uma ação sinérgica com a fluoxetina (antidepressivo inibidor seletivo da recaptação da serotonina), N_G-nitro-L-arginina (inibidor da óxido nítrico sintase (NOS)), 7-nitroindazol (7-NI, inibidor seletivo da NOS neuronal), azul de metileno (inibidor da NOS e da guanilato ciclase (GC)), 1H- 1,2,4]oxadiazolo [4,3-a]quinoxalin-1-ona (ODQ, inibidor seletivo da GC) (artigo 2), tetraetilamônio (TEA, bloqueador dos diferentes tipos de canais de K^+, inclusive os dependentes de voltagem), glibenclamida (bloqueador de canais de K^+ dependente de ATP), caribdotoxina (bloqueador de canais de K^+ de alta condutância ativados por cálcio) e apamina (bloqueador de canais de K^+ de baixa condutância ativados por cálcio) (artigo 3). A administração do SVBS (0,1-5 mg/kg, p.o.) também não modificou a atividade das enzimas monoamina oxidase-A e B (MAO-A e MAO-B) e Na^+K^+ATPase no cérebro dos camundongos. Os níveis de nitrito/nitrato no cérebro foram reduzidos com o tratamento com SVBS (1 mg/kg, p.o.). O tratamento com SVBS (1 e 5 mg/kg) diminui o comportamento do tipo depressivo e a dor nos camundongos submetidos à injúria por constrição crônica (ICC) (artigo 4). Além disso, a administração do composto SVBS (5 e 10 mg/kg, p.o.) demonstrou uma redução na hiperalgesia e no edema induzidos pela administração intraplantar (i.pl.) de Adjuvante Completo de Freund (ACF), carragenina (Cg) e prostaglandina E_2 (PGE_2) (artigo 5). A hiperalgesia mecânica induzida pela administração i.pl. de ACF ou pela avulsão do plexo braquial (ABP) foi atenuada com a administração crônica do SVBS, sem causar tolerância durante o tratamento. A administração concomitante de SVBS (5 e 10 mg/kg, p.o.) com vincristina durante 7 dias atenuou a hiperalgesia mecânica do 3º ao 28º dia de teste. De acordo com o presente estudo pode-se sugerir que os mecanismos responsáveis pela ação do tipo antidepressiva do SVBS envolvem a modulação dos receptores serotonérgicos (5-$HT_{2A/C}$ e 5-HT_3), PPARγ e via do NO/GMPc/K^+. Além disso, o tratamento com SVBS reduziu o comportamento depressivo induzido pela ICC e a hiperalgesia mecânica em modelos inflamatórios (ACF, Cg e PGE_2) e neuropáticos (ABP e vincristina). Desta forma, o presente estudo demonstrou que o tratamento com SVBS apresentou efeitos do tipo antidepressivo e anti-hiperalgésico em diferentes modelos em camundongos.

Palavras-chave: selênio, depressão, nocicepção, hiperalgesia, dor, mecanismos farmacológicos.

ABSTRACT

Thesis of Doctor's Degree
Graduativy Course in Toxicological Biochemistry
Federal University of Santa Maria, RS, Brazil

MECHANISMS INVOLVED IN THE EFFECTS OF BIS SELENIDE IN ANIMAL MODELS OF DEPRESSION AND PAIN

AUTHOR: Cristiano Ricardo Jesse
ADVISOR: Cristina Wayne Nogueira
CO-ADVISOR: Gilson Zeni
DATE AND PLACE OF THE DEFENSE: Santa Maria, March, 2011

The association between depression and chronic pain is frequent and bidirectional. The high number of patients with depression and chronic pain who are refractory to currently available drugs makes it important search for new drugs to treat these disease. The bis selenide is an organic selenium compound with activities such as antioxidant, antinociceptive and anti-inflammatory in mice. Thus, this study investigated the effect of bis selenide in models of depression and pain in mice, besides the pharmacological mechanisms involved. Oral administration of bis selenide (5mg/kg, p.o.) reduced the immobility time in tail suspension test (TST) with a maximal effect at 1 hour and it remained up to 6 hours in relation to control group. The effect caused by bis selenide in reducing the immobility time was observed at doses of 0.5, 1 and 5 mg/kg in the TST and the forced swimming test (FST) (article 1). The antidepressant-like activity of bis selenide was reversed by pretreatment with p-chlorophenylalanine ethyl ester (PCPA, an inhibitor of synthesis of serotonin), ketanserin (5-$HT_{2A/2C}$ receptor antagonist), ondansetron (5 -HT_3 receptor antagonist) (article 1), L-arginine (an amino acid precursor of nitric oxide (NO)), sildenafil (phosphodiesterase inhibitor type 5, PDE-V), S-nitroso-N-acetyl-penicillamine (SNAP, an NO donor) (article 2), cromakalim (potassium channels - K^+- opener), minoxidil (K^+ channels opener) and GW 9662 (antagonist of peroxisome proliferator activated receptors gamma (PPARγ) (article 3). However, the antidepressant-like effect of SVBS (1 mg / kg, po) was not prevented by pretreatment with prazosin (α_1-receptor antagonist), yohimbine (α_2-receptor antagonist), propranolol (β-receptor antagonist), SCH 23390 (D_1 receptor antagonist), sulpiride (D_2 receptor antagonist) and WAY 100635 (selective antagonist of 5-HT_{1A}) (article 1). Furthermore, when administered in a sub-active dose, the bis selenide (0.1 mg / kg, p.o.) showed a synergistic action with fluoxetine (an selective serotonin inhibitor reuptake), N^G-

nitro-L-arginine (an inhibitor of the NO synthase (NOS)), 7-nitroindazole (7-NI, a specific inhibitor of NOS neurornal), methylene blue (inhibitor of NOS and guanylate cyclase (GC)), 1H-1,2,4] oxadiazole [4, 3-a] quinoxaline-1-one (ODQ, a specific inhibitor of GC) (article 2), tetraethylammonium (TEA, a non-specific inhibitor of K(+) channels), glibenclamide (an ATP-sensitive K(+) channel inhibitor) charibdotoxin (a large and intermediate conductance calcium-activated K(+) channel inhibitor) and apamin (a small-conductance calcium-activated K(+) channel inhibitor) (article 3). The administration of bis selenide (0.1 – 5 mg/kg, p.o.) also did not modify the activity of monoamine oxidase –A and B (MAO-A and MAO-B) and Na^+K^+ATPase in mice. The levels of nitrite/nitrate in the brain of mice were reduced by treatment of bis selenide (1 mg/kg). Treatment with bis selenide (1 and 5 mg/kg) decreased the depressive-like behavior and mechanical hypernociception in mice subjected to chronic constriction injury (CCI) (article 4). Moreover, the administration of bis selenide (5 and 10 mg/kg, po) showed a reduction in mechanical hyperalgesia and edema in inflammatory models induced by intraplantar (i.pl.) injection of Complete Freund Adjuvant (CFA), carrageenan (Cg) and prostaglandin E_2 (PGE_2) (article 5). The mechanical hyperalgesia induced by i.pl. injection of CFA or the brachial plexus avulsion (BPA) was attenuated by chronic administration of bis selenide without causing tolerance during treatment. Concomitant administration of bis selenide (5 and 10 mg/kg) with vincristine for 7 days attenuated the mechanical hyperalgesia from 3 to 28 days. According to this study, it may suggest that the mechanisms responsible for the antidepressant-like effect of bis selenide involve the modulation of serotonin (5-$HT_{2A/C}$ and 5-HT_3) and PPARγ receptors and the NO/GMPc/K^+ pathway. Furthermore, treatment with bis selenide reduced the depressive-like behavior induced by CCI and the mechanical hyperalgesia in inflammatory (CFA, Cg and PGE_2) and neuropathic (BPA and vincristine) models. Thus, this study demonstrated that treatment with bis selenide showed antidepressant-like and anti- hyperalgesic effects in different models in mice.

Key words: selenium, depression, nociception, hyperalgesia, pain, pharmacological mechanism

LISTA DE FIGURAS

REVISÃO BIBLIOGRÁFICA

DISCUSSÃO

ARTIGO 1

LISTA DE TABELAS

REVISÃO BIBLIOGRÁFICA

ARTIGO 2

ARTIGO 4

LISTA DE ABREVIATURAS

5-HIAA	Ácido 5-Hidroxi-3-Indol Acético
5-HT	Serotonina
ACF	Adjuvante Completo de Freund
AINEs	antiinflamatórios não esteroidais
AMPA	ácido α-amino-3-hidróxi-5-metil-4-isoxazolopropiônico
AMPc	adenosina monofosfato-cíclico
ANOVA	análise de variância
ATP	adenosina trifosfato
ABP	Avulsão ao plexo braquial
BK	bradicinina
Cg	Carragenina
CGRP	peptídeo relacionado ao gene da calcitonina
COX	ciclooxigenase
DA	dopamina
DAG	diacilglicerol
EROs	espécies reativas de oxigênio
ERNs	espécies reativas de nitrogênio
FAD	flavina-adenina
DI_{50}	Dose inibitória 50%
DSM-IV	diagnóstico da associação norte-americana de Psiquiatria
DALY	Disability Adjusted Life Years
GC	Guanilato ciclase
GMPc	guanosina monofosfato-3'-5'-cíclico
GTP	guanosina trifosfato
HHA	hipotalâmico-hipofisário-adrenal
i.c.v.	intracerebroventricular
ICC	injúria por constrição crônica
IL-1β	interleucina-1 beta
IMAOs	inibidores da monoamina oxidase
iNOS	sintase do óxido nítrico induzida
i.p.	intraperitoneal

IP3	inositol trifosfato
ISRS	inibidores seletivos da recaptação da serotonina
i.t.	intratecal
L-NAME	N^G-nitro-L-arginina metil éster
L-NOARG	N^{ω}-nitro-L-arginina
LTP	Long-term potentiation
MAO	Monoamina oxidase
MHPG	3-metoxi-4-hidroxifenilglicol
NA	Noradrenalina
NADPH	nicotinamida adenina dinucleotídeo fosfato
NGF	fator de crescimento do nervo
NMDA	ácido N-metil-D-aspártico
NMR	núcleo magno da rafe
NO	óxido nítrico
NOSe	óxido nítrico sintase endotelial
NOSi	óxido nítrico sintase induzível
NOSn	óxido nítrico sintase neuronal
NOS	Óxido nítrico sintase
ODQ	1H-[1,2,4]oxadiazolo[4,3- a]quinoxalin-1-one
PAG	substância cinzenta periaquedutal
PCPA	paraclorofenilalanina
PGE_2	prostaglandina E_2
PKA	proteína quinase A
PKC	proteína quinase C
PKG	proteínas quinases dependentes de GMPc
PPARγ	Receptores ativados da proliferação de peroxissomos-gama
p.o	*per* oral
RVM	medula rostroventromedial
s.c.	subcutânea
SNAP	S-nitroso-N-acetil-penicilamina
SNC	sistema nervoso central
SNP	sistema nervosa periférico
SP	substância P

SVBS	seleneto vinílico bis substituído
TCA	Teste do campo aberto
TNF-α	fator de necrose tumoral-alfa
trans-ACPD	ácido (±)-1-aminociclopentano-trans-1,3-dicarboxílico
TEA	tetraetilamônio
TNF	Teste do nado forçado
TSC	Teste da suspensão da cauda
TZD	tiazolidinadionas

SUMÁRIO

APRESENTAÇÃO

Os resultados que fazem parte desta tese estão apresentados sob a forma de artigos, os quais encontram-se no item ARTIGOS CIENTÍFICOS E MANUSCRITO. As seções Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se nos ARTIGOS CIENTÍFICOS e representam a íntegra deste estudo. Os itens, DISCUSSÃO E CONCLUSÕES, encontram-se no final desta tese e apresentam interpretações e comentários gerais sobre os artigos científicos neste trabalho. As REFERÊNCIAS BIBLIOGRÁFICAS referem-se somente às citações que aparecem nos itens INTRODUÇÃO, REVISÃO BIBLIOGRÁFICA e DISCUSSÃO desta tese.

1. INTRODUÇÃO

A prevalência de transtorno depressivo maior em pacientes com dor persistente é de aproximadamente 30 a 50% (World Health Organization, 2010), uma taxa muito maior do que a encontrada na população geral (World Health Organization, 2010) e em pacientes com outras doenças clínicas (Fleck, 2006). Em torno de dois terços dos pacientes com dores persistentes apresentam histórico de transtorno depressivo (World Health Organization, 2010). A literatura descreve que a dor é uma queixa muito comum em pacientes deprimidos (Wörz, 2003) e que mais de 50% destes a relatam como um sintoma (Wörz, 2003). A ocorrência desta comorbidade torna a frequência de suicídio 2 a 3 vezes maior do que na população geral e também aumenta a ideação suicida e as tentativas de suicídio (World Health Organization, 2001).

A depressão é uma das condições neuropsiquiátricas mais comum e altamente incapacitante com uma prevalência de 10-30 % em mulheres e 7-15 % em homens (Nair e Vaidya, 2006; Tierney, 2007). Estima-se que na população geral, a prevalência da depressão na vida de um indivíduo se situa em torno de 15-17 % (Holtzheimer e Nemeroff, 2006) e pacientes que sofrem de depressão severa apresentam altas taxas de morbidade e mortalidade, com conseqüências econômicas e sociais profundas (Nemeroff, 2006). A depressão é a segunda causa de incapacitação no mundo, sendo a primeira a doença cardíaca (Holtzheimer e Nemeroff, 2006).

A depressão resulta, pelo menos em parte, de uma deficiência na atividade monoaminérgica na fenda sináptica (Elhwuegi, 2004). Além desse, vários outros sistemas de neurotransmissores e mecanismos de transdução de sinal estão envolvidos, como os receptores glutamatérgicos e a via da L-arginina-óxido nítrico (NO)/guanilato ciclase cíclica (GMPc)/canais de potássio (K^+) (Kaster et al., 2005; Savegnago et al., 2008). Alguns fármacos disponíveis para o tratamento da depressão foram descobertos a mais de 50 anos, como os antidepressivos tricíclicos e inibidores da enzima monoamina oxidase (Kaster et al., 2005). Estes fármacos ainda encontram-se no mercado farmacêutico, apesar de apresentarem muitos efeitos adversos os quais limitam o seu uso. Além desses, outros medicamentos foram desenvolvidos e estão disponíveis no mercado para a utilização no tratamento da depressão, como os inibidores seletivos de recaptação de serotonina (5-HT) e/ou noradrenalina (NA) e dopamina (DA), que são tão efetivos quanto os tricíclicos, mas são mais seletivos e produzem menos efeitos colaterais (Holtzheimer e Nemeroff, 2006).

A sensação de dor é um mecanismo de alerta do organismo que indica a presença de um estímulo lesivo e que aciona respostas protetoras apropriadas (Julius e Basbaum, 2001). Dessa maneira, o funcionamento adequado do sistema nociceptivo é essencial para proteger o organismo de danos teciduais. Entretanto, sob condições patológicas, este sistema se torna sensibilizado e a dor transforma-se em uma doença (Zeilhofer, 2005).

A dor neuropática foi definida pela Associação Internacional para o Estudo da dor como “dor iniciada ou causada por lesão primária ou disfunção do sistema nervoso” (Zimmermann, 2001). A etiologia da dor neuropática é heterogênea e pode ser ocasionada por um insulto primário ao sistema nervoso periférico ou central (Zimmermann, 2001). As neuropatias originam-se quando ocorre uma lesão nos nervos ou nas demais estruturas que transmitem a sensação dolorosa e podem resultar de trauma mecânico, lesão nervosa (amputação ou compressão), efeitos tóxicos de drogas, doenças como diabetes ou síndrome da imunodeficiência adquirida (HIV/AIDS) (Mendell e Sahenk, 2003). Em uma lesão tecidual, como um trauma mecânico ou invasão por agentes infecciosos, o organismo aciona mecanismos cujo propósito é limitar o dano e regenerá-lo. Esses mecanismos fazem parte da resposta inflamatória, caracterizada por quatro sinais: rubor, calor, dor e tumor; e em alguns casos pode acometer o membro com perda da função (Julius e Basbaum, 2001).

O arsenal farmacológico para o tratamento da dor é composto, basicamente, por dois grandes grupos de medicamentos analgésicas: os opióides, que abolem diretamente a transmissão nociceptiva, no sistema nervoso central, através de sua ligação a receptores opióides (Hoskin e Hanks, 1991), e antiinflamatórios, que previnem a sensibilização de receptores periféricos de dor inibindo a ciclooxigenase (COX) (Cashman, 1996). Embora os opióides sejam muito efetivos, sua utilização é limitada pelo fato dessas drogas apresentarem muitos efeitos indesejados (Hoskin e Hanks, 1991). Os antiinflamatórios convencionais são indicados para dores agudas, porém sua toxicidade renal e gastrointestinal pode resultar em grande morbidade, limitando seu uso para o tratamento da dor (Hoskin e Hanks, 1991).

Os principais instrumentos farmacológicos no tratamento das dores crônicas são os anticonvulsivantes e os antidepressivos. Os anticonvulsivantes estudados em ensaios clínicos randomizados e controlados foram carbamazepina e gabapentina com resultados positivos e fenitoína e lamotrigina com resultados conflitantes (Backonja, 2001). Historicamente, os antidepressivos tricíclicos foram os primeiros utilizados no controle da dor crônica por se considerar, na época, a importância da depressão na dor (Watson et al., 1982). Os antidepressivos tricíclicos (amitriptilina, nortriptilina, clomipramina, imipramina, desipramina) têm se mostrado eficazes em condições neuropáticas, como polineuropatia

dolorosa, neuralgia pós-herpética, neuropatia traumática, dor central pós-ictal (Sindrup, Jensen, 1999). Mais recentemente, novos antidepressivos não – tricíclicos têm surgido e com algumas vantagens por terem menos efeitos colaterais. Os novos antidepressivos com ação incluem os inibidores da recaptação seletiva da 5-HT (paroxetina e citalopram) e os tetracíclicos (miaserina).

Recentemente vem crescendo o interesse e o estudo de compostos orgânicos de selênio (Se), os quais possuem síntese química muito simples e várias atividades farmacológicas (Nogueira et al., 2004; Savegnago et al., 2006, 2007 a,b,c; Nogueira e Rocha, 2010). Em destaque, o seleneto vinílico bis substituído (SVBS), um composto orgânico de selênio, possui atividade antioxidante *in vitro*, antinociceptiva em testes químicos e térmicos em camundongos (Savegnago et al., 2006; Jesse et al., 2007, 2008, 2009). O presente estudo teve como objetivo determinar se o composto SVBS modula a depressão e a hiperalgesia mecânica em camundongos, além dos mecanismos moleculares envolvidos.

2. REVISÃO BIBLIOGRÁFICA

2.1. Depressão

O humor de um indivíduo reflete seu estado emocional. Mudanças de humor ocorrem em resposta a eventos externos e são adaptativas. Porém, quando tais mudanças assumem um caráter inadequado, em termos de intensidade e persitência, alterando de modo constante os estados emocionais e interferindo na rotina do indivíduo, configuram o chamado transtorno de humor (Guimarães et al., 2006).

Conforme o Manual estatístico e diagnóstico da associação norte-americana de Psiquiatria (DSM-IV), que compreende a nomenclatura oficial e oferece critérios diagnósticos específicos para os transtornos mentais, os transtornos de humor constituem uma categoria diagnóstica que abrange sinais e sintomas clinicamente reconhecíveis, persistentes e geralmente com padrão recorrente ou cíclico. Nele, os transtornos de humor estão divididos em transtornos depressivos, transtornos bipolares e outros transtornos de humor. Há duas manifestações básicas dentro dos transtornos de humor: episódios depressivo – com predomínio de sintomas de tristeza e perda de interesse; e episódios de mania – com predomínio de sintomas de excitação e euforia. Com base no tipo de episódio é feita a classificação. A tabela 1 apresenta, resumidamente, esta classificação.

Nesse contexto, o termo “depressão” tem sido utilizado para designar uma síndrome cuja principal queixa é o humor deprimido na maior parte do dia. Porém, as manifestações clínicas dos transtornos depressivos receberam outras denominações ao longo dos tempos. O termo depressão era usado para designar sintomas ou estados mentais de uma doença chamada melancolia – *bile negra* (segundo a classificação hipocrática das doenças, baseada nos humores). Como um dos traços que caracterizava a melancolia era tristeza ou abatimento, surgiu a tradução *para de-premere* (pressionar para baixo), a partir da versão latina das obras de Hipócrates. Embora a descrição remonte aos manuscritos hipocráticos, nosso atual entendimento sobre o transtorno emergiu na segunda metade do século XIX, com o Tratado de Kraepelin sobre os transtornos psiquiátricos que criou um sistema de classificação das desordens psiquiátricas (Licino e Wong, 2001). Na psiquiatria, o termo depressão é usado para designar entidades nosológidas, como o transtorno depressivo maior ou episódios de transtornos de humor (Sonenreich et al., 1995).

Tabela 1- Classificação dos transtornos de humor de acordo com o DSM-IV

Categoria	Substipos principais	Características
Transtornos depressivos	Transtorno depressivo maior Transtorno distímico Transtorno depressivo sem outra especificação	Predomínio de humor depremido e anedonia, mas sem histórico de episódios maníacos, mistos ou hipomaníacos
Transtornos bipolares	Transtorno bipolar I Transtorno bipolar II Transtorno ciclotímico Transtorno bipolar sem outra especificação	Crises depressivas acompanhadas de episódios maníacos, mistos ou hipomaníacos
Outros	Transtorno de humor devido a uma condição médica geral	Perturbação de humor como consequência fisiológica de uma condição médica geral
	Transtorno de humor induzido por substância	Perturbação de humor como consequência fisiológica de uso de droga

Pelo critério diagnóstico atual, os episódios depressivos maiores (Tabela 2) caracterizam o tipo de transtorno depressivo.

Tabela 2 – Critérios diagnósticos do episódio depressivo maior conforme DSM-IV

1. Humor deprimido durante a maior parte do dia
2. Perda marcante do interesse ou prazer em toda ou quase todas as atividades durnate a maior parte do dia
3. Irritabilidade
4. Perda ou ganho de peso (mudança maior que 5% do peso em um mês) ou diminuição ou aumento do apetite
5. Insônia ou sono excessivo
6. Agitação psicomotora ou lentidão dos movimentos
7. Fadiga ou perda de energia
8. Sentimento de falta de valor, culpa excessiva ou inapropriada
9. Redução da capacidade de pensar ou se concentrar, indecisão
10. Sentimento de falta de esperança
11. Pensamentos recorrentes de morte, recorrente ideação suicida sem um plano específico, tentativa de suicídio ou plano específico para se matar

Adaptado do DSM-IV

Para o diagnóstico de episódio depressivo maior, é necessário um período mínimo de duas semanas de humor deprimido ou perda de interesse, acompanhado por, pelo menos, quatro sintomas adicionais de depressão durante o mesmo período, alterando o estado habitual do indivíduo. Um ou mais episódios depressivos maiores, sem histórico de episódios maníacos, mistos ou hipomaníacos, caracterizam o curso clínico da depressão maior. A distimia é caracterizada por, no mínimo, dois anos de humor deprimido acompanhados por sintomas depressivos adicionais, mas sem completar o critério de diagnóstico de depressão maior.

A depressão é uma enfermidade crônica de alto custo socioeconômico, principalmente por sua alta prevalência na população geral, por acarretar incapacidade e prejuízo no funcionamento global do indivíduo, pelo maior risco de desenvolvimento de doenças de alta

mortalidade (cardiovasculares e tumores) e pelas altas taxas de suicídio (Sartorius, 2001; Hyman et al., 2006).

De acordo com os resultados do Projeto "Global Burden of Disease", o transtorno depressivo unipolar é a quarta doença que mais contribui para a maior carga (Burden) global de doenças e a primeira causa de incapacidade (Lopez et al., 2006). A carga total de doenças é mensurada pelo indicador "Disability Adjusted Life Years" (DALY) (Anos de vida perdidos por incapacidade) (Lopez et al., 2006). Enquanto todos os transtornos mentais contribuem para 13% dos DALYs, o transtorno depressivo unipolar corresponde a 4,4% (Lopez et al., 2006). Já entre os indivíduos na faixa etária entre 15 a 44 anos, esse número aumenta para 8,6%.

O custo total da depressão varia de acordo com região geográfica, prevalência da doença, contexto socioeconômico do país, tipo de serviço de saúde, tipo de tratamento disponível, tipo de desenho de estudo, medida de custo utilizada e análise empregada (Konohue e Pincus, 2007). No Reino Unido, por exemplo, o custo total da depressão é maior do que com diabete e hipertensão juntos (Konohue e Pincus, 2007). Entretanto, os custos indiretos representam a maior parcela do impacto econômico atribuído à depressão. O estudo de Greengerg e colaboradores (2003) comparou os custos indiretos em indivíduos com depressão entre os anos de 1990 e 2000, nos Estados Unidos. Constatou-se que esses custos dobraram neste período. Os custos indiretos representam 62% dos custos totais com a depressão, demonstrando que o maior impacto econômico da depressão é causado pela perda da produtividade no trabalho, pelo absenteísmo e pelo desemprego.

2.1.1. Anormalidades neuroquímicas

2.1.1.1. Monoaminas

A hipótese monoaminérgica da depressão tem evoluído nas últimas décadas e contribuído para o entendimento dos eventos celulares mediados por receptores aminérgicos e para a aplicação deste conhecimento no tratamento dos transtornos depressivos (Lanni et al., 2009). O primeiro relato clínico da relação entre alterações das monoaminas cerebrais (5-HT, NA e DA) e depressão foi descrito a partir de um estudo com cinco pacientes hipertensos que desenvolveram depressão após o tratamento com altas doses de reserpina, um inibidor do transporte ativo de NA e 5-HT para seus respectivos sítios de armazenamento (Schildkraut, 1965). As aminas livres são, então, rapidamente inativadas pela monoamina oxidase (MAO),

sofrendo uma acentuada depleção no sistema nervoso central (SNC) e no periférico. Desde então, a comprovação da eficácia antidepressiva de substâncias que induzem uma elevação de monoaminas na fenda sináptica, tais como inibidores da monoamina oxidase (IMAOs), antidepressivos heterocíclicos e inibidores seletivos da recaptação da 5-HT (ISRS) tem representado o principal argumento para validação da hipótese monoaminérgica (Scholoss e Henn, 2004).

As monoaminas interagem com segundos mensageiros a partir da membrana pós-sináptica. Tais receptores, então ativados, causam uma mudança na maneira como o neurônio responde aos neurotransmissores ubíquos (ácido gama-aminobutírico- GABA e glutamato) no cérebro, os quais se ligam a canais dependentes de ligantes (Golan et al., 2009). Substâncias de ação central que causam depleção ou inativação da NA (p. ex, reserpina) produzem sedação ou depressão, enquanto substâncias que potencializam ou aumentam o tônus noradrenérgico cerebral (antidepressivos) estão associados à estimulação e à excitação comportamental (Golan et al., 2009).

2.1.1.2. Serotonina (5-HT)

Neurônios serotonérgicos se projetam do núcleo dorsal da rafe para o córtex cerebral, o hipotálamo, o tálamo, os núcleos da base, o septo pelúcido e o hipocampo (Ramamoorthy et al., 2010). Suas vias têm funções inibitórias e facilitadoras no cérebro. A 5-HT é um regulador importante do sono, do apetite, da temperatura corporal, do metabolismo e da libido, inibe a agressividade e ajuda a regular o ritmo circadiano e em conjunto com a NA e DA, modula o comportamento direcionado. A 5-HT é modulada diferentemente em situações de estresse específicas, com aumento transitório de sua liberação relacionada ao estresse agudo e à redução de atividade eventualmente relacionada ao estresse (Pytliak et al., 2010).

Alterações no sistema serotonérgico, tais como redução dos níveis plasmáticos de triptofano, redução do metabólito ácido 5-hidroxi-3-indol acético (5-HIAA) no líquido cerebroespinhal, redução da recaptação da 5-HT plaquetária e respostas neuroendócrinas em estudos de desafio de diferentes receptores serotonérgicos sugerem uma redução na responsividade serotonérgica e têm sido relatadas em pacientes depressivos, quando comparados com controles saudáveis (Oquendo et al., 2003). Estudos utilizando modernas técnicas de neuroimagem molecular têm também sugerido um comprometimento geneneralizado da função serotonérgica nos transtornos depressivos (Oquendo et al., 2003).

A 5-HT e seus metabólitos encontram-se diminuídos na urina e no líquido cerebroespinhal de indivíduos com depressão. Sua concentração no cérebro de vítimas de suicídio violento encontra-se reduzido em comparação a controles (Mann et al., 1995). Estudos *pos-mortem* examinando pessoas deprimidas que cometeram suicídio constataram alterações em inúmeros marcadores adicionais de 5-HT, incluindo níveis de transmissores e metabólitos regionais, densidade de transportador e receptor pós-sináptico e de transcrição (Bonkale et al., 2004; Sanchez-Bahillo et al., 2008).

Ainda que o uso do precursor serotonérgico triptofano tenha se mostrado ineficaz no tratamento da depressão, a restrição dietética desse aminoácido essencial causa recaída em pacientes remitidos em uso de inibidores seletivos da recaptação da 5-HT ou sem medicação (Delgado et al., 1999).

O sistema serotoninérgico é amplamente reconhecido como especificamente envolvido na etiologia da depressão. Drogas que agem sobre este sistema têm sido largamente utilizadas no tratamento dos distúrbios depressivos (Wong et al., 2005). Uma série de estudos relaciona os receptores serotoninérgicos, especialmente dos sub-tipos 5-$HT_{1A/1B}$, 5-$_{HT2A/C}$ e 5-HT_3 na ação central dos antidepressivos (Redrobe e Bourin, 1997; Hensler, 2002).

2.1.1.3. Noradrenalina (NA)

Os corpos celulares dos neurônios noradrenérgicos cerebrais ficam situados no *locus ceruleus* (LC) do tronco cerebral e se projetam rostralmente para o hipotálamo, os núcleos basais, o sistema límbico e o córtex cerebral (Ramamoorthy et al., 2010). Essa distribuição difusa é consistente com o papel do sistema noradrenérgico de neurotransmissão, ou seja, iniciar e manter a ativação límbica e cortical, assim como a modulação de outros sistemas.

Estudos de animais utilizando o radiotraçador nisoxetina, um ligante do transportador noradrenérgico, têm demonstrado que a administração crônica de desipramina reduz o seu potencial de ligação em diferentes regiões cerebrais, incluindo o hipocampo (Bauer e Tejani-Butt, 1992). As concentrações do metabólito da NA 3-metoxi-4-hidroxifenilglicol (MHPG) foram medidas na urina, no plasma e no líquido cerebroespinhal de sujeitos com depressão, porém os resultados têm sido contraditórios, com estudos relatando aumento, redução ou ausência de alterações nos níves desse metabólito (MHPG) (Muscettola et al., 1984). O agonista do receptor α_2, clonidina, têm sido utilizado para investigar alterações da densidade e na sensibilidade de adrenoceptores α_2 na depressão, mas os resultados também foram inconsistentes (Georgotas et al., 1987). No entanto, uma modificação na expressão de

adrenoceptores β (auto-receptores) tem sido consistentemente descrita na depressão, e uma *downregulation* desses receptores foi relatada com um marcador de eficácia antidepressiva (Leonard, 1997).

Reduções no potencial de ligação do radiotraçador nisoxetina também foram descritas no LC de pacientes deprimidos em estudo *post-mortem* (Klimek et al., 1997). Estudos *post-mortem* também apontaram um aumento na densidade e na afinidade de adrenoceptores α_2 no córtex frontal de vítimas de suicídio (Meana et al., 1992). Os auto-receptores adrenérgicos estariam hipersensíveis nos transtornos depressivos e teriam a sensibilidade reduzida pela administração crônica de desipramina, um inibidor potente da recaptação da NA (Charney et al., 1981).

Estudos têm demonstrado também alterações na transmissão noradrenérgica na depressão (Wong e Licinio, 2001). Várias evidências mostram a participação dos α-adrenoceptores e β-adrenoceptores na ação de fármacos antidepressivos. O bloqueio de α_1-adrenoceptores está associado a estados depressivos, enquanto que o tratamento crônico com antidepressivos causa um aumento na densidade e na atividade de α_1-adrenoceptores no córtex frontal e no hipocampo. O tratamento crônico com antidepressivos também causa uma *downregulation* dos autoreceptores α_2-adrenérgicos e dos β_1- adrenoceptores (Millan, 2004).

2.1.1.4. Dopamina (DA)

Está bem estabelecido o relato de uma elevada prevalência de depressão em pacientes com doenças cerebrais que têm a perda dopaminérgica como mecanismo fisiopatológico central (p. ex., doença de Parkinson e coréia de Huntington) (Huot e Parent, 2007. Ainda que um mecanismo dopaminérgico primário para depressão seja considerado improvável, o papel da DA em alguns aspectos do transtorno depressivo é corroborado por diversas observações experimentais. As propriedades de elevação do humor e a utilidade clínica do metilfenidato, um inibidor do transportador dopaminérgico, no tratamento de alguns pacientes deprimidos estão documentados de forma consistente. Embora a estimulação dopaminérgica isolada geralmente não alivie todos os sintomas depressivos (Volkow et al., 2002).

Foi observada uma *upregulation* dos transportadores dopaminérgicos no estriado de pacientes com depressão maior (Laasonen-Balk et al., 1999), o que pode ser o fator primário que determina uma menor disponibilidade de DA na fenda sináptica (Dailly et al., 2004). O tratamento com antidepressivos aumenta a transmissão dopaminérgica preferencialmente no sistema mesolímbico, deste modo induzindo uma melhora nos sintomas da depressão, tal

como a anedonia (Dailly et al., 2004). Portanto o efeito de antidepressivos sobre a transmissão dopaminérgica parece ter um papel importante no seu efeito terapêutico.

2.1.1.5. Monoamina oxidase (MAO)

A MAO é uma flavoproteína localizada na membrana mitocondrial externa de diversas células, como neurônios e células da glia. Ela é uma enzima que utiliza o dinucleotídeo de flavina-adenina (FAD) como cofator e catalisa a desaminação oxidativa de diversas aminas, incluindo a 5-HT, a histamina, a tiramina e as catecolaminas DA e NA (Shih et al., 1999).

Duas isoformas da MAO (MAO-A e MAO-B) são encontradas na maioria dos tecidos de mamíferos, as quais foram originalmente distinguidas devido à sua sensibilidade aos inibidores irreversíveis clorgilina e selegilina (L-deprenil) e à sua especificidade por diferentes substratos (Magyar e Knoll, 1977). A MAO-A desamina preferencialmente a 5-HT e é inibida seletivamente por baixas concentrações de clorgilina, enquanto que a MAO-B é inibida seletivamente por baixas concentrações de selegilina e metaboliza a benzilamina. As aminas DA, NA, adrenalina, triptamina e tiramina são oxidadas por ambas as isoformas da enzima na maioria das espécies (Youdim et al., 2006).

Devido à sua função no metabolismo dos neurotransmissores 5-HT, DA e NA, a MAO exerce um papel importante na patofisiologia de diversas desordens neurológicas e psiquiátricas. Logo, a diminuição da neurotransmissão monoaminérgica relacionada à depressão, faz dos inibidores da MAO-A agentes terapêuticos potenciais para serem utilizados no tratamento deste distúrbio afetivo (Berton e Nestler, 2006). Além disso, Meyer e colaboradores (2006) demonstraram que pacientes com depressão apresentaram uma densidade aumentada de MAO-A em diversas regiões do cérebro quando comparados a indivíduos saudáveis. Os autores sugerem que esse aumento na quantidade de MAO-A poderia ser a causa dos baixos níveis de monoaminas encontrados em pacientes deprimidos. De fato, os inibidores da MAO (IMAOs) foram os primeiros fármacos antidepressivos descritos e continuam sendo utilizados até hoje com grande sucesso (Berton e Nestler, 2006). Apesar da primeira geração de IMAOs irreversíveis e não-seletivos (por exemplo, fenelzina e tranilcipromina) apresentar atividade antidepressiva, seu uso acarretava uma série de efeitos colaterais, como as crises hipertensivas agudas quando ingeridos juntamente com alimentos ricos em tiramina. Essas limitações impulsionaram o desenvolvimento dos IMAOs de segunda geração, os quais ainda apresentavam um perfil de atividade irreversível, porém

seletivo para MAO-A (clorgilina) ou MAO-B (selegilina). Contudo, as indesejáveis crises hipertensivas continuavam a limitar o uso dos inibidores seletivos da MAO-A.

2.1.1.6. Estresse Oxidativo na Depressão

As espécies reativas de oxigênio (EROs) e as de nitrogênio (ERNs) são um importante fator de dano em muitos processos patológicos e toxicológicos, incluindo os distúrbios psiquiátricos. Vários estudos mostram o envolvimento do estresse oxidativo na patogênese da depressão. Maes et al. (2000), bem como outros pesquisadores (Tsuboi et al., 2006; Sarandol et al., 2007) mostraram a co-existência de estresse oxidativo aumentado e sintomas de depressão em pacientes. Esse resultado foi evidenciado pela diminuição das defesas antioxidantes e pelo aumento da peroxidação lipídica no plasma destes pacientes. Dessa forma, a utilização de antioxidantes pode representar uma alternativa para o tratamento da depressão.

2.1.1.7. Na^+ K^+ ATPase

Alguns estudos mostram que a atividade da Na^+ K^+ ATPase no cérebro está associada à depressão (El-Mallakh e Wyatt, 1995; Gamaro et al., 2003). A Na^+ K^+ ATPase é a enzima responsável pelo transporte ativo dos íons Na^+ e K^+, mantendo assim o gradiente iônico necessário para a excitabilidade neuronal. Está presente em altas concentrações nas membranas das células cerebrais, consumindo 40-50% da adenina trifosfato (ATP) produzido nesse tecido (Erecinska e Silver, 1994). Dessa forma, alterações na atividade dessa enzima podem causar um distúrbio na função neuronal. Além disso, a 5-HT que está envolvida na fisiopatologia das desordens afetivas (Van Praag et al., 1990), é removida da fenda sináptica pelos transportadores localizados nos neurônios pré-sinápticos por captação dependente de Na^+ (Lesch et al., 1993) o que está diretamente relacionado com a atividade da Na^+ K^+ ATPase (Lesch et al., 1993).

2.1.1.8. Via da L-arginina-óxido nítrico(NO) / guanosina monofosfato-3’-5’-cíclico (GMPc)

O conceito de NO como molécula de sinalização celular começou a surgir na década de 70 em trabalhos que procuravam pelo transmissor gerado em resposta à ativação de receptores do ácido N-metil-D-aspártico (NMDA) e capaz de aumentar os níveis de GMPc (Ferrendelli et al., 1974). Desde então, seu papel tem sido relacionado à regulação de vários processos comportamentais, cognitivos e emocionais, incluindo memória e aprendizado, agressão, ansiedade e depressão (Da Silva et al., 2000). O NO é produzido durante a conversão do aminoácido L-arginina em citrulina pela enzima óxido nítrico sintase (NOS). Três isoformas da NOS foram caracterizadas, e incluem uma isoforma expressa em células endoteliais (NOSe), uma isoforma induzida, usualmente expressa após ativação do sistema imunológico, especialmente nas células microgliais (NOSi), e a isoforma neuronal (NOSn) (Paakkari e Lindsberg, 1995). A NOSn é a forma mais abundante, presente tanto no sistema nervoso periférico (SNP) quanto no SNC, especialmente nas espinhas dendríticas (Dreyer et al. 2004). Ela é ativada pelo complexo Ca^{2+}/calmodulina em resposta ao influxo de Ca^{2+} induzido principalmente pela estimulação dos receptores NMDA. Sua associação física a sub-unidade NR2B dos receptores NMDA e a proteína da densidade pós-sináptica PSD-95 (Brenman et al., 1996), ajuda a explicar a intima ligação entre ativação de receptores NMDA e produção de NO. As ações fisiológicas do NO são mediadas principalmente pela estimulação da enzima Guanilato Ciclase (GC), responsável pela produção de GMPc. O aumento nos níveis de GMPc modula a atividade de vários alvos intracelulares como proteínas quinases dependentes de GMPc (PKG) e canais iônicos (Garthwaite e Boulton, 1995).

Vários estudos pré-clínicos demonstram que inibidores da NOS como o L-NAME e 7-nitrindazol (7-NI), e inibidores da GC, como o 1H-[1,2,4]oxadiazolo[4,3- a]quinoxalin-1-one (ODQ), tem propriedades antidepressivas em modelos animais (Da Silva et al., 2000; Volke et al., 2003). Além disso, o estresse crônico moderado causa um aumento na expressão da NOSn no hipocampo, associado a um perfil depressivo e diminuição da neurogênese em camundongos. Já a depleção genética desta enzima ou o
tratamento com inibidores específicos restauram a neurogênese e o perfil comportamental dos animais submetidos ao estresse (Zhou et al., 2007).

2.1.1.9. Canais de K^+

Os canais de K^+ são membros de uma família de proteínas que permite a rápida difusão de íons K^+ pela membrana plasmática, a favor de um gradiente de concentração. Eles desempenham um papel fundamental no controle da excitabilidade neural e propagação de sinal pelo sistema nervoso. A abertura destes canais leva a uma hiperpolarização da membrana plasmática, resultando em uma redução na excitabilidade da célula (MacKinnon, 2003). A diversidade entre os membros desta família está relacionada principalmente com o tipo de estímulo capaz de causar a abertura do canal e difusão dos íons K^+, processo que é regulado por uma variedade de estímulos como: alterações na voltagem da membrana plasmática, níveis intracelulares de certos íons e moléculas orgânicas e proteínas (Ca^{2+}, ATP, AMPc, sub-unidades de proteinas G). Baseado nestas diferenças estruturais os canais de K^+ foram divididos em quatro subtipos: dependentes de voltagem, ativados por cálcio, canais de K^+ retificadores de influxo e canais de K^+ de dois poros. A manipulação farmacológica destes canais pode representar uma ferramenta no controle de uma série de doencas do SNC, como a epilepsia, demencia, ansiedade e depressão (Wickenden, 2002).

O envolvimento dos canais de K^+ na modulação da depressão tem sido sugerido por alguns estudos pré-clínicos. A administração de inibidores de diferentes tipos de canais de K^+ como tetraetilamonio (TEA), apamina, caribdotoxina, gliquidona e glibenclamida produz um efeito antidepressivo no TNF (Galeotti et al., 1999), enquanto
ativadores destes canais, como o cromacalim e o minoxidil, promovem um efeito depressogênico, aumentando o tempo de imobilidade dos animais no mesmo teste (Galeotti et al., 1999). Inan e colaboradores (2004) demonstraram que a administração de sertralina, um inibidor seletivo da recaptação de 5-HT, potencializa o efeito antidepressivo do TEA e da 3,4-diaminopiridina, dois bloqueadores de canais de K^+ no TNF. Além disso, a administração de gliburida, um inibidor de canais de K^+ modulados por ATP ou quinina, um bloqueador de canais de K^+ ativados por Ca^{2+}, produz um efeito sinérgico com antidepressivos no TNF (Guo et al., 1995).

2.1.1.10. Receptores ativados da proliferação de peroxissomos-gama (PPARγ)

Estruturalmente podem ser incluídos como membros da subfamília de receptores que incluem o receptor do hormônio da tireóide, receptor do ácido retinóico e o receptor da vitamina D3. Três proteínas, codificadas por genes distintos, têm sido identificadas: PPARα, PPARβ e PPARγ. Os receptores PPARs são fatores de transcrição ligantes dependentes que regulam a expressão do gene-alvo pela ligação a elementos responsivos aos proliferadores de peroxissoma (PPREs) situados em sítios regulatórios de cada gene. O receptor liga-se ao PPRE como um heterodímero, juntamente com um fator protéico adicional, o receptor do ácido 9-*cis* retinóico. Sob atuação de agonistas, a conformação do receptor PPAR é alterada e estabilizada, criando um sítio de ligação, com posterior recrutamento de coativadores transcricionais, resultando em aumento na transcrição gênica. Os PPAR são conhecidos como fatores de transcrição que regulam a expressão de genes envolvidos em importantes processos fisiológicos como o metabolismo lipídico, homeostase da glicose e atividades inflamatórias (Desvergne andWahli, 1999). Com isso, o desenvolvimento de ligantes que atuem de forma agonista nesses receptores aponta como um promissor alvo de estudos para o tratamento de desordens como o Diabetes mellitus, dispilidemia, arteriosclerose e doença do SNC (Collino et al., 2006). Os receptores PPARγ estão localizados no hipocampo, estriado e cortex pré-frontal (Lee et al., 2010). Desta forma, estes receptores podem contribuir na modulação do humor já que estão localizados em áreas do SNC envolvidas com depressão. Atualmente, no mercado farmacêutico, o uso das tiazolidinadionas (TZD) - ligantes sintéticos do PPARγ, é bem difundido na prática clínica. Esta classe de fármacos promove sensibilidade à insulina e melhora no Diabetes mellitus tipo 2, embora apresente limitações terapêuticas e efeitos indesejáveis. Além disso, os PPARγ estão envolvidos no mecanismo de ação de antidepressivos, pois os efeitos antidepressivos da rosiglitazona, NP031115 e ARA041418 no teste do nado forçado (TNF) em camundongos foram revertidos com o antagonista de PPARγ GW-9662 (Rosa et al., 2008).

2.1.2. Modelos preditivos de depressão em animais

Os modelos animais preditivos são ferramentas amplamente utilizadas para compreensão dos mecanismos responsáveis pela etiologia e tratamento da doença. Estes modelos sofrem com algumas restrições inerentes ao fato de não reproduzir fidedignamente algumas características dos transtornos humanos, como o sentimento de culpa, pensamento de

morte e suicídio. No entanto, são responsáveis, em grande parte, pelo desenvolvimento das hipóteses que relacionam as possíveis bases biológicas dos transtornos mentais e pelo que se sabe atualmente sobre as ações dos psicofármacos em diversas etapas dos processos de neurotransmissão.

Embora seja impossível recriar todos os aspectos de uma determinada doença, especialmente aquelas que envolvem condições complexas e multifatoriais como as doenças psiquiátricas, estes modelos mimetizam um ou alguns dos sintomas associados à doença ou são sensíveis aos fármacos utilizados clinicamente. Desta maneira, é possível estabelecer um paralelo entre os efeitos comportamentais induzidos pelos fármacos com os sinais clínicos ou neurofisiológicos em humanos, visando contribuir para a elucidação das bases etiológicas das várias doenças mentais.

Modelos com validade preditiva estão entre os mais utilizados para a seleção de novos fármacos antidepressivos, são de fácil uso e de boa reprodutibilidade (Cryan et al., 2002). Entre eles, podemos destacar o teste do nado forçado (TNF) e o teste da suspensão da cauda (TSC) (Nestler et al., 2002a,b). Estes modelos são assim designados por serem baseados exclusivamente no efeito comportamental dos fármacos utilizados clinicamente, contudo não mimetizam sintomas ou bases neurobiológicas da doença.

No entanto, outros modelos animais para depressão apresentam validade fenomenológica além da validade preditiva. Isto é, além de serem sensíveis aos fármacos utilizados clinicamente, mimetizam em animais sintomas ou efeitos neurobiológicos associados à doença. Entre eles, pode-se destacar a bulbectomia olfatória, o modelo do desamparo aprendido, a separação materna, o isolamento social e o estresse crônico (McArthur e Borsini, 2006).

O TNF é um dos modelos comportamentais mais utilizados para detectar atividade antidepressiva de fármacos. O método original foi descrito por Porsolt (1977) e baseia-se na observação de que quando os animais são submetidos a uma situação onde não há possibilidade de escape, após um período de agitação inicial eles adotam uma postura de imobilidade. O camundongo é considerado imóvel quando flutua ou faz apenas movimentos necessários para manter sua cabeça acima da água. O tempo de imobilidade foi cronometrado durante 6 minutos em um cilindro plástico de 10 cm de diâmetro e 24 cm de altura contendo 19 cm de altura de água, a temperatura de 25 °C±1°C. A redução no tempo de imobilidade é o efeito observado após a administração aguda de várias classes de fármacos antidepressivos (Porsolt et al., 1977), já o aumento deste tempo caracterizara um estado “depressivo” dos animais ou um efeito depressogênico de fármacos.

O TSC foi descrito por Steru et al., (1985). Os camundongos acústica e visualmente isolados foram suspensos 50 cm acima do chão por fita adesiva e a imobilidade foi registrada durante 6 minutos Os antidepressivos reduzem o tempo de imobilidade neste teste (Steru et al., 1985; Cryan et al., 2005).

O teste do campo aberto (TCA) foi proposto por Hall (1936) para a avaliação da atividade locomotora dos animais. O aparato consiste em uma caixa de madeira medindo 40x30x40 cm, com o chão dividido em 9 quadrantes iguais. O número de quadrantes cruzados em um período de 6 minutos é o parâmetro utilizado para avaliar a atividade locomotora. O teste é feito em uma sala acusticamente isolada e com baixa luminosidade. Como fármacos que apresentam um efeito psicoestimulante podem representar um resultado "falso positivo" no TNF e no TSC, o TCA é imprescindível para se determinar a especificidade do efeito antidepressivo.

2.2. Dor e nocicepção

Dor é uma experiência complexa, difícil de ser definida, descrita ou interpretada. Segundo o Comitê de Taxonomia da Associação Internacional para o Estudo da Dor, pode ser conceituada como uma experiência sensorial e emocional desagradável associada a um dano tecidual atual ou potencial (Merskey e Bogduk, 1994). A dor é extensivamente influenciada por ansiedade, depressão, expectativa e outras variáveis psicológicas. É uma experiência multifacetada, um entrelaçamento das características físicas dos estímulos com as funções motivacionais, afetivas e cognitivas do indivíduo.

A dor desempenha um papel de alerta, comunicando ao indivíduo que algo está errado, podendo gerar estresse acentuado e incapacidade física. É, sem sombra de dúvida, a maior causa de afastamento do trabalho, gerando um enorme ônus para diferentes nações. As sensações dolorosas induzem respostas urgentes para seu alívio provocando nos animais comportamentos como massagear ou lamber a área lesada. A dor manifesta-se com intensidade diferente entre os indivíduos, variando de acordo com o sexo, idade e estado de humor (Chapman e Gavrin, 1999; Sharp, 2001).

A dor, além de uma sensação, é uma experiência. Isso é importante porque as sensações possuem vias neuroanatômicas, com receptores específicos que permitem a detecção de estímulos. Já as experiências incorporam componentes sensoriais com influências pessoais e ambientais importantes. No entanto, clínica e experimentalmente se faz necessária a distinção entre a dor percebida e a resposta ao dano tecidual ou nocicepção (Kandel e Mack,

2003). Assim, o termo nocicepção refere-se somente à percepção do sinal no sistema nervoso central, evocado pela ativação de receptores sensoriais especializados (nociceptores), provenientes de um tecido danificado (Fürst, 1999). Os animais não são capazes de verbalizar os componentes subjetivos da dor, ou seja, não é possível avaliar a dor, mas nocicepção. Sendo assim, termos como dor e analgesia são mais adotados para humanos assim como nocicepção e antinocicepção para animais (Jones, 1992). Evidências sugerem que a hiperexcitabilidade e mudanças em descargas dos aferentes nociceptivos primários levam a alodínia, hiperalgesia ou dor espontânea devido ao aumento da atividade de uma variedade de canais iônicos (Millan, 1999). Sendo que a hiperalgesia é a resposta aumentada a um estímulo doloroso, enquanto a alodínia é a dor resultante de estímulo que é normalmente não nocivo (Millan, 1999). Contudo, em metodologias como o de estímulo no método de Von Frey, detecta-se comportamento nociceptivo antes da administração de um estímulo inflamatório.

2.2.1. Classificação da dor

A dor pode ser considerada como um sintoma ou manifestação de uma doença ou afecção orgânica, mas também pode vir a constituir um quadro clínico mais complexo. Existem muitas maneiras de se classificar a dor. Considerando a duração da sua manifestação, ela pode se apresentar nas formas transitória, aguda ou crônica. Na dor transitória, a ativação dos nociceptores acontece na ausência de qualquer dano tecidual e contribui para proteger o organismo de potenciais danos físicos, causados pelo ambiente ou por estresse de tecidos corporais. Contudo, a dor aguda é uma resposta causada por uma lesão de tecido com conseqüente ativação dos nociceptores, no local da lesão, caracterizando-se por ser de curta duração, desaparecendo, até mesmo, antes da cura do dano tecidual. Além disso, a dor aguda é geralmente de fácil identificação e tratamento, possuindo também caráter protetor (Loeser e Melsack, 1999). Já a dor crônica é causada por uma lesão tecidual ou doença e geralmente ultrapassa o tempo de recuperação do organismo, ou seja, esse tipo de dor pode não desaparecer mesmo quando o trauma inicial (lesão) foi resolvido. A dor crônica se estende por meses ou anos. É de difícil identificação, sendo uma doença que geralmente necessita de tratamento complexo (Tracey e Mantyh, 2007). Não é apenas a duração que distingue a dor aguda da dor crônica, mas também a capacidade do organismo de reparar o sítio da lesão e restaurar os disparos aferentes e o processamento central normal (Loeser e Melsack, 1999). Pode-se destacar ainda alterações adaptativas como a neuroplasticidade, em vários níveis do sistema nervoso, tais como sensibilização, desinibição dos neurônios inibitórios do corno

dorsal, reorganização do circuito neuronal do corno dorsal e alterações na facilitação e inibição descendente da dor. Tendo em vista que estes eventos são dependentes da intensidade e da duração do estímulo, quanto mais persistente for o processo doloroso, mais difícil se torna o tratamento do quadro patológico (Woolf e Salter, 2000). Quanto a sua origem, existem quatro tipos principais de dor: 1) a "dor nociceptiva", que se origina devido à estimulação excessiva dos nociceptores localizados na pele, vísceras e outros órgãos; 2) a "dor neurogênica", que reflete o dano de tecido neuronal, na periferia ou no sistema nervoso central ("dor central"); 3) a "dor neuropática", que acontece devido a uma disfunção ou dano de um nervo ou grupo de nervos e 4) a "dor psicogênica", que não é oriunda de uma fonte somática identificável e que pode refletir fatores psicológicos (Millan, 1999).

2.2.2. Mecanismos neurais da dor

A transmissão da dor envolve uma interação complexa de estruturas periféricas e centrais desde a pele, vísceras e outros tecidos até o córtex cerebral. Um nervo periférico consiste em axônios de três diferentes tipos de neurônios: sensoriais primários, motores e pós-ganglionares simpáticos. As terminações livres de fibras aferentes primárias sensíveis a estímulos nocivos são chamados de nociceptores (ou receptores da dor) (Millan, 1999). A sensibilização dos nociceptores se deve a diferentes estímulos, tais como mudança de temperatura (estímulo nocivo térmico), diferença osmótica ou distensão tecidual (estímulo nocivo mecânico), hipóxia ou lesão tecidual seguida de inflamação (estímulo nocivo químico) (Julius e Basbaum, 2001). Esses estímulos nocivos ativam fibras aferentes sensoriais delgadas do tipo C e Aδ. Além dessas, outro tipo de fibra que pode estar envolvida na transmissão do estímulo sensorial são as fibras do tipo Aβ, que normalmente respondem a estímulos inócuos, aplicados à pele, que em condições especiais são capazes de conduzir rapidamente o estímulo doloroso (30-100 m/s). As fibras Aβ são mielinizadas e de grande diâmetro. Já as fibras do tipo Aδ, de condução intermediária (12 a 30 m/s), são pouco mielinizadas, enquanto que as fibras do tipo C são não-mielinizadas e transmitem o estímulo nociceptivo de forma mais lenta (0,5 a 2,0m/s) (Figura 1) (Julius e Basbaum, 2001).

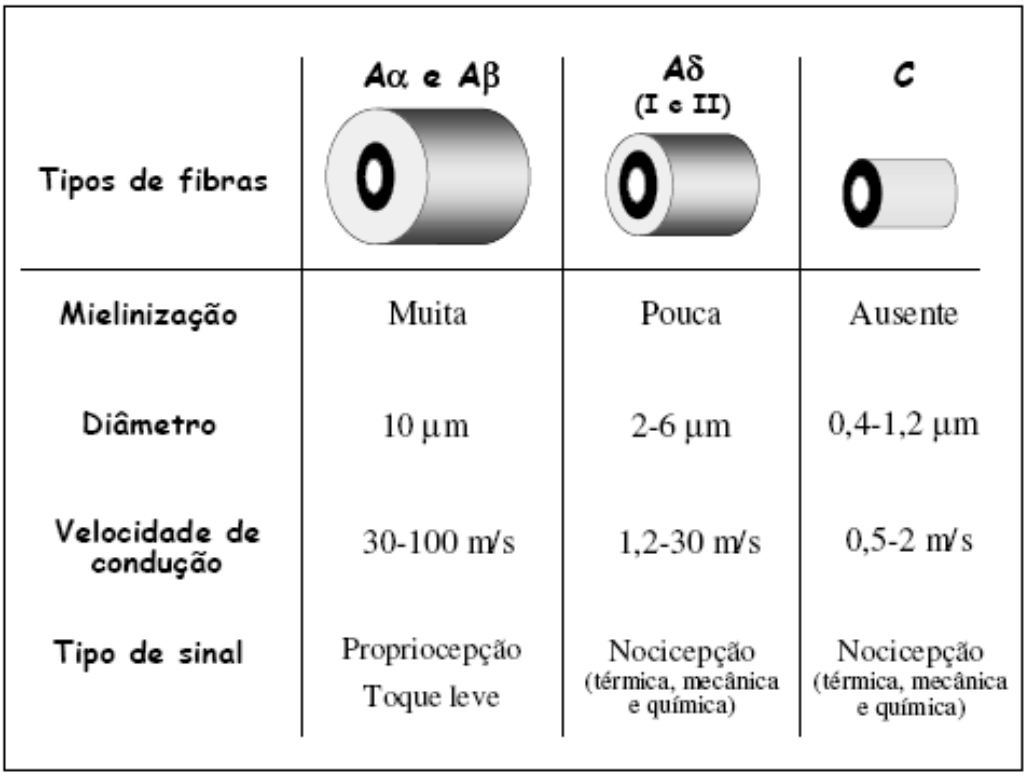


Figura 1: Representação esquemática dos diferentes tipos de neurônio sensoriais primários responsáveis pela condução do sinal nociceptivo da periferia ao SNC. Adaptado a partir de Julius e Basbaum, 2001.

Em grande parte do organismo as fibras aferentes primárias C e Aδ transmitem a informação nociceptiva da periferia até o corno dorsal da medula espinhal e suas terminações encontram-se principalmente nas lâminas I (zona marginal) e lâmina II (substância gelatinosa). No corno dorsal da medula espinhal neurônios de primeira ordem fazem sinapse com neurônios de segunda ordem, que formam as vias ascendentes. Conseqüentemente, esses neurônios recebem seus sinais sensoriais pela liberação de glutamato e substância P (SP) dos neurônios aferentes primários. Este processo excitatório envolve canais de Ca^{2+}, sendo os principais reguladores da liberação de neurotransmissores (Millan, 1999).

Os neurônios de segunda ordem cruzam a medula espinhal para ascender ao trato espinotalâmico, projetando seus corpos celulares ao tálamo. No tálamo, neurônios de terceira ordem emitem axônios, através da cápsula interna do córtex somatosensoria, ocorrendo a somatização do estímulo nocivo e/ou emitindo axônios ao giro cingulado anterior, responsável pelo componente emocional da dor (Russo e Brose, 1998).

O tálamo e o córtex são regiões finais da projeção das vias de nocicepção, sendo que o tálamo é um dos responsáveis por informar que existe sensação nociceptiva. Já o córtex é

responsável pela discriminação do tipo de sensação nociceptiva e por identificar, de forma pouco fiel, de onde provém (Millan, 1999). Além dessa modulação ascendente, existe uma modulação descendente da nocicepção. As vias descendentes originam-se no tronco cerebral e outras estruturas como hipotálamo, córtex, tálamo, núcleo magno da rafe (NMR), substância cinzenta periaquedutal (PAG) e estruturas adjacentes da medula rostroventromedial (RVM), que exercem importante papel na integração e modulação das mensagens nociceptivas, no corno dorsal da medula espinhal (Millan, 2002). Os mecanismos descendentes modulam a resposta nociceptiva por exercer suas ações, em nociceptores presentes nas fibras aferentes primárias, bem como em neurônios intrínsecos do corno dorsal, como interneurônios excitatórios, interneurônios inibitórios e neurônios de projeção (Millan, 2002). Uma das descobertas mais interessantes a respeito do circuito modulatório da dor é que esse pode tanto facilitar, quanto inibir a transmissão nociceptiva (Julius e Basbaum, 2001). Por exemplo, na RVM estão presentes dois tipos de neurônios, as chamadas células "liga" (on) e as células "desliga "(off), as quais estão envolvidas na modulação nociceptiva. É proposto que as células "liga" (on) medeiam a facilitação da condução de estímulos nociceptivos quando ativadas, e as células "desliga" (off) medeiam a inibição da transmissão nociceptiva, provocada pela estimulação da substância cinzenta periaquedutal (PAG).

De maneira geral, a substância cinzenta periaquedutal deve excitar as células off e inibir as células on na medula rostroventromedial (Millan, 2002). Logo, o balanço entre a ativação dessas duas subpopulações de neurônios determina a resposta a um estímulo nociceptivo periférico. No entanto, em situações de dor persistente, as alterações da neuroplasticidade podem resultar em uma estimulação facilitatória sustentada, o que ocasiona respostas persistentes e exageradas à dor (Millan, 2002).

Além da modulação descendente da informação nociceptiva envolver uma série de estruturas cerebrais, conforme mencionado anteriormente, os sistemas de neurotransmissores também estão envolvidos nesta conexão. Todos os neurotransmissores, envolvidos na inibição descendente (tais como opióides endógenos, 5-HT, NA e DA), parecem inibir a excitação de neurônios de segunda ordem na presença de estímulo nocivo (Millan, 2002).

2.2.3. Mediadores químicos na via nociceptiva

A atividade dos nociceptores é mediada pela ação de substâncias algogênicas liberadas e/ou sintetizadas em elevada concentração, no ambiente tecidual, na decorrência de processos inflamatórios (Cotran et al., 1994). Substâncias endógenas, como prostaglandinas, neuropeptídeos, cininas, aminoácidos excitatórios, entre outros, são produzidas e/ou liberadas pelo tecido lesionado e estimulam os receptores presentes na membrana dos neurônios. Além disso, os mediadores inflamatórios liberados facilitam a neurotransmissão e sensibilizam o nociceptor (Björkman, 1995). Existem várias fontes importantes de mediadores químicos que participam da resposta dolorosa, entre os quais se destacam os tecidos lesionados e adjacentes, sistema vascular, células do sistema imunológico, nervos simpáticos e sensoriais, entre outros. Dessa forma, há liberação local de diversos mediadores químicos celulares, resultantes de lesão tecidual capazes de desencadear uma reação inflamatória local, atraindo macrófagos e linfócitos. Essas células liberam mediadores inflamatórios como: cininas (bradicina e calidina); produtos das células imunes (citocinas, como as interleucinas e o fator de necrose tumoral-TNFα); aminas (5-HT e histamina) e prostanóides. Desse modo, a lesão celular e a reação inflamatória advindas de tal lesão, expõem as fibras aferentes primárias a um grande número de substâncias capazes de estimular o nociceptor. O aferente primário transmite o impulso nociceptivo a neurônios específicos para a nocicepção ou a neurônios que também transmitem outras sensações no corno dorsal da medula (Millan, 1999; Calixto et al., 2001).

A transferência sináptica de informação nociceptiva é comandada pela liberação de neurotransmissores como glutamato, presente em todos os tipos de aferentes primários. O glutamato é um aminoácido excitatório, podendo ser encontrado em concentrações na faixa de μM na medula espinhal, originado de fibras aferentes primárias mielinizadas e não-mielinizadas, em adição a interneurônios intrínsecos e projeção de neurônios (Battaglia e Rustioni, 1988). O glutamato e alguns neuropeptídeos são liberados juntos de terminais aferentes primários e têm ações fisiológicas distintas nos neurônios pós-sinápticos, atuando coordenadamente para regular as propriedades desses neurônios (Kandel et al., 2003).

As ações de aminoácidos excitatórios são mediadas principalmente por receptores ionotrópicos NMDA e não-NMDA. Os receptores não-NMDA consistem de dois receptores, ácido α-amino-3-hidróxi-5-metil-4-isoxazolopropiônico (AMPA) e cainato (Dickenson, 1995). A transmissão de fibras C, depois de estímulos agudos mecânicos ou térmicos, parece envolver receptores AMPA para produzir excitações curtas e constantes. Os estímulos são mantidos e/ou sua freqüência é aumentada pela liberação de transmissores que contribuem

para a transmissão nociceptiva quando o receptor NMDA é ativado, aumentando resposta de hiperalgesia (Dickenson, 1995). Uma ação excitatória direta, do tipo retroalimentação positiva, do glutamato, nas fibras aferentes primárias, seria consistente com a evidência de que a ativação dos receptores NMDA causa a liberação de SP e peptídeo relacionado ao gene da calcitonina (CGRP) por seus terminais centrais (Liu et al., 1994). Essa ação do NMDA pode ser mediada pelo NO dos terminais das fibras aferentes primárias (Sorkin, 1993), e o NO poderia também interferir nas ações periféricas mediadas pelas fibras aferentes primárias (Jackson et al., 1995).

2.2.4. Dor inflamatória

Numa lesão tecidual, como um trauma mecânico ou invasão por agentes infecciosos, o organismo aciona mecanismos cujo propósito é limitar o dano e regenerá-lo. Esses mecanismos fazem parte da resposta inflamatória, caracterizada por quatro sinais: rubor, calor, dor e tumor; e em alguns casos pode acometer o membro com perda da função. A inflamação pode ser classificada como aguda ou crônica, de acordo com o tempo de duração e características patológicas (Sherwood e Toliverkinsky, 2004). A inflamação aguda apresenta duração (horas a dias) e muitos mediadores como o NO e prostaglandinas promovem principalmente a vasodilatação, um dos sinais clássicos do processo inflamatório (Sherwood e Toliver-Kinsky, 2004). Outro sinal precoce da inflamação aguda é a formação do edema, que ocorre devido ao fluxo transvascular de fluido, rico em proteínas (plasma) dos compartimentos intravasculares para o interstício devido ao aumento de permeabilidade vascular de capilares e vênulas, como resultado da liberação de histamina, bradicinina, leucotrienos, fatores do complemento, SP e fator de agregação plaquetária (PAF) no sítio inflamatório (Sherwood e Toliver-Kinsky, 2004). A progressão da resposta tecidual para inflamação crônica caracteriza-se por infiltração de células mononucleares, que incluem macrófagos, linfócitos e plasmócitos. Os processos inflamatórios crônicos são prolongados, estendendo-se de semanas a meses. Durante a inflamação crônica, ocorrem simultaneamente: a presença de processo inflamatório ativo, tentativas de reparo tecidual com conseqüente destruição do tecido e formação de fibrose (Sherwood e Toliver-Kinsky, 2004).

Tanto a liberação de mediadores primários, quanto à síntese de novos mediadores são responsáveis pela ativação e/ou sensibilização de nociceptores adjacentes à lesão. A sensibilização dos nociceptores diminui o limiar de ativação e aumenta a probabilidade de que esses disparem com estímulos de menor intensidade (Khasar et al., 1999; Holden e Pizzi,

2003). Dessa forma, esses nociceptores são ativados por estímulos, que em condições normais, seriam inócuos (Millan, 1999). Isso é bem evidenciado, através de modelos experimentais, cuja injeção de um agente pró-inflamatório como o Adjuvante Completo de Freund (ACF) sensibiliza os locais injetados, tornando esses animais responsivos a estímulos térmicos e mecânicos inócuos a um animal não injetado. De fato, a dor é uma característica peculiar da inflamação e a dor inflamatória é o maior problema clínico em vários distúrbios inflamatórios, como, por exemplo, a artrite reumatóide (MacMahon et al., 2005). Portanto, nessa situação, encontra-se um quadro de estimulação constante dos nociceptores, que é responsável por alterações plásticas, não somente no tecido nervoso periférico, mas também em nível central isso é muito comum em casos de neuropatia periférica, cuja lesão nervosa gera um processo inflamatório crônico, com alterações plásticas no SNP e SNC (Ji e Strichartz, 2004). De fato, do ponto de vista clínico, um dos aspectos mais problemáticos da dor de origem inflamatória é a possibilidade da progressão de um estado agudo para um estado prolongado, podendo, dessa forma, aumentar a susceptibilidade de instalação de um quadro de dor inflamatória crônica (Woolf e Mannion, 1999). Nessas condições, a inflamação perde sua característica de proteção ao organismo e torna-se uma patologia. A grande preocupação na busca dos mecanismos que envolvem a inflamação é devido ao seu envolvimento em doenças crônicas, incluindo câncer, diabetes, doença neurodegenerativas, cardiovasculares e reumáticas (MacMhon et al., 2005).

2.2.5. Dor neuropática

A dor neuropática foi definida em 1994 pela Associação Internacional para o Estudo da Dor como “dor iniciada ou causada por lesão primária ou disfunção do sistema nervoso”. A etiologia da dor neuropática é heterogênea e pode ser ocasionada por um insulto primário ao sistema nervoso periférico ou central (Zimmermann, 2001). As neuropatias originam-se quando ocorre uma lesão nos nervos ou nas demais estruturas que transmitem a sensação dolorosa e podem resultar de trauma mecânico, lesão nervosa (amputação ou compressão), efeitos tóxicos de drogas (anti-neoplásicos), doenças como diabetes ou síndrome da imunodeficiência adquirida (HIV/AIDS) (Mendell e Sahenk, 2003). A lesão de nervos periféricos é frequentemente acompanhada de inflamação local transitória, contribuindo para o início da sensação de dor. Nesse sentido, assim como na dor inflamatória, na dor neuropática também estão envolvidos múltiplos mediadores inflamatórios (Ji e Strichartz, 2004).

Corroborando com isso, estudos mostram que a extensão da hiperalgesia está diretamente relacionada com a extensão da resposta inflamatória ao sítio da lesão do nervo (Clatworthy et al., 1995). Os mecanismos exatos da instalação do quadro de dor neuropática ainda não são inteiramente compreendidos. No entanto, MacFarlane e colaboradores (1997) sugerem que o desenvolvimento de dor crônica após lesão de nervo ocorra através de alterações, na medula espinhal, como excitabilidade aumentada, inibição diminuída, restruturação organizacional das células e, eventualmente, mudança no fenótipo. Essas alterações ocorrem principalmente devido a uma estimulação excessiva dos nociceptores, uma vez que estes estão com limiar de ativação mais baixo (hipersensibilidade) (Coutaux et al., 2005). A excitabilidade aumentada ocorre em função de estimulação repetitiva sobre as fibras não mielinizadas do tipo C, resultando em prolongada descarga no corno dorsal da medula espinhal. Esse fenômeno, conhecido como "Wind up" significa aumento progressivo no número de potenciais de ação por estímulo e ocorre em neurônios do corno dorsal. Esses episódios repetitivos podem levar à potenciação a longo prazo ("long-term potentiation", LTP), que envolve o aumento prolongado na transmissão sináptica. Ambos "Wind up" e LTP fazem parte do processo de sensibilização, envolvido na maioria dos estados de dor crônica (Millan, 2002). Por ser um evento crônico, a principal característica dessa patologia são mudanças plásticas, causadas por alterações na expressão gênica de receptores, canais iônicos, proteínas intracelulares, neuromoduladores, mediadores de sinalização extracelular entre outros (Woolf e Mannion, 1999). Por conseguinte, os mecanismos patofisiológicos da dor neuropática refletem, em grande parte, aqueles ocorridos na inflamação, embora alguns autores relatem que a lesão de nervo produz, em neurônios aferentes primários, alterações neuroquímicas, ligeiramente distintas daquelas produzidas na inflamação (Woolf e Mannion, 1999).

2.2.6. Tratamento farmacológico

O arsenal farmacológico para o tratamento da dor é composto, basicamente, por dois grandes grupos de drogas analgésicas: os opióides, que abolem diretamente a transmissão nociceptiva, no sistema nervoso central, pela ligação em receptores opióides (Hoskin e Hanks, 1991), e antiinflamatórios, que previnem a sensibilização de receptores periféricos da dor inibindo a ciclooxigenase (Cashman, 1996).

Analgésicos opióides, como a morfina e a codeína, são indicadas no tratamento de dores agudas, moderadas ou intensas, que não respondem a analgésicos menos eficazes.

Embora os opióides sejam muito efetivos, sua utilização é limitada pelo fato dessas drogas apresentarem muitos efeitos indesejados, como constipação, náuseas, vômitos, broncoconstrição, hipotensão, bradicardia e depressão respiratória e tolerância (Hoskin e Hanks, 1991).

Analgésicos não-opióides, como os antiinflamatórios convencionais (indometacina, paracetamol, AAS entre outros), são indicados para dores agudas, porém sua toxicidade renal e gastrointestinal pode resultar em grande morbidade, limitando seu uso para o tratamento da dor (Henry et al., 1996). Estudos farmacológicos, eletrofisiológicos e anatômicos têm contribuído para o descobrimento de múltiplos mediadores químicos envolvidos na dor, o que facilita o entendimento dos mecanismos de ação dos neurotransmissores e das drogas envolvidas, na modulação central e periférica da dor (Millan, 1999). Muito esforço tem sido dedicado, buscando compreender os mecanismos celulares e moleculares envolvidos na origem da dor, com o objetivo de encontrar drogas eficazes, com baixos efeitos colaterais e que possam ser empregadas nessas circunstâncias. De fato, atualmente, não existe tratamento satisfatório e nem medidas adequadas e específicas para o controle da dor (Mendell e Sahenk, 2003).

Os componentes psicológicos da dor neuropática são de fundamental importância e deverão ser abordados paralelamente às outras estratégias, incluindo avaliação e métodos de tratamento psicológico. Um passo importante é a identificação dos possíveis sintomas e mecanismos, os quais se apresentam atualmente como alvos terapêuticos. O grande obstáculo é que um sintoma pode representar vários mecanismos, e um mecanismo pode dar origem a múltiplos sintomas. Assim, o tratamento farmacológico tem a grande vantagem de ser avaliado por ensaios clínicos bem controlados e desenhados, o que, entretanto, tem sido feito em menor escala em relação aos métodos físicos, psicológicos e, principalmente, aos métodos cirúrgicos e invasivos, nos quais as limitações éticas são maiores (Rowbotham, 2005). As drogas atualmente em uso para o tratamento da dor neuropática incluem: anticonvulsivantes, antidepressivos, opióides, agentes tópicos e antiarrítmicos, gabaérgicos, antagonistas de NMDA, bloqueios anestésicos e neurolépticos (Dworkin et al., 2003a).

Historicamente, os antidepressivos tricíclicos foram os primeiros a serem utilizados no controle da dor crônica por se considerar, na época, a importância da depressão na dor (Watson et al., 1982). Os antidepressivos tricíclicos (amitriptilina, nortriptilina, clomipramina, imipramina, desipramina) têm se mostrado eficazes em condições neuropáticas, como polineuropatia dolorosa, neuralgia pós-herpética, neuropatia traumática, dor central pós-ictal (Sindrup e Jensen, 2002). Mais recentemente, novos antidepressivos não – tricíclicos têm

surgido e com algumas vantagens por terem menos efeitos colaterais. Os novos antidepressivos incluem os inibidores da recaptação seletiva da serotonina (paroxetina e citalopram), os inibidores balanceados da recaptação de serotonina e noradrenalina (bupropion e maprotilina) e os tetracíclicos (miaserina).

2.3. Selênio

2.3.1. Características químicas

O elemento selênio (Se) foi descoberto em 1817, pelo químico sueco Jöns Jacob Berzelius. Esse elemento químico é um calcogênio do grupo 16 da tabela periódica, que pode se apresentar sob quatro estados de oxidação: selenato (Se^{+6}), selenito (Se^{+4}), selênio elementar (Se^{0}) e seleneto (Se^{-2}). O Se compartilha propriedades químicas e físicas com o enxofre (S). Esta similaridade permite que o Se substitua o S, promovendo interações Se-S nos sistemas biológicos. Por outro lado, as diferenças nas propriedades físico-químicas entre Se e S constituem a base de seus papéis biológicos específicos (Stadtman, 1980). Os selenóis (R-SeH) são as formas correspondentes aos tióis (R-SH), onde ocorre a substituição do átomo de S pelo átomo de Se (Nogueira et al., 2004).

2.3.2. Biodisponibilidade do Se

Nos mamíferos, o Se parece ser rapidamente absorvido no duodeno, seguido pelo jejuno e íleo. Além do trato gastrintestinal, o Se pode ser absorvido por tecidos cutâneos e inalação. Estas duas últimas vias de absorção de Se estão relacionadas com a exposição e intoxicação ocupacional por compostos de selênio (Wanger et al.,1976). Após a absorção, os maiores níveis de Se estão localizados nos eritrócitos, fígado, baço, coração, unha e esmalte de dentes (Martin e Gerlack, 1972). Em animais intoxicados cronicamente, o Se é depositado principalmente nos rins e fígado, seguido pelo pâncreas, baço e pulmões (Wilber, 1980).

2.3.3. Atividade biológica

O interesse em relação ao Se tem crescido nos últimos anos, devido à descoberta do Se como um elemento traço essencial (Nogueira et al., 2004; Savegnago et al., 2006; Jesse et al., 2009) cuja concentração pode ocasionar deficiência ou toxicidade. De fato, quando a ingesta diária excede a capacidade corporal de eliminação, algum tipo de intoxicação e/ou patologia pode surgir.

No ano de 1930, o Se foi reconhecido como uma substância tóxica quando cavalos do oeste da China que se alimentaram de plantas com grande potencial de acumular este elemento e apresentaram sintomas de envenenamento, como perda de cascos, pêlos e anemia

(Spallholz, 1993). As patologias mais comuns associadas à toxicidade do Se são: a doença alcalina (*alkali disease*), uma doença de caráter crônico, e o falso cambalear (*blind staggers*), uma doença de caráter agudo que compromete o SNC (Martin e Gerlack, 1972).

O Se também exerce influência em patologias associadas aos transtornos de humor, uma vez que a carência de Se parece levar a um estado de humor mais deprimido (Hawkes e Hornbostel, 1996) e o aumento no consumo deste elemento estabiliza o humor e diminui o estado depressivo e outros sintomas negativos do humor como ansiedade, confusão e hostilidade (Sher, 2008). Neste contexto, a suplementação de dietas com Se, tanto para animais quanto para humanos, tem sido aceita pela comunidade científica. Para humanos, a Junta de Alimentação e Nutrição da Academia de Ciências dos Estados Unidos propõe uma ingestão diária de 50 - 200 μg (Sher, 2008), podendo ser encontrado nos seguintes alimentos: castanha-do-pará, alho, cebola, brócolis, cogumelos, cereais, pescados, ovos e carnes (Dumont et al., 2006).

Este calcogênio apresenta um grande número de funções biológicas, sendo a mais importante como antioxidante. Além disso, o Se está presente como resíduo de selenocisteína no sítio ativo das enzimas glutationa peroxidase (Flohé et al., 1973), tioredoxina redutase (Holmgren, 1985), 5'-deiodinase (Behne e Kyriakopoulos, 1990) e selenoproteina P (Ursini et al., 1990), sendo que a atividade redox do Se tem fundamental importância para o sítio catalítico dessas enzimas. Portanto, a partir da descoberta do papel essencial do Se no sítio ativo de selenoproteínas e do conceito de que moléculas contendo Se podem ser melhores nucleófilos (e, portanto antioxidantes) que os clássicos antioxidantes, (Arteel e Sies, 2001) se intensificou a síntese e o interesse em compostos orgânicos contendo Se.

2.3.4. Compostos orgânicos de Se

Nos últimos anos, os compostos orgânicos de Se têm sido alvos de interesse em síntese orgânica em virtude da descoberta de suas aplicações sintéticas, de suas propriedades farmacológicas e por apresentar menor toxicidade em relação às espécies inorgânicas (Nogueira et al., 2004; Nogueira e Rocha, 2010). Dentre os compostos orgânicos de Se estudados no nosso grupo de pesquisa se destacam o ebselen e o disseleneto de difenila.

2.3.4.1. Ebselen

O ebselen (2-fenil-1,2-benzilsoselenazol-3(2H)-ona) (Figura 2) é um composto orgânico de selênio que apresenta propriedade antioxidante similar à selenoenzima glutationa peroxidase (Parnhan e Graf, 1991). Esse composto possui baixa toxicidade, pois ele não libera selênio de sua molécula (Parnhan e Graff, 1987). De fato, Wendel e colaboradores (1984) demonstraram que em animais deficientes de selênio, a atividade da enzima glutationa peroxidase não aumentava pela suplementação com ebselen. O ebselen apresentou importantes efeitos como antioxidante na redução da peroxidação lipídica e pelas reações com grupos sulfidrílicos (-SH) em diferentes tecidos (Rossato et al., 2002; Davis et al., 2004; Nowak et al., 2006). Além disso, esse composto apresenta efeitos protetores em diversos modelos de isquemia tais como: cerebral, cardíaca, hepática e em um modelo *in vitro*, pela privação de glicose e oxigênio (Osaki et al., 1997; Imai et al., 2001).

O ebselen possui atividades antiinflamatórias, as quais podem ser devido a sua capacidade de inibir algumas enzimas envolvidas no processo inflamatório e por reduzir a síntese e a liberação de citocinas pró-inflamatórias (Parnhan e Graf, 1987; 1991). O ebselen também inibe a formação de leucotrienos sintetizados pela via da lipoxigenase 5, e em granulócitos inibe a atividade da proteína cinase c (PKC) e da NADPH oxidase (Mugesh et al., 2001). Além disso, o ebselen inibe *in vivo* e *in vitro* a enzima óxido nítrico sintase induzível (NOS)i, isoforma da óxido nítrico sintase que se torna ativa nos processos inflamatórios (Porciúncula et al., 2003). Recentemente, estudos têm investigado a habilidade deste composto na supressão de enzimas envolvidas no processo apoptótico e vias de sinalização, colaborando para a elucidação de outros mecanismos que possam estar envolvidos nas propriedades farmacológicas do ebselen (Yoshizumi et al., 2002, 2004). Em estudos *in vitro* com tecidos e células do SNC o ebselen apresentou efeito protetor contra a excitotoxidade provocada pelo glutamato, bem como demonstrou alterar alguns parâmetros do sistema glutamatérgico (Porciúncula et al., 2001, 2004; Nogueira et al., 2002). Além disso, Herin et al (2001) sugeriram que o efeito neuroprotetor do ebselen em injúrias provocadas pelo glutamato e seus agonistas, pode ser em parte devido a sua direta interação com o sítio modulatório redox do receptor de glutamato do tipo NMDA.

O ebselen possui efeito do tipo antidepressivo em modelo animal de depressão (TNF) na dose de 10 mg/kg (s.c.) sem alteração comportamental no teste do campo aberto (Posser et al., 2009). Os mecanismos envolvidos neste efeito estão relacionados aos receptors α_1 e α_2 adrenérgicos e D_1 e D_2 dopaminérgicos, mas não aos 5-HT_{1A} ou 5-$HT_{2A/2C}$ serotonérgicos.

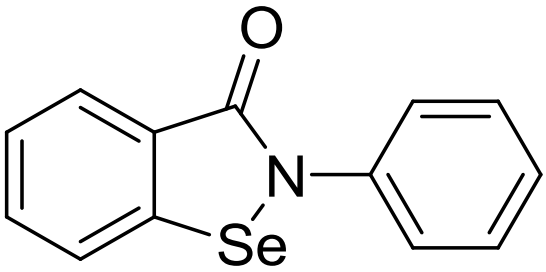


Figura 2: Estrutura química do ebselen

2.3.4.2. Disseleneto de difenila

O composto disseleneto de difenila (figura 3) é conhecido por ser mais ativo como mimético da selenoenzima glutationa peroxidase (Meotti et al., 2004) que o ebselen. De acordo com isso, em diversos trabalhos o disseleneto de difenila tem-se apresentado como um bom agente antioxidante na redução da peroxidação de lipídios provocada por diversos agentes e em diferentes tecidos (Meotti et al., 2004). Dados da literatura também indicam que esse composto orgânico de selênio apresenta várias propriedades farmacológicas tais como antinociceptiva (Savegnago et al., 2007; 2008) e também protege contra a discinesia orofacial induzida por reserpina e haloperidol (Burger et al., 2004, 2006).

Estudos recentes do nosso grupo relataram que o disseleneto de difenila quando administrado pela via subcutânea ou pela via oral apresenta ação antinociceptiva, antiinflamatória e anti-hiperalgésico mecânico e térmico (modelos como a administração de ACF, constrição do nervo ciático e injeção intratecal de glutamato, NMDA, bradicinina e prostaglandina E_2- PGE_2) em camundongos e ratos (Zasso et al., 2005; Savegnago et al., 2007 a,b,c). Sendo que, o possível mecanismo envolvido na ação antinociceptiva do disseleneto de difenila parece envolver a via glutamatérgica (receptores NMDA) adenosinérgica (receptores A_{2B}), canais de K^+, NO e serotoninérgica (Savegnago et al., 2007 a,b,c).

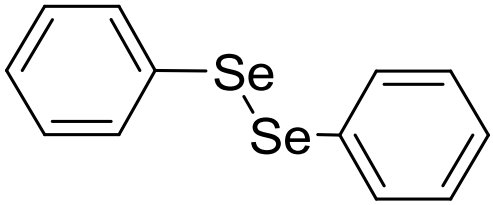
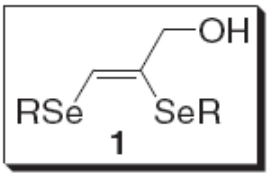


Figura 3 - Estrutura química do disseleneto de difenila

O disseleneto de difenila apresenta efeito do tipo antidepressivo em modelos em ratos e camundongos (Savegnago et al., 2007d, 2008). Os mecanismos envolvidos são a modulação dos receptores 5-HT_{1A}, 5-$HT_{2A/2C}$, 5-HT_3, D_1, D_2, D_3, α_1 e α_2 e da via L-arginina-NO/GMPc/K^+ (Savegnago et al., 2007; 2008).

2.3.4.3. Selenetos vinílicos bis substituídos

Os derivados selenetos bis alcenos foram avaliados em estudos de atividade antinociceptiva e antioxidante. Os compostos 1c, 1d e 1e (Figura 4) demonstraram os melhores resultados nos estudos da atividade antioxidante *in vitro* (peroxidação lipídica) e o composto 1d apresentou a menor CI_{50}. No trabalho realizado por Savegnago e colaboradores (2006) os compostos mais promissores (1c e 1d) foram administrados pela via subcutânea nas doses de 5 a 50 mg/kg em testes nociceptivos. Os compostos apresentaram efeito antinociceptivo no teste das contorções induzidas por ácido acético, como DI_{50} maior que 50 e 14.61 mg/kg, respectivamente. Além disso, os compostos 1c e 1d inibiram de maneira significativa a nocicepção induzida pela capsaicina. Os compostos também foram avaliados no teste de nocicepção térmica da imersão da cauda em água a 55°C e mostraram efeito antinociceptivo que não foi prevenido pela pré-administração de naloxona (antagonista opióide).



1a - R = C_4H_9
1b - R = C_6H_5
1c - R = 2,4,6-$Me_3C_6H_2$
1d - R = 4-ClC_6H_4
1e - R = 4-$MeOC_6H_4$

Figura 4 - Estrutura química dos derivados selenetos bis alcenos

Após o trabalho de seleção dos compostos mais promissores, nosso grupo avaliou os compostos 1c (figura 5) e 1d (figura 6, SVBS) em novas metodologias de testes nociceptivos. O composto 1c foi administrado oralmente em modelos nociceptivos químicos em ratos e camundongos (Jesse et al., 2007). O composto apresentou atividade antinociceptiva na primeira e na segunda fase do teste da formalina em ratos a partir de 10 mg/kg (p.o). Este efeito foi bloqueado pela pré-administração de N^G-L-nitro-arginina-metil Ester (L-NAME, inibidor da NOS), azul de metileno (inibidor da NOS/GC) e glibenclamida (inibidor dos canais de K^+ sensíveis ao ATP) e não pela atropina (antagonista muscarínico). Além disso, o composto inibiu o tempo de lambida e diminuiu o edema induzido por 5-HT, histamina, composto 48/80 e glutamato.

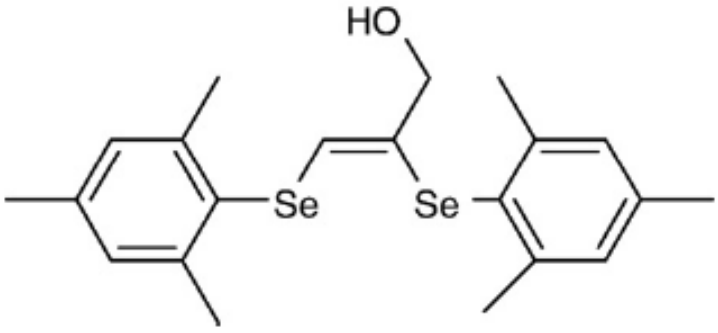


Figura 5 - Estrutura química do composto 1c

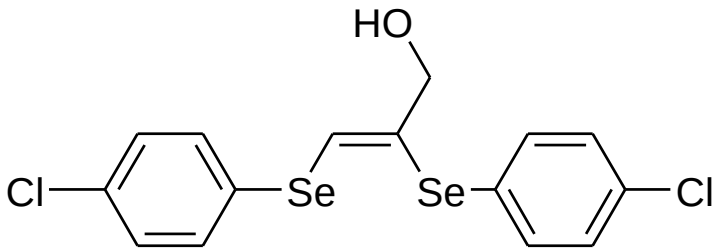


Figura 6 - Estrutura química do composto 1d (SVBS)

O SVBS apresentou efeito antinociceptivo e antiinflamatório em modelos agudos (injeção i.pl. de formalina, glutamato, histamina e capsaicina) a partir de 5 mg/kg em camundongos (Jesse et al., 2008; 2009). Os mecanismos envolvidos no efeito antinociceptivo do SVBS são a modulação de canais de K^+ sensíveis à ATP, receptores glutamatérgicos do tipo cainato e metabotrópico, vanilóides, neuropeptidérgicos e citocinas pró-inflamatórias (Jesse et al., 2008). Em outro estudo, a modulação dos receptores do tipo serotonérgico 5-HT_{2A}, 5-HT_3, histaminérgico H_2 e prostanóides também está envolvida no efeito antinociceptivo e antiinflamatório do SVBS em camundongos. Além disso, neste estudo o SVBS modulou as vias proteína cinase A (PKA) e PKC na resposta antinociceptiva (Jesse et al., 2009).

3. OBJETIVOS

3.1. Objetivo geral

Avaliar a atividade do composto SVBS em testes do tipo antidepressivo e anti-hiperalgésico mecânico em camundongos, bem como os mecanismos de ação envolvidos nestes efeitos.

3.2. Objetivos específicos

1) Investigar a ação do SVBS em testes de atividade do tipo antidepressiva em camundongos através do TNF e do TSC;
2) Avaliar a participação dos receptores serotoninérgicos, dopaminérgicos e noradrenérgicos, do sistema nitrérgico e a atividade das enzimas MAO-A, MAO-B e Na^+K^+ATPase no efeito do tipo antidepressivo do SVBS em camundongos;
3) Determinar a participação dos receptores PPARγ e dos canais de K^+ no efeito do tipo antidepressivo do SVBS;
4) Investigar a ação do tipo antidepressiva do SVBS em camundongos submetidos à injúria por constrição crônica (ICC) do nervo ciático;
5) Determinar o efeito do SVBS sobre a hiperalgesia mecânica nos modelos de dor neuropática causada pela avulsão do plexo braquial (ABP) e pela administração do antineoplásico vincristina;
6) Avaliar a ação do SVBS sobre a hiperalgesia mecânica nos modelos de dor inflamatória induzida pela administração intraplantar (i.pl.) de ACF, carragenina (Cg) e PGE_2.

4- ARTIGOS CIENTÍFICOS E MANUSCRITO

Os resultados que fazem parte desta tese estão apresentados sob a forma de artigos científicos e de manuscrito, os quais estão organizados em introdução, materiais e métodos, resultados, discussão e referências bibliográficas.

Os **Artigos 1, 2, 3 e 4** estão dispostos da mesma forma que foram publicados na edição das revistas científicas. O **Artigo 5** (forma de manuscrito) está submetido e se encontra no formato exigido pela revista.

4.1. Evidência do envolvimento dos receptores serotoninérgicos 5-$HT_{2A/C}$ e 5-HT_3 no efeito do tipo antidepressivo causado pela administração oral de bis seleneto em camundongos

4.1.1. Artigo 1

Evidência do envolvimento dos receptores serotoninérgicos 5-$HT_{2A/C}$ e 5-HT_3 no efeito do tipo antidepressivo causado pela administração oral de bis seleneto em camundongos

Cristiano R. Jesse, Ethel A. Wilhelm, Cristiani F. Bortolatto, Cristina W. Nogueira

Progress in Neuro-Psychopharmacology& Biological Psychiatry 34 (2010) 294-302

Progress in Neuro-Psychopharmacology & Biological Psychiatry 34 (2010) 294–302

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Evidence for the involvement of the serotonergic 5-$HT_{2A/C}$ and 5-HT_3 receptors in the antidepressant-like effect caused by oral administration of bis selenide in mice

Cristiano R. Jesse, Ethel A. Wilhelm, Cristiani F. Bortolatto, Cristina W. Nogueira *

Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

ARTICLE INFO

Article history:
Received 4 July 2009
Received in revised form 26 November 2009
Accepted 26 November 2009
Available online 2 December 2009

Keywords:
Antidepressant
Bis selenide
Noradrenergic
Selenium
Serotonergic and dopaminergic receptors

ABSTRACT

The present study investigated a possible antidepressant-like activity of bis selenide using two predictive tests for antidepressant effect on rodents: the forced swimming test (FST) and the tail suspension test (TST). Bis selenide (0.5–5 mg/kg, p.o.) decreased the immobility time in the mouse FST and TST. The anti-immobility effect of bis selenide (1 mg/kg, p.o.) in the TST was prevented by the pretreatment of mice with p-chlorophenylalanine methyl ester (PCPA; 100 mg/kg, i.p., an inhibitor of serotonin synthesis), ketanserin (1 mg/kg, i.p., a 5-$HT_{2A/2C}$ receptor antagonist), and ondasentron (1 mg/kg, i.p., a 5-HT_3 receptor antagonist). Pretreatment of mice with prazosin (1 mg/kg, i.p., an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist), propranolol (2 mg/kg, i.p., a β-adrenoceptor antagonist), SCH23390 (0.05 mg/kg, s.c., a dopamine D_1 receptor antagonist), sulpiride (50 mg/kg, i.p., a dopamine D_2 receptor antagonist), or WAY 100635 (0.1 mg/kg, s.c., a selective 5-HT_{1A} receptor antagonist) did not block the antidepressant-like effect of bis selenide (1 mg/kg, p.o.) in the TST. Administration of bis selenide (0.1 mg/kg, p.o.) and fluoxetine (1 mg/kg), at subeffective doses, produced an antidepressant-like effect in the TST. Bis selenide did not alter Na^+ K^+ ATPase, MAO-A and MAO-B activities in whole brains of mice. Bis selenide produced an antidepressant-like effect in the mouse TST and FST, which may be related to the serotonergic system (5-$HT_{2A/2C}$ and 5-HT_3 receptors).

1. Introduction

Depression is a common illness associated with high rates of chronicity, relapse, and recurrence, psychosocial and physical impairments and suicide. Depression is also considered a significant risk factor for the development of coronary artery disease and stroke (Musselman et al., 1998). Regarding treatment, about 65% of patients ultimately respond to antidepressant drug therapy (Steffens et al., 1997), which has numerous side effects associated (Nemeroff and Owens, 2002).

Although the molecular alterations underlying the pathogenesis of depression remain to be clearly established, preclinical and clinical studies have suggested the involvement of monoamines, particularly serotonergic and noradrenergic systems (Millan, 2004). There are evidences indicating that serotonergic and noradrenergic neurotransmissions are involved in the expression of an antidepressant-like effect in the behavioral despair models of depression (Elhwuegi, 2004). Moreover, pharmacological studies have reported the efficacy of antidepressants with dopaminergic effects on the treatment of depression (Papakostas, 2006) as well as antidepressant-like responses in preclinical models of depression (D'Aquila et al., 2000).

Monoamine oxidase (MAO) is the key enzyme that is associated with the metabolism of these monoamines regulating their intracellular concentrations in the brain. Therefore, the abnormal function of this enzyme is thought to be involved in several psychiatric disorders, such as depression (Deniker, 1983). Na^+ K^+ ATPase is the enzyme responsible for the active transport of sodium and potassium ions in the nervous system, maintaining the ionic gradient necessary for neuronal excitability and regulation of neuronal cell volume. Moreover, its activity is decreased in patients with bipolar affective disorder and other psychiatric disorders (Reddy et al., 1992; El-Mallakh and Wyatt, 1995). Some studies have reported that psychoactive drugs such as haloperidol, carbamazepine, and lithium modify Na^+ K^+ ATPase activity (Reddy et al., 1992; El-Mallakh and Wyatt, 1995). The clinical therapy of depression is based on classical antidepressant drugs such as monoamine oxidase inhibitors (MAOi; e.g. tranylcypromine), catecholamine reuptake inhibitors (e.g. imipramine), and selective inhibitors of serotonin reuptake (SSRIs; e.g. fluoxetine) (Wong and Licinio, 2001).

Abbreviations: ANOVA, analysis of variance; bis selenide, [(*Z*)-2,3-Bis(4-chlorophenylselanyl)prop-2-en-1-ol]; DA, dopamine; DMSO, dimethylsulfoxide; FST, forced swimming test; 5-HT, serotonin; i.p., intraperitoneal; MAO, monoamine oxidase; MAOi, monoamine oxidase inhibitors; NA, noradrenaline; NE, norepinephrine; OFT, open field test; PCPA, p-chlorophenylalanine methyl ester; p.o., per oral; SCH23390, (R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; TST, tail suspension test; WAY100635, N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridynyl) cyclohexanecarboxamide.

* Corresponding author. Tel.: +55 55 32209611; fax: +55 55 32208978.
E-mail address: criswn@quimica.ufsm.br (C.W. Nogueira).

0278-5846/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2009.11.023

Several studies have demonstrated the role of selenium in mood disorders (Hawkes and Hornbostel, 1996; Burk, 2002; Sher, 2008). The low-selenium status (low-selenium diet contains 32–36 μg per day) has been associated with a significant increased incidence of depression, anxiety, confusion, and hostility. In addition, high dietary and/or supplementary selenium (226 μg per day) improve mood (Rayman, 2000). Under this point of view, bis selenide, an organo-selenium compound, could be an attractive target for the treatment of depression because it is a non-toxic drug when acutely administered to rodents at doses with pharmacological effects (Savegnago et al., 2006, Jesse et al., 2007, 2008).

Considering the need of discovering compounds that could improve conventional therapies, we sought to investigate the effect of a single oral administration of bis selenide in the TST and FST, which are two animal models of depression used to screen new antidepressant drugs (Bourin et al., 2005). The positive results obtained in the TST have prompted us to investigate the involvement of the monoaminergic system in the antidepressant-like effect of bis selenide in this model. The next step of our study was to investigate the possible antidepressant-like effect of subeffective doses of bis selenide and conventional antidepressants in the TST. The activity of Na^+, K^+ ATPase, MAO-A, and MAO-B has been implicated in the pathogenesis of depression. Therefore, we also investigate the activity of Na^+, K^+ ATPase, MAO-A, and MAO-B in mice treated with bis selenide.

2. Materials and methods

2.1. Animals

The behavioral experiments were conducted using male adult Swiss mice (25–35 g) maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle, with lights on at 6:00 a.m. 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. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

2.2. Chemicals

Bis selenide [(*Z*)-2,3-Bis(4-chlorophenylselanyl)prop-2-en-1-ol] was prepared and characterized in our laboratory by the method previously described (Moro et al., 2005). 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 bis selenide (99.9%) was determined by GC/HPLC. The following drugs were used: prazosin, yohimbine, propranolol, SCH 23390, sulpiride, PCPA, WAY 100635, ketanserin, ondansetron, fluoxetine, imipramine, ATP, ouabain, selegiline, clorgiline, kynuramine and 4- hydroxyquinoline (Sigma Chemical Co, USA). Drugs were diluted in saline 0.9%. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.3. In vivo experiments

To assess time-course of the antidepressant-like effect of bis selenide, mice were pretreated with bis selenide (5 mg/kg, p.o.) or vehicle (canola oil, 10 ml/kg, p.o.) 0.5–8 h before the TST. In order to assess the antidepressant-like effect of bis selenide, this compound (dose range: 0.1–5 mg/kg, p.o.) or vehicle was administered 1 h before the FST or TST. The doses of antagonists, which demonstrate effect on pharmacological and biochemical studies, and the doses of antidepressants, which did not modify the basal response in the TST and the locomotor activity in the OFT, were chosen on the basis of published studies. Several authors confirm the selectivity and efficacy of the above mentioned treatments at the doses used (Kaster et al., 2007; Brocardo et al., 2008; Cunha et al., 2008; Kulkarni et al., 2008; Binfaré et al., 2009).

To test the hypothesis that the antidepressant-like effect of bis selenide is mediated through an interaction with the noradrenergic system, animals were pretreated with prazosin (vehicle, 1 mg/kg, i.p., an α_1-adrenoceptor antagonist), yohimbine (vehicle, 1 mg/kg, i.p., an α_2-adrenoceptor antagonist) or propranolol (vehicle, 2 mg/kg, i.p., a β-adrenoceptor antagonist) and after 1 h, animals received bis selenide (1 mg/kg, p.o.) or vehicle and were tested in the TST 1 h later.

To assess the possible involvement of the dopaminergic system in the antidepressant-like effect of bis selenide in the TST, independent groups of animals were pretreated with SCH23390 (vehicle, 0.05 mg/kg, subcutaneous (s.c.), a dopaminergic D_1 receptor antagonist) or sulpiride (vehicle, 50 mg/kg, i.p., a dopaminergic D_2 receptor antagonist) and after 1 h, they received bis selenide (1 mg/kg, p.o.) or vehicle and were tested in the TST 1 h later.

To investigate the possible contribution of the serotonergic system to the effect of bis selenide on reducing the immobility time in the TST, animals were pretreated with PCPA (vehicle, 100 mg/kg, i.p., an inhibitor of serotonin synthesis), once a day, for 3 consecutive days. Twenty-four hours after the last PCPA injection, animals were treated with bis selenide (1 mg/kg, p.o.) or vehicle and were tested in the TST. In a separate series of experiments, the involvement of the serotonin (5-HT) receptor subtypes in the effect caused by bis selenide in the TST was studied. Mice were pretreated with WAY (vehicle, 0.1 mg/kg, s.c., a selective 5-HT_{1A} receptor antagonist), ketanserin (vehicle, 1 mg/kg, i.p., a 5-$HT_{2A/2C}$ receptor antagonist) or ondasentron (vehicle, 1 mg/kg, i.p., a 5-HT_3 receptor antagonist) and after 15 min they received bis selenide (1 mg/kg, p.o.) or vehicle and were tested in the TST 1 h later.

We investigated the antidepressant-like effect of subeffective doses of bis selenide with conventional antidepressants on the TST. To this end, mice received by p.o. route fluoxetine (vehicle, 1 mg/kg, a 5-HT reuptake inhibitor), imipramine (vehicle, 0.1 mg/kg, a 5-HT and noradrenaline reuptake inhibitor) and immediately after bis selenide (0.1 mg/kg, p.o.) or vehicle was administered. Sixty minutes later, the TST was carried out.

The open field test (OFT) was carried out to rule out any psychostimulant effect of the interaction of prazosin, yohimbine, propranolol, SCH 23390, sulpiride, PCPA, WAY 100635, ketanserin, ondasentron, fluoxetine, imipramine and bis selenide. These behavioral tests were performed by an observer blind to the drug treatment.

2.3.1. Forced swimming test (FST)

The test was conducted using the method described by Porsolt et al. (1977). Briefly, mice were individually forced to swim in open cylinders (25 cm height × 10 cm diameter) containing 19 cm of water at 25 ± 1 °C. The duration of immobility was scored during the 6 min test period as described previously (Rodrigues et al., 2002). Each mouse was recorded as immobile when floating motionless or making only those movements necessary to keep its head above water.

2.3.2. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period (Rodrigues et al., 2002).

2.3.3. Open field test (OFT)

The OFT was carried out to determine if the compounds or combination of compounds produced effects on locomotor and exploratory activities. The open field was made of polywood and

surrounded by walls 30 cm in height. The floor of the open field, 45 cm in length and 45 cm in width, was divided by masking tape markers into 09 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 6 min to record the locomotor (number of segments crossed with the four paws) and exploratory activities (expressed by the number of time rearing on the hind limbs) (Zomkowski et al., 2002; Brocardo et al., 2008).

2.4. Ex vivo experiments

To test the hypothesis that the antidepressant effects of bisselenide is mediated through an inhibition of MAO-A, MAO-B or NA^+ K^+ ATPase activity, mice were pretreated with bis selenide (dose range: 0.1–5 mg/kg, p.o.) or vehicle (canola oil, p.o.) after 1 h, animals were killed and the whole brains removed.

2.4.1. Monoamine oxidase (MAO) activity

2.4.1.1. Mitochondria preparations. A preparation of brain mitochondria was used for MAO assay (Soto-Otero et al., 2001). Cerebral cortices were immediately removed and washed in ice-cold isolation medium (Na_2PO_4/KH_2PO_4 isotonized with sucrose, pH 7.4). Cerebral mitochondria were then obtained by differential centrifugation. Briefly, after removing blood vessels and pial membranes, cerebral cortices were manually homogenized with four volumes (w/v) of the isolation medium. Then, the homogenate was centrifuged at 900× *g* at 4 °C for 5 min. The supernatant was centrifuged at 12,500×*g* for 15 min. The mitochondria pellet was then washed once with isolation medium and recentrifuged under the same conditions. Finally, the mitochondrial pellet was reconstituted in a buffer solution (Na_2PO_4/KH_2PO_4 isotonized with KCl, pH 7.4) and stored in aliquots.

2.4.1.2. Enzyme assay. MAO activity was determined by Krajl (1965) with some modifications (Matsumoto et al., 1984). An aliquot of 100 µl of each sample was incubated at 37 °C for 5 min in a medium containing buffer solution and specific inhibitors, selegiline (a MAO- B inhibitor, 250 nM) or clorgiline (a MAO-A inhibitor, 250 nM) at a final volume of 600 µl. Then 20 µl of kynuramine dihydrobromide was added to the reaction mixture (90 µM (MAO-A) and 60 µM (MAO-B)) as substrate. Samples were then incubated at 37 °C for 30 min. After incubation, the reaction was terminated by adding 10% of trichloroacetic acid. After cooling and centrifugation at 3000×*g* for 15 min, an aliquot of 800 µl of the supernatant was added to 1 ml of 1 M NaOH. The fluorescence intensity was detected spectrofluorimetrically with excitation at 315 nm and emission at 380 nm. Clorgiline (100 nM) and selegiline (100 nM) were used as positive controls in MAO-A and MAO-B assays, respectively. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve of 4-hydroxyquinoline. MAO activity was expressed as nmol of 4-hydroxyquinoline formed/mg protein.

2.4.2. Na^+ K^+ ATPase activity

The sample of whole brain was homogenized in 50 mM Tris–HCl, pH 7.4 (1:5, w/v) and centrifuged at 2400×*g* for 10 min to obtain the low-speed supernatant (S_1).

For Na^+ K^+ ATPase activity assay, a reaction mixture containing S_1 in a final volume of 500 µl was used. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Control samples were carried out under the same conditions with the addition of 0.1 mM ouabain. The samples were incubated at 37 °C for 30 min, the incubation was stopped by adding trichloroacetic acid solution (10%) with 10 mM $HgCl_2$. Na^+ K^+ ATPase activity was calculated by the difference between the two assays. SKF 82526 (1 mM) was used as a positive control. Released inorganic phosphate (Pi) was measured by the method of Fiske and Subbarow (1925). Enzyme activity was expressed as nmol Pi/mg protein/min.

2.4.3. Protein quantification

Protein concentration was measured by the method of Bradford (1976), using bovine serum albumin as the standard.

2.5. Statistical analysis

All experimental results are given as the mean ± S.E.M. Comparisons between experimental and control groups were performed by one-way (bis selenide treatment) or two-way ANOVA (prazosin, yohimbine, propranolol, SCH 23390, sulpiride, PCPA, WAY 100635, ketanserin, ondasentron, fluoxetine and imipramine X bis selenide treatment) followed by Newman–Keuls test for post hoc comparison when appropriate. A value of $P<0.05$ was considered to be significant. Main effects of first order interactions are presented only when interaction was not significant.

3. Results

3.1. Effect of bis selenide in the mouse FST, TST and OFT

A time-course analysis of the antidepressant profile of bis selenide was accomplished in the TST. The antidepressant-like effect of bis selenide reached its peak at 1 h and remained significant up to 6 h after p.o. administration (Fig. 1) ($F(6,51) = 28.02$, $P<0.001$). Thus, the time point (1 h) of the maximum effect of bis selenide was chosen for all further studies.

The immobility time in the FST and TST of animals treated with bis selenide is shown in Fig. 2A and B, respectively. One-way ANOVA revealed a significant main effect of treatment (canola oil, bis selenide) in the FST ($F(4,35) = 9.47$, $P<0.001$) and TST ($F(4,35) = 12.64$, $P<0.001$). Bis selenide at doses of 0.5, 1 and 5 mg/kg reduced the immobility time in the FST and TST.

Bis selenide given by p.o. route, at all doses tested, did not produce any change in the number of crossings ($F(4,35) = 0.11$, $P<0.972$) and rearing ($F(4,35) = 0.35$, $P<0.843$) in the mouse OFT (data not shown).

3.2. Involvement of the noradrenergic system

Results depicted in Fig. 3A show that the pretreatment of mice with prazosin (1 mg/kg, i.p., an α_1-adrenoceptor antagonist, a subeffective dose in the immobility time in the TST) did not reverse the reduction in immobility time elicited by bis selenide (1 mg/kg, p.o., an effective dose) in the TST. Two-way ANOVA revealed a main effect of treatment (saline, bis selenide) ($F(1,24) = 36.79$, $P<0.001$), but not

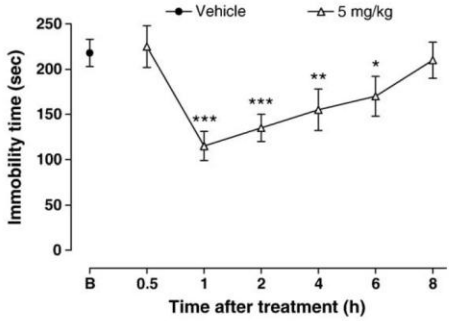


Fig. 1. Time-course of the antidepressant-like effect caused by bis selenide (5 mg/kg, p.o.) in the mouse TST. Bis selenide was administered 0.5, 1, 2, 4, 6 and 8 h before the TST. Each point represents the mean of 7 animals and the error bars indicate the S.E.M. Vehicle indicates mice treated with canola oil. Asterisks denote the significance levels, when compared to the vehicle group (one-way ANOVA followed by Newman–Keuls). $^*P<0.05$ and $^{**}P<0.01$ and $^{***}P<0.001$ when compared to the vehicle treated group. B, baseline values to TST.

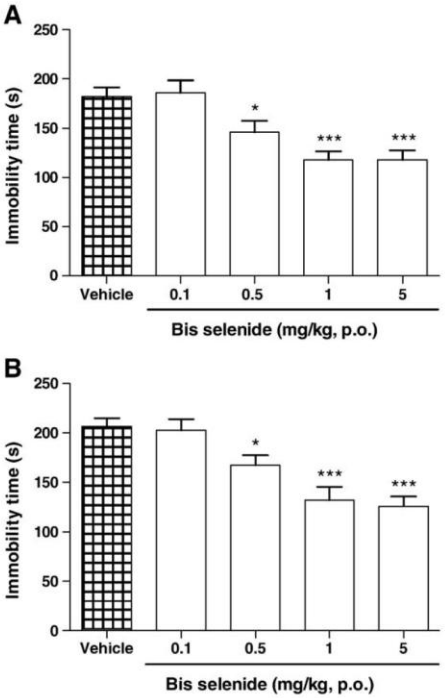


Fig. 2. Effect of acute administration of bis selenide in the mouse FST (panel A) and in the TST (panel B). Bis selenide (dose range: 0.1–5 mg/kg) was orally administered 1 h before the test. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). $^{*}P<0.05$ and $^{***}P<0.001$ when compared to the vehicle treated group.

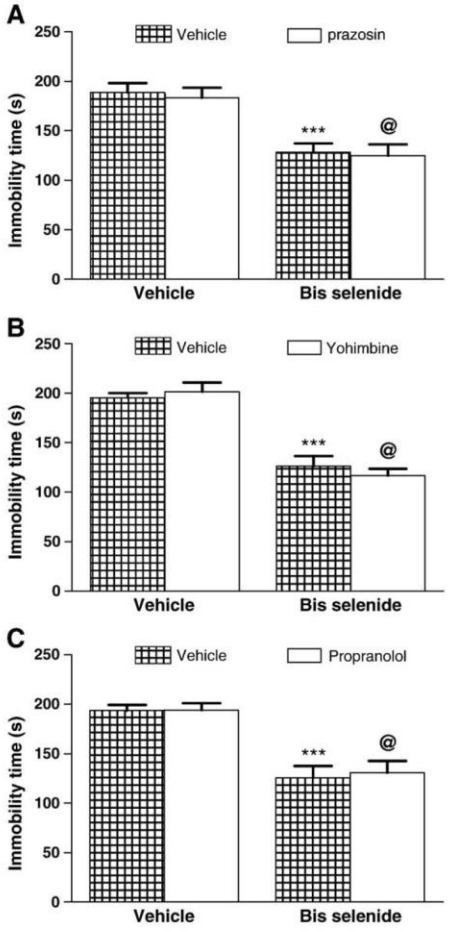


Fig. 3. Effect of pretreatment of mice with prazosin (1 mg/kg, i.p., panel A), yohimbine (1 mg/kg, i.p., panel B) or propranolol (2 mg/kg, panel C) on the action of bis selenide (1 mg/kg, p.o.) in the mouse TST. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by Two-Way Analysis of Variance (ANOVA) followed by Newman–Keuls test. $^{***}P<0.001$ compared to the vehicle group and @ compared to vehicle group pretreated with antagonist.

of the pretreatment with α_1-adrenoceptor antagonist (prazosin, canola oil) ($F(1,24) = 0.33$, $P<0.569$) nor pretreatment × treatment interaction (prazosin, bis selenide) ($F(1, 24) = 0.01$, $P<0.906$). No significant effect was observed on the number of crossings in the OFT in mice treated with bis selenide and prazosin (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1, 24) = 0.07$, $P<0.799$), pretreatment (prazosin, canola oil) ($F(1,24) = 0.01$, $P<0.938$) nor pretreatment × treatment interaction (prazosin, bis selenide) ($F(1,24) = 0.09$, $P<0.764$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and prazosin (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.33$, $P<0.591$), pretreatment (prazosin, canola oil) ($F(1,24) = 0.08$, $P<0.775$) nor pretreatment × treatment interaction (prazosin, bis selenide) ($F(1,24) = 0.02$, $P<0.916$).

Fig. 3B shows that the pretreatment with yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist, a subeffective dose in the immobility time in the TST) was not able to reverse the antidepressant-like effect of bis selenide (1 mg/kg, p.o.) in the TST. Two-way ANOVA revealed a main effect of treatment (saline, bis selenide) ($F(1,24) = 90.62$, $P<0.001$), but not of the pretreatment with α_2-adrenoceptor antagonist (yohimbine, canola oil) ($F(1,24) = 0.05$, $P<0.827$) nor pretreatment × treatment interaction (yohimbine, bis selenide) ($F(1,24) = 0.86$, $P<0.362$). No significant effect was observed on the number of crossings in the OFT in mice treated with bis selenide and yohimbine (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.57$, $P<0.458$), pretreatment (yohimbine, canola oil) ($F(1,24) = 0.07$, $P<0.793$) nor pretreatment × treatment interaction (yohimbine, bis selenide) ($F(1,24) = 0.63$, $P<0.435$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and yohimbine (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1, 24) = 0.42$, $P<0.520$), pretreatment (yohimbine, canola oil) ($F(1,24) = 0.29$, $P<0.593$) nor pretreatment × treatment interaction (yohimbine, bis selenide) ($F(1,24) = 0.18$, $P<0.671$).

Fig. 3C shows that the pretreatment of mice with propranolol (2 mg/kg, i.p., a β-adrenoceptor antagonist, a subeffective dose in the immobility time in the TST) did not prevent the antidepressant-like effect of bis selenide (1 mg/kg, p.o.) in the TST. Two-way ANOVA revealed a main effect of treatment (saline, bis selenide) ($F(1,24) = 46.78$, $P<0.001$), but not of the pretreatment with β-adrenoceptor antagonist (propranolol, canola oil) ($F(1,24) = 0.02$, $P<0.896$) nor pretreatment × treatment interaction (propranolol, bis selenide) ($F(1,24) = 1.16$, $P<0.293$). Administration of bis selenide with propranolol to mice did not modify the number of crossings in the OFT (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.01$, $P<0.937$), pretreatment (propranolol, canola oil) ($F(1,24) = 0.01$, $P<0.993$) nor pretreatment ×

treatment interaction (propranolol, bis selenide) ($F(1,24)=0.28$, $P<0.604$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and propanolol (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24)=0.01$, $P<0.951$), pretreatment (propranolol, canola oil) ($F(1,24)=0.30$, $P<0.587$) nor pretreatment × treatment interaction (propranolol, bis selenide) ($F(1,24)=0.04$, $P<0.840$).

3.3. Involvement of the dopaminergic system

Fig. 4A shows that pretreatment of mice SCH 23390 (0.05 mg/kg, s.c., a D_1 receptor antagonist, a subeffective dose in the immobility time in the TST) did not block the effect of bis selenide (1 mg/kg, p.o.) in the TST. Two-way ANOVA revealed a main effect of treatment (saline, bis selenide) ($F(1,24)=80.43$, $P<0.001$), but not of the pretreatment with D_1 receptor antagonist (SCH 23390, canola oil) ($F(1,24)=0.01$, $P<0.925$) nor pretreatment × treatment interaction (SCH 23390, bis selenide) ($F(1,24)=0.02$, $P<0.883$). Administration of bis selenide with SCH 23390 to mice did not modify the number of crossings in the OFT (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24)=0.01$, $P<0.907$), pretreatment (SCH 23390, canola oil) ($F(1,24)=0.11$, $P<0.743$) nor pretreatment × treatment interaction (SCH 23390, bis selenide) ($F(1,24)=0.01$, $P<0.954$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and SCH 23390 (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24)=0.25$, $P<0.622$), pretreatment (SCH 23390, canola oil) ($F(1,24)=0.11$, $P<0.742$) nor pretreatment × treatment interaction (SCH 23390, bis selenide ($F(1,24)=0.51$, $P<0.483$).

Fig. 4B shows that the pretreatment with sulpiride (50 mg/kg, i.p., a D_2 receptor antagonist, a subeffective dose in the immobility time in the TST) did not prevent the action of bis selenide (1 mg/kg, p.o.) in the TST. Two-way ANOVA revealed a main effect of treatment (saline, bis selenide) ($F(1,24)=101.8$, $P<0.001$), but not of the pretreatment with D_2 receptor antagonist (sulpiride, canola oil) ($F(1,24)=0.21$, $P<0.652$) nor pretreatment × treatment interaction (sulpiride, bis selenide) ($F(1,24)=0.01$, $P<0.977$). Administration of bis selenide with sulpiride to mice did not modify the number of crossings in the OFT (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24)=0.19$, $P<0.664$), pretreatment (sulpiride, canola oil) ($F(1,24)=0.42$, $P<0.523$) nor pretreatment × treatment interaction (sulpiride, bis selenide ($F(1,24)=1.26$, $P<0.274$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and sulpiride (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24)=0.21$, $P<0.649$), pretreatment (sulpiride, canola oil) ($F(1,24)=0.02$, $P<0.923$) nor pretreatment × treatment interaction (sulpiride, bis selenide ($F(1,24)=0.03$, $P<0.868$).

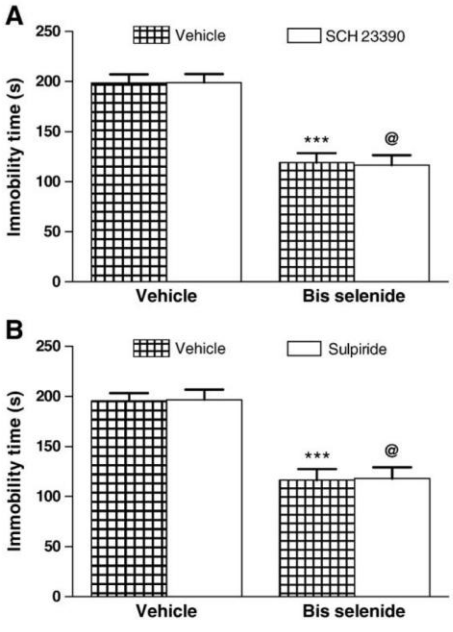


Fig. 4. Effect of pretreatment of mice with SCH23390 (0.05 mg/kg, s.c., panel A) or sulpiride (50 mg/kg, i.p., panel B) on the action of bis selenide (1 mg/kg, p.o.) in the mouse TST. Values are expressed as mean ± S.E.M. ($n=7$ mice/group). Data were analyzed by Two-Way Analysis of Variance (ANOVA) followed by Newman–Keuls test. ***$P<0.001$ compared to the vehicle group and @ compared to the vehicle group pretreated with antagonist.

3.4. Involvement of the serotonergic system

Results depicted in Fig. 5A show that the pretreatment of mice with an inhibitor of 5-HT synthesis, PCPA (100 mg/kg, i.p., once a day for 3 consecutive days, a subeffective dose on the immobility time in the TST), was able to reverse the antidepressant-like effect of bis selenide (1 mg/kg, p.o.) in the TST ($F(1,24)=25.47$, $P<0.001$). Two-way ANOVA revealed a main effect of treatment (saline, bis selenide) ($F(1,24)=16.03$, $P<0.002$), pretreatment with an inhibitor of 5-HT synthesis (PCPA, canola oil) ($F(1,24)=20.40$, $P<0.001$) and pretreatment × treatment interaction (PCPA, bis selenide) ($F(1,24)=25.47$, $P<0.001$). Administration of bis selenide with PCPA to mice did not modify the number of crossings in the OFT (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24)=0.32$, $P<0.575$), pretreatment (PCPA, canola oil) ($F(1,24)=0.19$, $P<0.664$) nor pretreatment × treatment interaction (PCPA, bis selenide ($F(1,24)=0.02$, $P<0.879$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and PCPA (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24)=0.37$, $P<0.551$), pretreatment (PCPA, canola oil) ($F(1,24)=0.59$, $P<0.448$) nor pretreatment × treatment interaction (PCPA, bis selenide ($F(1,24)=0.07$, $P<0.791$).

Fig. 5B shows that the pretreatment of mice with WAY100635 (0.1 mg/kg, s.c., a selective 5-HT_{1A} receptor antagonist, a subeffective dose in the immobility time in the TST) did not prevent the effect of bis selenide (1 mg/kg, p.o.) in the TST. Two-way ANOVA revealed a main effect of treatment (saline, bis selenide) ($F(1, 24)=105.11$, $P<0.001$), but not of the pretreatment with a selective 5-HT_{1A} receptor antagonist (WAY100635, canola oil) ($F(1,24)=0.54$, $P<0.469$) nor pretreatment × treatment interaction (WAY100635, bis selenide) ($F(1,24)=0.04$, $P<0.849$). Administration of bis selenide with WAY100635 to mice did not modify the number of crossings in the OFT (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24)=1.94$, $P<0.176$), pretreatment (WAY100635, canola oil) ($F(1,24)=0.14$, $P<0.709$) nor pretreatment × treatment interaction (WAY100635, bis selenide ($F(1,24)=0.07$, $P<0.793$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and WAY100635 (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24)=0.64$, $P<0.430$), pretreatment (WAY100635, canola oil) ($F(1,24)=0.03$, $P<0.865$) nor pretreatment × treatment interaction (WAY100635, bis selenide ($F(1,24)=0.04$, $P<0.845$).

As shown in Fig. 5C the pretreatment of mice with ketanserin (1 mg/kg, i.p., a 5-$HT_{2A/2C}$ receptor antagonist, a subeffective dose in the immobility time in the TST) prevented the effect of bis selenide (1 mg/kg, p.o.) in the TST. Two-way ANOVA revealed a main effect of

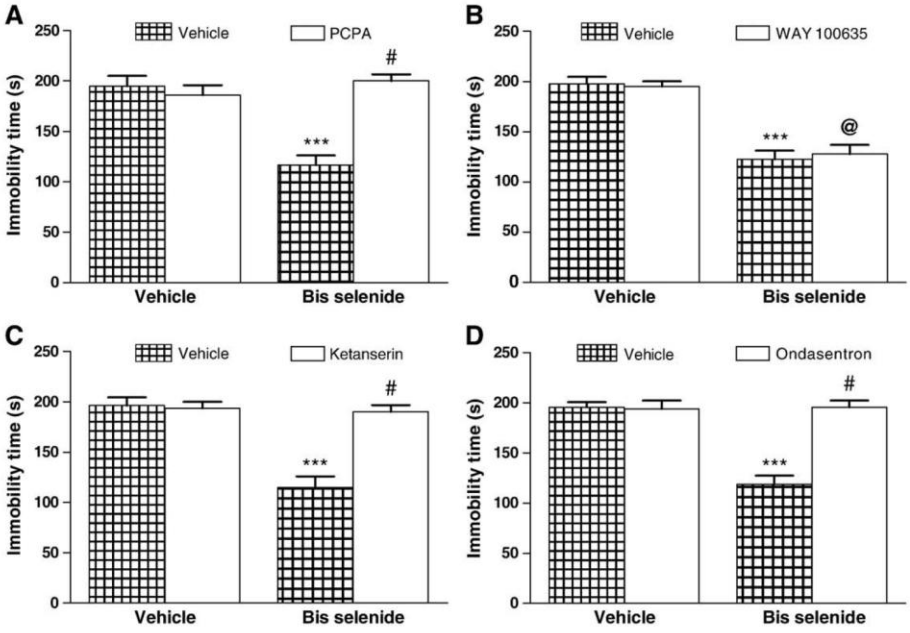


Fig. 5. Effect of pretreatment of mice with PCPA (100 mg/kg, i.p., panel A), WAY100635 (0.1 mg/kg, s.c., panel B), ketanserin (1 mg/kg, i.p., panel C) or ondasentron (1 mg/kg, i.p., panel D) on the action of bis selenide (1 mg/kg, p.o.) in the mouse TST. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by Two-Way Analysis of Variance (ANOVA) followed by Newman–Keuls test. ***$P<0.001$ compared to the vehicle group, @ compared to the vehicle group pretreated with antagonist and # compared to the bis selenide group pretreated with vehicle.

treatment (saline, bis selenide) ($F(1, 24) = 22.68$, $P<0.001$), pretreatment with a 5-$HT_{2A/2C}$ receptor antagonist (ketanserin, canola oil) ($F(1,24) = 19.57$, $P<0.001$) and pretreatment × treatment interaction (ketanserin, bis selenide) ($F(1,24) = 1.86$, $P<0.041$). The number of crossings was unmodified by the administration of bis selenide and ketanserin (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.98$, $P<0.333$), pretreatment (WAY100635, canola oil) ($F(1,24) = 0.23$, $P<0.636$) nor pretreatment × treatment interaction (WAY100635, bis selenide ($F(1,24) = 0.35$, $P<0.561$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and WAY100635 (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.02$, $P<0.891$), pretreatment (WAY100635, canola oil) ($F(1,24) = 0.01$, $P<0.984$) nor pretreatment × treatment interaction (WAY100635, bis selenide ($F(1,24) = 0.06$, $P<0.808$).

Results depicted in Fig. 5D show that the pretreatment of mice with ondasentron (1 mg/kg, i.p., a 5-HT_3 receptor antagonist, a subeffective dose in the immobility time in the TST) blocked the reduction in immobility time elicited by bis selenide (1 mg/kg, p.o.) in the TST. Two-way ANOVA revealed a main effect of treatment (saline, bis selenide) ($F(1,24) = 27.15$, $P<0.001$), pretreatment with a 5-HT_3 receptor antagonist (ondasentron, canola oil) ($F(1,24) = 33.26$, $P<0.001$) and pretreatment × treatment interaction (ondasentron, bis selenide) ($F(1,24) = 29.69$, $P<0.001$). The number of crossings was unmodified by the administration of bis selenide and ondasentron (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.15$, $P<0.698$), pretreatment (ondasentron, canola oil) ($F(1,24) = 0.1$, $P<0.903$) nor pretreatment × treatment interaction (ondasentron, bis selenide ($F(1,24) = 0.06$, $P<0.803$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and ondasentron (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.84$, $P<0.368$), pretreatment (ondasentron, canola oil) ($F(1,24) = 1.43$, $P<0.242$) nor pretreatment × treatment interaction (ondasentron, bis selenide ($F(1,24) = 0.92$, $P<0.348$).

3.5. Interaction of bis selenide with antidepressants in the TST

Results depicted in Fig. 6A show that administration of subeffective doses of bis selenide (0.1 mg/kg, p.o.) and fluoxetine (1 mg/kg, p.o.) elicited an antidepressant-like effect in the mouse TST. Two-way ANOVA revealed a main effect of treatment (saline, bis selenide) ($F(1, 24) = 13.92$, $P<0.001$), pretreatment (fluoxetine, canola oil) ($F(1,24) = 18.16$, $P<0.003$) and pretreatment × treatment interaction (fluoxetine, bis selenide) ($F(1,24) = 15.41$, $P<0.02$). The number of crossings was unmodified by the administration of bis selenide and fluoxetine (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.88$, $P<0.357$), pretreatment (fluoxetine, canola oil) ($F(1,24) = 0.40$, $P<0.532$) nor pretreatment × treatment interaction (fluoxetine, bis selenide ($F(1,24) = 0.63$, $P<0.435$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and fluoxetine (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.02$, $P<0.880$), pretreatment (fluoxetine, canola oil) ($F(1,24) = 0.21$, $P<0.652$) nor pretreatment × treatment interaction (fluoxetine, bis selenide ($F(1,24) = 0.12$, $P<0.731$).

Fig. 6B shows that administration of subeffective doses of imipramine (0.1 mg/kg, p.o.) and bis selenide (0.1 mg/kg, p.o.) did not elicit an antidepressant-like effect in the mouse TST. Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.81$, $P<0.3765$), pretreatment (imipramine, canola oil) ($F(1,24) = 0.11$, $P<0.744$) nor pretreatment × treatment interaction (imipramine, bis selenide) ($F(1,24) = 0.02$, $P<0.879$). The number of crossings was unmodified by the administration of bis selenide and imipramine (data not shown). Two-way ANOVA did not reveal a

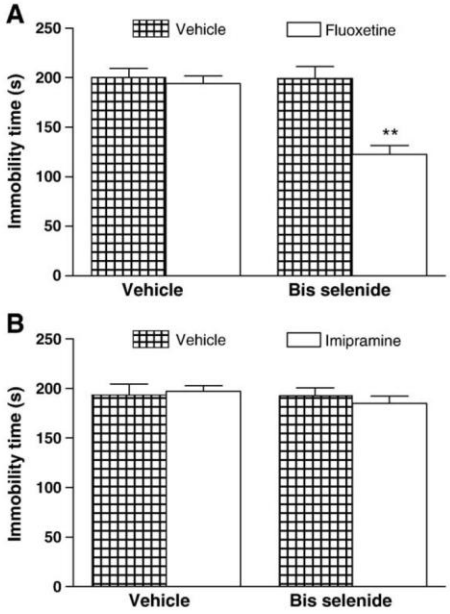


Fig. 6. Effect of subeffective doses of bis selenide (0.1 mg/kg, p.o.) and fluoxetine (1 mg/kg, p.o., panel A) or imipramine (0.1 mg/kg, p.o., panel B) on the immobility time in the TST. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by Two-Way Analysis of Variance (ANOVA) followed by Newman–Keuls test. $^{**}P<0.01$ compared to the vehicle group and to the bis selenide group pretreated with vehicle.

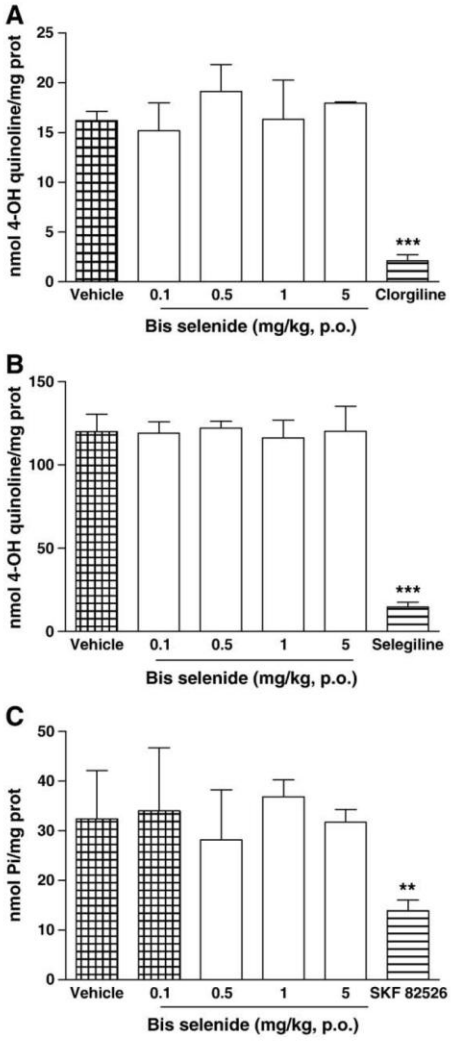


Fig. 7. Effect of bis selenide on MAO-A (panel A), MAO-B (panel B) and Na^+ K^+ ATPase (panel C) activities. Mice were pretreated with bis selenide (0.1–5 mg/kg, p.o.) or vehicle (canola oil, p.o.) for MAO-A, MAO-B and Na^+ K^+ ATPase activities measurement. Values are expressed as mean ± S.E.M. of 4 animals. Clorgiline (a MAO- A inhibitor, 100 nM), selegiline (a MAO-B inhibitor, 100 nM) or SKF 82526 (1 mM) as positive control. $^{**}P<0.01$ and $^{***}P<0.001$ compared to the vehicle group (canola oil, p.o.).

main effect of treatment (saline, bis selenide) ($F(1,24) = 1.15$, $P<0.293$), pretreatment (imipramine, canola oil) ($F(1,24) = 0.02$, $P<0.893$) nor pretreatment × treatment interaction (imipramine, bis selenide ($F(1,24) = 0.87$, $P<0.359$). No significant effect was observed on the number of rearing in the OFT in mice treated with bis selenide and imipramine (data not shown). Two-way ANOVA did not reveal a main effect of treatment (saline, bis selenide) ($F(1,24) = 0.57$, $P<0.458$), pretreatment (imipramine, canola oil) ($F(1,24) = 0.01$, $P<0.925$) nor pretreatment × treatment interaction (imipramine, bis selenide ($F(1,24) = 0.02$, $P<0.887$).

3.6. Effect of bis selenide on MAO-A and MAO-B activities

One-way ANOVA of MAO-A ($F(4,15) = 0.99$, $P<0.351$) (Fig. 7A) or MAO-B ($F(4,15) = 1.45$, $P<0.255$) (Fig. 7B) activity demonstrated that treatment (canola oil, bis selenide) did not alter the activities of these enzymes in brains of mice.

3.7. Effect of bis selenide on Na^+ K^+ ATPase activity

One-way ANOVA of Na^+ K^+ ATPase activity ($F(4,15) = 0.88$, $P<0.341$) (Fig. 7C) revealed that treatment (canola oil, bis selenide) did not modify the enzyme activity in brains of mice.

4. Discussion

In the present study we demonstrate that bis selenide, orally administered, was effective in producing a significant antidepressant-like effect, which was dependent on an interaction with the serotonergic system (5-$HT_{2A/2C}$ and 5-HT_3 receptors), but not with dopaminergic and noradrenergic systems. Bis selenide did not alter the activity of Na^+ K^+ ATPase, MAO-A or MAO-B demonstrating that these enzymes did not directly contribute to the antidepressant-like effect of bis selenide in the FST and TST. In addition, administration of subeffective doses of bis selenide and fluoxetine (a conventional antidepressant) produced an antidepressant-like effect in the TST, a predictive test of antidepressant action.

Bis selenide, given by p.o. route, caused an antidepressant-like effect when assessed in two animal models of depression used to screen new antidepressant drugs (Porsolt et al., 1977; Steru et al., 1985; Zomkowski et al., 2002). Thus, the fact that bis selenide administration is active in both tests supports the hypothesis that this compound may play a role in the modulation of depression. However, it is important to point out that the TST and FST are models of antidepressant efficacy with poor face validity. The main limitation of

these models is that they may poorly reflect the mechanisms involved in the human situation. As a result, the biological basis of the animal behavior in these tests (climbing, swimming and diving) may be very different from the biological basis of human symptoms (Nestler et al., 2002).

Depression seems to be associated with a hypofunction of the noradrenergic system. In fact, some antidepressants such as reboxetine and mirtazapine act by increasing the synaptic availability of noradrenaline (NA) (Brunello et al., 2003). The decrease in the immobility time elicited by bis selenide was not reversed by prazosin, yohimbine and propranolol suggesting that α1-, α2- and β-adrenoceptors are not involved in the action of bis selenide in the mouse TST. Although the experimental data support the idea that α1-, α2- and β-adrenoceptors are not involved in the bis selenide effect, without dose–response curves we cannot establish firm conclusions regarding the complete contributions of noradrenergic receptors in the behavioral response to bis selenide in the mouse TST.

A role for dopamine (DA) deficiency in the pathophysiology of depression is supported by studies demonstrating reduced levels of DA and its metabolite homovanillic acid (Papakostas, 2006) as well as increased DA D_1/D_2 receptor binding (Shah et al., 1997) in depressed patients when compared to normal individuals. Our results demonstrate that a selective DA D_1 receptor antagonist, SCH23390, and a selective DA D_2 receptor antagonist, sulpiride, did not prevent the anti-immobility effects caused by bis selenide in the TST. The decrease in the immobility time elicited by bis selenide was not reversed by dopaminergic antagonists suggesting that this neurotransmitter system is not involved in the antidepressant-like action of bis selenide in the mouse TST. However, single doses of pharmacological compounds were used as pretreatment to bis selenide therefore we cannot draw more concrete conclusions regarding the roles of dopaminergic (D_1 and D_2) receptors in the behavioral response to bis selenide in the mouse TST.

A large number of experimental and clinical studies show that the 5-HT system is strongly associated to the neural regulation of mood and evidences indicate abnormalities in 5-HT neurotransmission in the pathophysiology of depression (Wong and Licinio, 2001). Drugs affecting 5-HT neurotransmission, such as those inhibiting 5-HT reuptake at nerve terminals or inhibiting monoamines metabolism (MAO inhibitors) are effective in treating depression (Wong and Licinio, 2001; Elhwuegi, 2004). In the present study, the depletion of endogenous 5-HT with a tryptophan hydroxylase inhibitor, PCPA, at a dose known to decrease the content of 5-HT, reversed the effect of bis selenide demonstrating the involvement of the 5-HT system in its antidepressant-like action. In addition, selective antagonists of 5-$HT_{2A/2C}$ and 5- HT_3 receptors blocked the antidepressant-like effect elicited by bis selenide in the TST. There are studies showing that 5-$HT_{2A/2C}$ antagonism has a role in the mechanism underlying the antidepressant-like effect of certain antidepressants in the FST (Elhwuegi, 2004; Redrobe and Bourin, 1997). The involvement of 5-HT_3 receptors in the mechanism of action of antidepressants was evidenced by the fact that different classes of antidepressants modulated 5-HT_3 receptors (Krishnan et al., 1991; Ishihara and Sasa, 2001; Eisensamer et al., 2003).

Although pharmacological evidences have pointed out the involvement of serotonergic receptors (5-$HT_{2A/2C}$ and 5-HT_3), but not of serotonergic (5-HT_{1A}) noradrenergic (α_1, α_2 and β), and dopaminergic (D_1 and D_2) receptors, in the antidepressant-like effect elicited by bis selenide in the mouse TST, we admit that the lack of complete full dose–response curves for all treatments is a shortcoming of this study.

An important finding of our study was that a single oral dose of bis selenide (0.1–5 mg/kg, p.o.) did not alter MAO-A, MAO-B and Na^+ K^+ ATPase activities in whole brain preparations. However, we should not rule out the altered activity of these enzymes in discrete brain regions such as hippocampus and mesocorticolimbic dopamine system, which may be masked or washed out by examining activity in the entire brain.

Finally, another finding of this study was that the classical antidepressant fluoxetine, a selective 5-HT reuptake inhibitor (SSRI), was able to potentiate the action of subeffective dose of bis selenide in the TST. This result suggests that the effect of bis selenide in the TST in mice seems to be similar to the effect of fluoxetine, regarding the interaction with receptor subtypes. Moreover this finding could suggest that bis selenide directly or indirectly stimulates 5-HT release in brain projection areas related to emotional integration to the augmentation of the antidepressant-like effect in the mouse TST. Fluoxetine is commonly used to treat major depression (Holtzheimer and Nemeroff, 2006). Considering the need for safer, more effective, and faster acting treatments for depression (Berton and Nestler, 2006), the antidepressant-like effect elicited by the association of bis selenide with a conventional antidepressant, fluoxetine, could indicate a new strategy to decrease the doses and side effects of the antidepressant prescribed.

Conversely, coadministration of subeffective doses of imipramine and bis selenide did not exert antidepressant-like effect in the mouse TST. This result indicates that there is no interaction between imipramine and bis selenide in that the mechanisms of antidepressant effects of each drug do not interfere. Rather, it may be that imipramine and bis selenide exert their effects in such a way that there is no link between the cascades of sub-cellular events. This result reinforces the hypothesis that the noradrenergic system is not involved in the mechanisms of the antidepressant-like action of bis selenide. Further studies are necessary to explore the biochemical basis of bis selenide/5-HT/NA modulation in other paradigms of depression.

5. Conclusion

Results demonstrate pharmacological evidences supporting the antidepressant-like action elicited by bis selenide in the FST and TST. Bis selenide antidepressant-like effect may be related to serotonergic receptors (5-$HT_{2A/2C}$ and 5-HT_3). Since no dose–response curves were carried out, we cannot draw more concrete conclusions regarding the roles of dopaminergic, noradrenergic and 5-HT_{1A} receptors in the behavioral response of mice treated with bis selenide. The present study demonstrate that the noradrenergic (α_1, α_2 and β), dopaminergic (D_1 and D_2), and serotonergic (5-HT_{1A}) receptors as well as MAO-A, MAO-B and Na^+ K^+ ATPase activities are not directly involved in the antidepressant-like effect of bis selenide in the mouse TST.

Acknowledgements

The financial support by UFSM, FAPERGS, CAPES and CNPq is gratefully acknowledged. This study was supported in part by the FINEP research grant "Rede Instituto Brasileiro de Neurociência (IBN-Net)" # 01.06.0842-00.

References

Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 2006;7:137–51.
Binfaré RW, Rosa AO, Lobato KR, Santos AR, Rodrigues AL. Ascorbic acid administration produced an antidepressant-like effect: evidence for the involvement of monoaminergic neurotransmission. Prog Neuropsychopharmacol Biol Psychiatry 2009;33:530–40.
Bourin M, Chenu F, Ripoll N, David DJP. A proposal of decision tree to screen putative antidepressants using forced swim and tail suspensions tests. Behav Brain Res 2005;164:266–9.
Burk RF. Selenium, an antioxidant nutrient. Nutr Clin Care 2002;5:75–9.
Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein–dye binding. Anal Biochem 1976;72:248–54.
Brocardo PS, Budni J, Kaster MP, Santos ARS, Rodrigues ALS. Folic acid administration produces an antidepressant-like effect in mice: evidence for the involvement of the serotonertic and noradrenergic systems. Neuropharmacology 2008;54:464–73.
Brunello N, Blier P, Judd LL, Mendlewicz J, Nelson CJ, Souery D, et al. Noradrenaline in mood and anxiety disorders: basic and clinical studies. Int Clin Psychopharmacol 2003;18:191–202.

Cunha MP, Machado DG, Bettio LEB, Capra JC, Rodrigues ALS. Interaction of zinc with antidepressants in the tail suspension test. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:1913–20.
D'Aquila PS, Collu M, Gessa GL, Serra G. The role of dopamine in the mechanism of action of antidepressant drugs. Eur J Pharmacol 2000;405:365–73.
Deniker P. Psychopharmacology and biological psychiatry, historical review. Soins Psychiatr 1983;37:5–6.
Eisensamer B, Rammes G, Gimpl G, Shapa M, Ferrari U, Hapfelmeier G, et al. Antidepressants are functional antagonists at the serotonin type 3 (5-HT3) receptor. Mol Psychiatry 2003;12:994-1007.
Elhwuegi AS. Central monoamines and their role in major depression. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:435–51.
El-Mallakh RS, Wyatt RJ. The Na^+ K^+ ATPase hypothesis for bipolar illness. Biol Psychiatry 1995;37:235–44.
Fiske CH, Subbarow YJ. The calorimetric determination of phosphorus. Biol Chem 1925;66:375–81.
Hawkes WC, Hornbostel L. Effects of dietary selenium on mood in healthy men living in a metabolic research unit. Biol Psychiatr 1996;39:121–8.
Holtzheimer PE, Nemeroff CB. Advances in the treatment of depression. J NeuroRx 2006;3:42–56.
Ishihara K, Sasa M. Potentiation of 5-HT3 receptor functions in the hippocampal CA1 region of rats following repeated electroconvulsive shock treatments. Neurosci Lett 2001;307:37–40.
Jesse CR, Savegnago L, Nogueira CW. Role of nitric oxide/ cyclic GMP/K^+ channel pathways in the antinociceptive effect caused by 2,3-bis(mesitylseleno)propenol. Life Sci 2007;81:1694–702.
Jesse CR, Savegnago L, Nogueira CW. Spinal mechanisms of antinociceptive effect caused by oral administration of bis selenide in mice. Brain Res 2008;1231:25–33.
Kaster MP, Raupp I, Binfaré RW, Andreatini R, Rodrigues AL. Antidepressant-like effect of lamotrigine in the mouse forced swimming test: evidence for the involvement of the noradrenergic system. Eur J Pharmacol 2007;565:119–24.
Kulkarni SK, Bhutani MK, Bishnoi M. Antidepressant activity of curcumin: involvement of serotonin and dopamine system. Psychopharmacology 2008;201:435–42.
Krajl M. A rapid microfluorimetric determination of monoamine oxidase. Biochem Pharmacol 1965;14:1683–5.
Krishnan KR, Doraiswamy PM, Figiel GS, Husain MM, Shah SA, Na C, et al. Hippocampal abnormalities in depression. J Neuropsychiatry Clin Neurosci 1991;3:387–91.
Matsumoto T, Furuta T, Nimura Y, Suzuki O. 3-(*p*-Hydroxyphenyl)propionic acid as a new fluorogenic reagent for amine oxidase assays. Anal Biochem 1984;138:133–6.
Millan MJ. The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. Eur J Pharmacol 2004;500:371–84.
Moro AV, Nogueira CW, Barbosa NBV, Menezes PH, Rocha JBT, Zeni G. Highly stereoselective one-pot producers to prepare bis- and tris chalcogenide alkenes via addition of disulfides and diselenides to terminal alkynes. J Org Chem 2005;70:5257–68.
Musselman DL, Evans DL, Nemeroff CB. The relationship of depression to cardiovascular disease: epidemiology, biology, and treatment. Arch Gen Psychiatry 1998;55:580–92.
Nemeroff CB, Owens MJ. Treatment of mood disorders. Nat Neurosci 2002;5:1068–70 Suppl.
Nestler EJ, Barrot M, Dileone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron 2002;34:13–25.
Papakostas GI. Dopaminergic-based pharmacotherapies for depression. Eur Neuropsychopharmacol 2006;16:391–402.
Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266:730–2.
Rayman MP. The importance of selenium to human health. Lancet 2000;356:23–41.
Reddy PL, Khanna S, Subhash MN, Channabasavanna SM, Rao BS. Erythrocyte membrane sodium–potassium adenosine triphosphatase activity in affective disorders. J Neural Transm Gen Sect 1992;89:209–18.
Redrobe JP, Bourin M. Partial role of 5-HT2 and 5-HT3 receptors in the activity of antidepressants in the mouse forced swimming test. Eur J Pharmacol 1997;325:129–35.
Rodrigues ALS, Da Silva GL, Mateussi AS, Fernandes ES, Miguel OG, Yunes RA, et al. Involvement of monoaminergic system in the antidepressant-like effect of the hydroalcoholic extract of *Siphocampylus verticillatus*. Life Sci 2002;70:1347–58.
Savegnago L, Jesse CR, Moro AV, Borges VC, Santos FW, Rocha JBT, Nogueira CW. Bis selenide alkene derivatives: a class of potential antioxidant and antinociceptive agents. Pharmacol Biochem Behav 2006;83:221–9.
Shah BH, Saeed SA, Rashid F, Gilani AH. Dopamine potentiation of calcium ionophore, A-23187-induced platelet aggregation. Adv Exp Med Biol 1997;407:537–40.
Sher L. Depression and suicidal behavior in alcohol abusing adolescents: possible role of selenium deficiency. Minerva Pediatr 2008;60:201–9.
Soto-Otero R, Mendez-Alvarez E, Hermida-Ameijeiras A, Sanchez-Sellero I, Cruz-Landeira A, Lamas LM. Inhibition of brain monoamine oxidase activity by the generation of hydroxyl radicals: potential implications in relation to oxidative stress. Life Sci 2001;69:879–89.
Steffens DC, Krishnan KR, Helms MJ. Are SSRIs better than TCAs? Comparison of SSRIs and TCAs: a meta-analysis. Depress Anxiety 1997;6:10–8.
Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 1985;85:367–70.
Wong ML, Licinio J. Research and treatment approaches to depression. Nat Rev Neurosci 2001;2:343–51.
Zomkowski ADE, Hammes L, Lin J, Calixto JB, Santos ARS, Rodrigues ALS. Agmatine produces antidepressant-like effects in two models of depression in mice. NeuroReport 2002;13:387–91.

4.2. Envolvimento da via L-arginina-óxido nítrico-guanosina monofosfato cíclico no efeito do tipo antidepressivo do bis seleneto no teste da suspensão da cauda em camundongos

4.2.1. Artigo 2

Envolvimento da via l-arginina-óxido nítrico-guanosina monofosfato cíclico no efeito do tipo antidepressivo do bis seleneto no teste da suspensão da cauda em camundongos

Cristiano R. Jesse, Ethel A. Wilhelm, Cristiani F. Bortolatto, João B.T. Rocha, Cristina W. Nogueira

European Journal of Pharmacology 635 (2010) 135-141

European Journal of Pharmacology 635 (2010) 135–141

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural Pharmacology

Involvement of L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effect of bis selenide in the mouse tail suspension test

Cristiano R. Jesse, Ethel A. Wilhelm, Cristiani F. Bortolatto, João B.T. Rocha, Cristina W. Nogueira *

Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

ARTICLE INFO

Article history:
Received 17 August 2009
Received in revised form 20 February 2010
Accepted 4 March 2010
Available online 20 March 2010

Keywords:
Bis selenide
Selenium
Antidepressant
Oxide nitric
Cyclic guanosine monophosphate
Immobility

ABSTRACT

The present study investigated a possible antidepressant-like effect of bis selenide by using the forced swimming and the tail suspension tests. The involvement of the L-arginine–nitric oxide–cyclic guanosine monophosphate signaling pathway in the antidepressant-like action of bis selenide was investigated. Bis selenide, given by oral route at doses of 0.5–5 mg/kg, decreased the immobility time in the forced swimming and tail suspension tests. Pretreatment with L-arginine (750 mg/kg, intraperitoneal, i.p., a nitric oxide precursor), sildenafil (5 mg/kg, i.p., a phosphodiesterase 5 inhibitor) or S-nitroso-N-acetyl-penicillamine (25 μg/site, intracerebroventricular, i.c.v., a nitric oxide donor) reversed the reduction in the immobility time elicited by bis selenide (1 mg/kg, p.o.) in the tail suspension test. Bis selenide (0.1 mg/kg, p.o., a subeffective dose) produced a synergistic antidepressant-like effect with N^G-nitro-L-arginine (0.3 mg/kg, i.p., an inhibitor of nitric oxide synthase) or 7-nitroindazole (25 mg/kg, i.p., a specific neuronal nitric oxide synthase inhibitor) in the tail suspension test. Pretreatment of animals with methylene blue (10 mg/kg, i.p., an inhibitor of nitric oxide synthase and soluble guanylate cyclase) or 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one (30 pmol, i.c.v., a specific inhibitor of soluble guanylate cyclase), at subeffective doses, caused a synergistic effect with bis selenide in the tail suspension test. Bis selenide (1 mg/kg, p.o.), at an effective dose in the forced swimming and tail suspension tests, caused a significant decrease in the mouse cerebral nitrate/nitrite levels. The antidepressant-like effect of bis selenide in the tail suspension test is dependent on the inhibition of the L-arginine–nitric oxide–cyclic guanosine monophosphate pathway.

1. Introduction

Depressive disorders represent a major public health problem due to their high prevalence and psychosocial impact. The World Health Organization (WHO) ranks unipolar depression as the fourth most important cause of mortality and disability (Murray and Lopez, 1997). Although underlying pathophysiological mechanisms of depression are not completely identified, novel targets have been identified for the development of new pharmacological and behavioural treatments (D'Aquila et al., 2000; Elhwuegi, 2004; Papakostas, 2006).

Numerous neural pathways are involved in the pathophysiology of depression. A great number of neurotransmitters participate in the underlying mechanisms of drugs (Palucha and Pilc, 2002). Nitric oxide donors and inhibitors have been shown to affect serotonin release in a dose-dependent manner in rodents (Lorrain and Hull, 1993; Kaehler et al., 1999). Studies have shown that the inhibition of nitric oxide synthase could be used as a strategy to enhance the clinical efficacy of serotonergic antidepressants (Smith and Whitton, 2000; Harkin et al., 2004). Further support for the hypothesis that the inhibition of nitric oxide synthase, with a subsequent decrease in the concentration of cyclic guanosine monophosphate (Snyder, 1992), may produce antidepressant-like effects, at least under certain conditions, comes from the reported reduction in the immobility time in the forced swimming test elicited either by the administration of methylene blue, which acts as a direct inhibitor of both nitric oxide synthase and soluble guanylate cyclase (Eroglu and Caglayan, 1997) or by the specific inhibitor of soluble guanylate cyclase activity, 1H-(1,2,4)-oxodiazolo (4,3-a)quinoxalin-1-one (Heiberg et al., 2002; Kaster et al., 2005; Ergün and Ergün, 2007).

Bis selenide has been reported to have pharmacological properties, such as antioxidant (Savegnago et al., 2006), antinociceptive and anti-inflammatory (Jesse et al., 2007, 2008a). There are also other organoselenium compounds (diphenyl diselenide and ebselen) whose therapeutic potential has been assessed in a variety of animal models such as the forced swimming and tail suspension tests (Savegnago et al., 2007, 2008; Posser et al., 2009). In addition, the inhibition of nitric oxide- cyclic guanosine monophosphate synthesis was previously shown to be involved in the diphenyl diselenide antidepressant-like effect (Savegnago et al., 2008).

In this way, it has been reported that low selenium status leads to depressed mood while high dietary and/or supplementary selenium improves mood. Moreover, several research groups have demonstrated that low selenium status has been associated with a significantly

* Corresponding author. Tel.: +55 55 32209611; fax: +55 55 32208978.
E-mail address: criswn@quimica.ufsm.br (C.W. Nogueira).

0014-2999/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2010.03.019

increased incidence of depression, anxiety, confusion, and hostility (Hawkes and Hornbostel, 1996; Rayman, 2000).

The primary aim of the present study was to investigate the antidepressant-like effect of bis selenide administered by per oral (p.o.) route to mice in the tail suspension and forced swimming tests, being that both tests used to screen new antidepressants (Cryan et al., 2002; Bourin et al., 2005). The second objective of this study was to investigate the involvement of the L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like activity of bis selenide in the mouse tail suspension test. Finally, the current study was also performed to examine cerebral nitrate/nitrite levels in mice treated with bis selenide.

2. Materials and methods

2.1. Animals

The behavioural experiments were conducted using male adults Swiss mice (25–35 g) maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle, with lights on at 6:00 a.m. 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. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

2.2. Chemicals

Bis selenide [(*Z*)-2,3-bis(4-chlorophenylselanyl)prop-2-en-1-ol] (Fig. 1) was prepared and characterized in our laboratory by the method previously described. For further details, refer to the following compound (2u) (Moro et al., 2005). The chemical purity of bis selenide (99.9%) was determined by gas chromatography/high-performance liquid chromatography. Chemo-physical properties: Yield: 0.285 g (65%). ^{1}H NMR, $CDCl_3$, 400 MHz, (ppm): 7.51–7.45 (m, 4H), 7.34 (s, 1H), 7.30–7.25 (m, 4H), 4.15 (d, *J*) (5.41 Hz, 2H), 1.83 (t, *J*) (6.28 Hz, 1H). ^{13}C NMR, $CDCl_3$, 100 MHz, (ppm): 134.5, 134.3, 133.9, 133.6, 133.4, 132.3, 129.5, 129.5, 128.2, 126.7, 67.5. MS (EI, 70 eV) *m/z* (relative intensity): 438 (10), 379 (17), 251 (62), 228 (52), 216 (100), 190 (77), 156 (58), 111 (38). HRMS: calcd for $C_{15}H_{12}Cl_2OSe_2$ 437.8596, found 437.8601. The following drugs were used: L-arginine, methylene blue, S-nitroso-N-acetyl-penicillamine, sildenafil, (1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one), N^G-nitro-L-arginine, 7-nitroindazole, VCl_3, N-(1-naphthyl) ethylene-diamine dihydrochloride, sulfanilamide and all other chemicals were purchased from Sigma Chemical USA.

2.3. Treatment

2.3.1. Antidepressant-like effect of bis selenide in the tail suspension and forced swimming tests

Time-course analysis of the antidepressant-like effect of bis selenide was performed. Mice were pretreated with bis selenide (5 mg/kg, p.o.) or with canola oil (10 ml/kg, p.o.) 0.5–8 h before the tail suspension test.

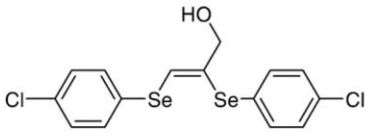


Fig. 1. Chemical structure of bis selenide (*Z*)-2, 3-bis(4-chlorophenylselanyl)prop-2-en-1-ol.

In order to assess the antidepressant-like effect of bis selenide, this compound was administered (dose range: 0.1–5 mg/kg, p.o.) 1 h before the forced swimming test, tail suspension test or open field test. We evaluated the locomotor activity in mice and immediately after the same mice were assessed in the forced swimming test or the tail suspension test.

2.3.2. Mechanisms involved in the antidepressant-like effect of bis selenide

The role played by the L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effect caused by bis selenide in the tail suspension test was investigated in distinct groups of animals. For this purpose, mice were pretreated with L-arginine, a precursor of nitric oxide (750 mg/kg, i.p., a dose that produces no effect in the tail suspension test), sildenafil, a specific type 5 phosphodiesterase inhibitor (5 mg/kg, i.p., a dose that produces no effect in the tail suspension test) or with the nitric oxide donor, S-nitroso-N-acetyl-penicillamine (25 μg/site, i.c.v., a nitric oxide donor, a dose that produces no effect in the tail suspension test). Thirty minutes after L-arginine, sildenafil or S-nitroso-N-acetyl-penicillamine, bis selenide (1 mg/kg, p.o., a dose effective in the tail suspension test) or vehicle was administered, and 1 h later the tail suspension test was carried out.

In another set of experiments, the synergistic effect of bis selenide (0.1 mg/kg, p.o., a subeffective dose) with a subeffective dose of N^G-nitro-L-arginine (0.3 mg/kg, i.p., an inhibitor of nitric oxide synthase), 7-nitroindazole (25 mg/kg, i.p., a specific neuronal nitric oxide synthase inhibitor), methylene blue (10 mg/kg, i.p., an inhibitor of nitric oxide synthase and soluble guanylate cyclase) or 1H-[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one (30 pmol/site, i.c.v., a specific soluble guanylate cyclase inhibitor) was investigated. Bis selenide (0.1 mg/kg, p.o.) or vehicle (p.o.) was administered 1 h before drugs. After 30 min of the i.p. administration of N^G-nitro-L-arginine, 7-nitroindazole, methylene blue or the i.c.v. injection of 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one the tail suspension test was carried out. The open field test was carried out to rule out any psychostimulant effect of the interaction of drugs and bis selenide.

2.3.3. Drug administration schedule

All drugs were dissolved in saline solution (0.9% NaCl), except 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one and 7-nitroindazole that were dissolved in 15% dimethyl sulfoxide and were made up to a final volume by the addition of 0.9% NaCl. Bis selenide was dissolved in canola oil. Mice received all drugs in a constant volume of 10 ml/kg body weight, except S-nitroso-N-acetyl-penicillamine and 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one which were injected by i.c.v. route (5 μl/site). Appropriate vehicle treated groups were also assessed simultaneously. Doses and the schedule of administration were chosen on the basis of experiments previously performed by our research group (Savegnago et al., 2008; Jesse et al., 2008b) and others (Almeida et al., 2006; Brocardo et al., 2008).

2.3.4. Intracerebroventricular injection technique

The i.c.v. administration was performed under light ether anesthesia. Briefly, a 0.4 mm external diameter hypodermic needle attached to a cannula, which was linked to a 25 μl Hamilton syringe, was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse. A volume of 5 μl was then administered in the left lateral ventricle. The injection was given over 30 s, and the needle remained in place for another 30 s in order to avoid the reflux of the substances injected. The injection site was 1 mm to the right or left from the mid-point on a line drawn through to the anterior base of the ears (Kaster et al., 2005). To ascertain that the drugs were administered exactly into the cerebral ventricle, the brains were dissected and examined macroscopically after the test. Control animals received a saline injection by i.c.v. route in a similar manner.

2.4. Behavioural analysis

2.4.1. Forced swimming test

The test was conducted using the method described by Porsolt et al. (1977). Briefly, mice were individually forced to swim in open cylinders (25 cm height × 10 cm diameter) containing 19 cm of water at 25 ± 1 °C. The duration of immobility was scored during the 6 min test period as described previously (Kaster et al., 2005). Each mouse was recorded as immobile when floating motionless or making only those movements necessary to keep its head above water.

2.4.2. Tail suspension test

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period (Kaster et al., 2005).

2.4.3. Open field test

To assess the possible effects of bis selenide on locomotor and exploratory activities, mice were evaluated in the open field test. The open field was made of plywood and surrounded by walls 30 cm in height. The floor of the openfield, 45 cm in length and 45 cm in width, was divided by masking tape markers into 09 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 6 min to record the locomotor (number of segments crossed with the four paws) (Rodrigues et al., 2002).

2.5. Ex vivo experiments

Mice were pretreated with bis selenide (a non-effective dose: 0.1 and an effective dose: 1 mg/kg, p.o.) or vehicle (canola oil, p.o.). The control group did not receive vehicle to exclude the vehicle effect on ex vivo experiments. Animals were killed after 1 h and the whole brain removed for the nitrate/nitrite determination.

2.5.1. Colorimetric determination of nitrate/nitrite content

The brain was dissected on an inverted ice-cold Petri dish and homogenized with $ZnSO_4$ (200 mM) and acetonitrile (96%), centrifuged at 16,000 × *g* at 4 °C for 30 min, and the supernatant was collected for assay of the nitrite plus nitrate content (Miranda et al., 2001). The resulting pellet was suspended in NaOH (6 M) for protein determination. NOx content was estimated in a medium containing 300 ml of 2% VCl_3 (in 5% HCl), 200 ml of 0.1% N-(1-naphthyl) ethylene-diamine dihydrochloride, 200 ml of 2% sulfanilamide (in 5% HCl). After incubating at 37 °C for 60 min, nitrite levels were determined spectrophotometrically at 540 nm, based on the reduction of nitrate to nitrite by VCl_3. Tissue nitrite and nitrate levels were expressed as nmol of nitrate/nitrite/mg of protein.

2.6. Statistical analysis

All experimental results are given as the mean (s) ± S.E.M. Comparisons between experimental and control groups were performed by one-way (bis selenide) or two-way ANOVA (inhibitors of nitric oxide synthase and/or soluble guanylate cyclase × bis selenide) followed by Newman–Keuls test for post hoc comparison when appropriate. A value of $P<0.05$ was considered to be significant. Main effects are presented only when the first order interaction was non-significant.

Table 1
Time course of the antidepressant-like effect caused by bis selenide (5 mg/kg, p.o.) in the mouse tail suspension test.

Time (h)	Immobility time (s)
Vehicle	218.8 ± 11.2
0.5	225.4 ± 9.02
1	118.9 ± 12.3[c]
2	136.2 ± 10.2[c]
4	156.8 ± 9.80[b]
6	171.2 ± 7.85[a]
8	210.2 ± 7.89

Bis selenide was administered 0.5, 1, 2, 4, 6 and 8 h before the tail suspension test. Results are expressed as mean + S.E.M. of 7 animals. One-way ANOVA followed by Newman–Keuls. [a]$P<0.05$ and [b]$P<0.01$ and [c]$P<0.001$ when compared to the vehicle treated control.

Table 2
Effect of acute administration of bis selenide in the mouse tail suspension and forced swimming tests.

Bis selenide (mg/kg, p.o.)	Immobility time (s) tail suspension test	Immobility time (s) forced swimming test
Vehicle	206.8 ± 8.40	192.3 ± 9.40
0.1	202.5 ± 11.42	185.8 ± 12.97
0.5	167.4 ± 9.96[a]	145.9 ± 11.72 [a]
1	131.8 ± 13.80[b]	117.8 ± 8.77[b]
5	126.0 ± 9.80[b]	116.0 ± 9.68[b]

Bis selenide (dose range 0.1–5 mg/kg) was administered orally 1 h before the tests. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). [a]$P<0.05$ and [b]$P<0.001$ when compared to the vehicle treated control.

3. Results

3.1. Effect of bis selenide orally administered in the mouse forced swimming, tail suspension and open field tests

A time-course analysis of the antidepressant-like profile of bis selenide was accomplished. The antidepressant-like effect of bis selenide reached its peak 1 h and remained significant up to 6 h after the compound administration (Table 1). Thus, the time point (1 h) of the maximum effect of bis selenide was chosen for all further studies.

The immobility time in the forced swimming and tail suspension tests of animals treated with bis selenide is shown in Table 2. One-way ANOVA revealed a significant effect of bis selenide, at doses of 0.5–5 mg/kg, in the forced swimming ($F(4,35) = 9.47$, $P<0.001$) and tail suspension ($F(4,35) = 12.64$, $P<0.001$) tests. Bis selenide, given by p.o. route at all doses tested, did not produce any change in the number of crossings ($F(4,35) = 0.11$, $P<0.972$) of mice in the open field (data not shown).

3.2. Analysis of the role played by the L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effect of bis selenide in the mouse tail suspension test

Results demonstrated in Fig. 2A show that the pretreatment of mice with L-arginine (750 mg/kg, i.p., a nitric oxide precursor) reversed the reduction in the immobility time elicited by bis selenide (1 mg/kg, p.o.) in the tail suspension test ($F(1,24) = 7.37$, $P<0.03$). No significant effects were observed on the number of crossings ($F(1,24) = 0.08$, $P<0.775$) in the open field test in mice treated with L-arginine and bis selenide (data not shown).

Fig. 2B shows that the pretreatment with sildenafil (5 mg/kg, i.p., a phosphodiesterase 5 inhibitor) was able to reverse the antidepressant-like effect of bis selenide (1 mg/kg, p.o.) in the tail suspension test ($F(1,24) = 23.47$, $P<0.001$). The number of crossings ($F(1,24) = 0.08$, $P<0.781$) in the open field was unmodified by the administration of sildenafil and bis selenide (data not shown).

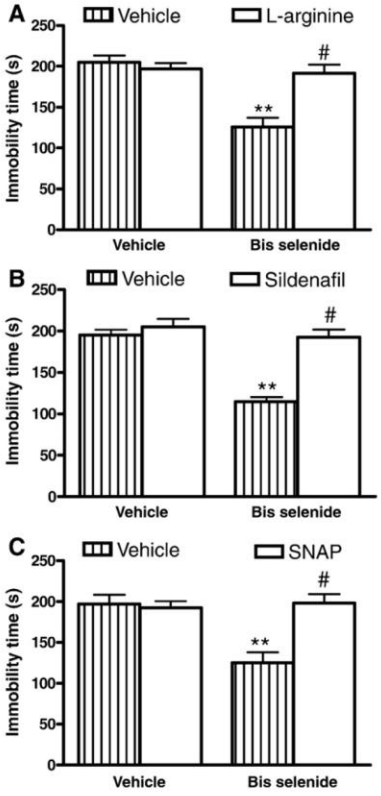


Fig. 2. Effect of pretreatment of mice with (A) L-arginine (750 mg/kg, i.p.), (B) sildenadil (5 mg/kg, i.p.) and (C) S-nitroso-N-acetyl-penicillamine (25 µl/site, i.c.v.) on bis selenide (1 mg/kg, p.o.)-induced reduction in the immobility time in the mouse tail suspension test. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by Two-Way Analysis of Variance (ANOVA) followed by Newman–Keuls test. $^{**}P<0.01$ compared to the vehicle treated group; $\#P<0.01$ compared to the bis selenide group pretreated with vehicle.

Fig. 2C shows that the pretreatment of mice with S-nitroso-N-acetyl-penicillamine (25 µg/kg, i.c.v., a nitric oxide donor) prevented the antidepressant-like effect of bis selenide (1 mg/kg, p.o.) in the tail suspension test ($F(1,24) = 5.60$, $P<0.02$). Administration of bis selenide with S-nitroso-N-acetyl-penicillamine did not modify the number of crossings ($F(1,24) = 0.52$, $P<0.477$) of mice in the open field test (data not shown).

Results illustrated in Fig. 3A show that the administration of N^G-nitro-L-arginine (an inhibitor of nitric oxide synthase, 0.3 mg/kg i.p., a subeffective dose) in combination with bis selenide (0.1 mg/kg, p.o., a subeffective dose) produced an antidepressant-like effect when compared to the administration of either drug alone ($F(1,24) = 13.53$, $P<0.001$). No significant effects were found on the number of crossings ($F(1,24) = 0.07$, $P<0.794$) in the open field test in mice treated with N^G-nitro-L-arginine and bis selenide (data not shown).

Fig. 3B shows that the administration of 7-nitroindazole (25 mg/kg i.p., a specific neuronal nitric oxide synthase inhibitor) in combination with bis selenide (0.1 mg/kg, p.o., a subeffective dose) produced an antidepressant-like effect when compared to the administration of either drug alone ($F(1,24) = 13.50$, $P<0.001$). Administration of 7-nitroindazole alone or in combination with bis selenide did not affect locomotor activity of mice in the open field test ($F(1,24) = 0.05$, $P<0.819$) (data not shown).

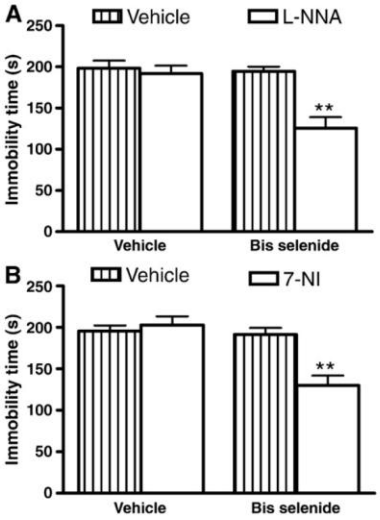


Fig. 3. The potentiating effect of (A) N^G-nitro-L-arginine (0.3 mg/kg, i.p.) and (B) 7-nitroindazole (25 mg/kg, i.p.) on the action of bis selenide (0.1 mg/kg, p.o.) in the mouse tail suspension test. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by Two-Way Analysis of Variance (ANOVA) followed by Newman–Keuls test. $^{**}P<0.01$ compared to the bis selenide group pretreated with vehicle.

Fig. 4A shows that methylene blue (10 mg/kg i.p., a subeffective dose, a direct inhibitor of both nitric oxide synthase and soluble guanylate cyclase) in combination with bis selenide (0.1 mg/kg, p.o., a subeffective dose) also produced an anti-immobility effect in the tail suspension test when compared with the administration of either drug alone ($F(1,24) = 15.12$, $P<0.007$). Administration of methylene blue (10 mg/kg i.p.) alone or in combination with bis selenide did not

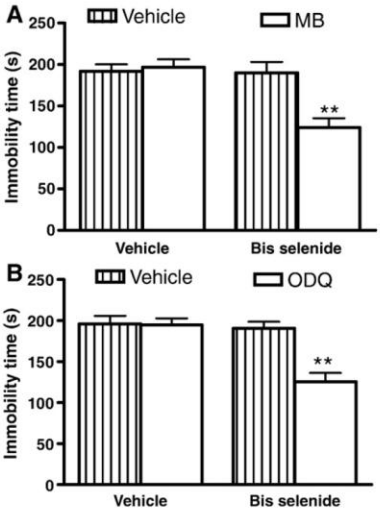


Fig. 4. The potentiating effect of (A) methylene blue (10 mg/kg, i.p.) and (B) 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one (30 pmol/site, i.c.v.) on the action of bis selenide (0.1 mg/kg, p.o.) in the mouse tail suspension test. Values are expressed as mean ± S.E.M. ($n = 7$ mice/group). Data were analyzed by Two-Way Analysis of Variance (ANOVA) followed by Newman–Keuls test. $^{**}P<0.01$ compared to the bis selenide group pretreated with vehicle.

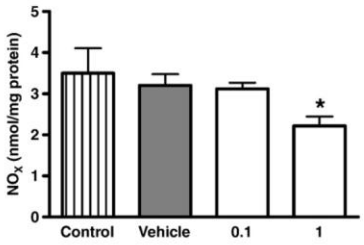


Fig. 5. Effect caused by oral administration of bis selenide on cerebral nitrate/nitrite levels in mice. Mice were pretreated with bis selenide (0.1 and 1 mg/kg, p.o.) or vehicle (canola oil, p.o.) for nitrate/nitrite measurement. Values are expressed as mean ± S.E.M. of 6 animals. Vehicle indicates the animal administered with canola oil and the asterisks denote the significance levels when compared to the vehicle group (one-way ANOVA followed by Newman–Keuls). *$P<0.05$ when compared to the vehicle treated group.

affect the ambulation of mice in the open field test ($F(1,24)=0.19$, $P<0.663$) (data not shown).

Fig. 4B shows that 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one (30 pmol, i.c.v., a subeffective dose, a direct inhibitor of soluble guanylate cyclase) in combination with bis selenide (0.1 mg/kg, p.o., a subeffective dose) produced an anti-immobility effect in the tail suspension test when compared to the administration of either drug alone ($F(1,24)=11.13$, $P<0.001$). Administration of 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one (30 pmol, i.c.v.) alone or in combination with bis selenide did not affect the number of crossings of mice in the open field test ($F(1,24)=1.44$, $P<0.241$) (data not shown).

3.3. Effect caused by bis selenide on cerebral nitrate/nitrite levels in mice

As shown in Fig. 5, treatment with an effective dose of bis selenide (1 mg/kg, p.o.) caused a significant decrease in the mouse cerebral nitrate/nitrite levels compared to that of the vehicle group ($F(3,20)=9.10$, $P<0.05$). Nitrate/nitrite levels in the brains of mice treated with a non-effective dose of bis selenide (0.1 mg/kg, p.o.) were similar to those in the vehicle group. Nitrate/nitrite levels remained unaltered in mice treated with vehicle (canola oil) and in non-treated (control) mice (Fig. 5).

4. Discussion

In this study we show that bis selenide produced an antidepressant-like effect in the mouse tail suspension and forced swimming tests. These findings support and extend published results demonstrating that organoselenium compounds produce an anti-immobility effect in the tail suspension and forced swimming tests in rodents (Savegnago et al., 2007, 2008; Posser et al., 2009). Besides, we demonstrate that the antidepressant-like effect of bis selenide in the mouse tail suspension test is probably due to the modulation of the L-arginine–nitric oxide–cyclic guanosine monophosphate pathway. The decrease in the cerebral nitrate/nitrite levels in mice treated with bis selenide, at an effective dose in the tail suspension and forced swimming tests, further support the involvement of the L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the bis selenide antidepressant-like effect.

It has become generally accepted that nitric oxide plays a significant neuromodulatory role in the nervous system and the pharmacological manipulation of the nitric oxide–cyclic guanosine monophosphate pathway may constitute a novel therapeutic approach for the treatment of depression (Da Silva et al., 2000; Yildiz et al., 2000; Volke et al., 2003; Kaster et al., 2005; Almeida et al., 2006; Jesse et al., 2008b). In this study, we show that the pretreatment of mice with L-arginine (a substrate for nitric oxide synthase) or with S-nitroso-N-acetyl-penicillamine (a nitric oxide donor) at doses that did not produce any effect in the tail suspension test, significantly inhibited the anti-immobility effect caused by bis selenide. Moreover, the reversal of the antidepressant-like effect of bis selenide in the tail suspension test by the pretreatment of mice with sildenafil (a selective type 5 phosphodiesterase inhibitor) reinforces the notion that the bis selenide administration produced a reduction in cyclic guanosine monophosphate levels, and that this target may be important for its effect in depressive-like tests. Sildenafil increases cyclic guanosine monophosphate levels in target tissues (Beavo, 1995) and reverses the anti-immobility effect of 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one in the forced swimming test (Kaster et al., 2005, Savegnago et al., 2008).

Furthermore, a synergistic antidepressant-like effect was demonstrated when bis selenide was administered with N^G-nitro-L-arginine, an inhibitor of nitric oxide synthase. Accordingly, some studies reported that, at least under some experimental conditions, the administration of nitric oxide synthase inhibitors, depending on their doses, produces antidepressant-like effect in the forced swimming and tail suspension tests (Da Silva et al., 2000; Yildiz et al., 2000; Volke et al., 2003; Harkin et al., 2004). In fact, Da Silva et al. (2000) have reported that N^G-nitro-L-arginine (0.3–10 mg/kg, i.p.), an inhibitor of nitric oxide synthase, significantly reduced the duration of immobility in both the forced swimming and tail suspension tests. Thus, these results indicate that the inhibition of nitric oxide synthesis may underlie the reduction in the immobility time elicited by bis selenide in the tail suspension test. Moreover, an antidepressant-like effect was produced when bis selenide was administered with 7-nitroindazole, which is a selective neuronal nitric oxide synthase inhibitor, or methylene blue, an inhibitor of both nitric oxide synthase and soluble guanylate cyclase.

In agreement with data obtained in the tail suspension test we show that an effective dose (1 mg/kg, p.o.) of bis selenide decreased cerebral nitrate/nitrite levels in mice. This result could further suggest a role for nitric oxide system in the antidepressant-like effect of bis selenide in the tail suspension test. Evidences in the literature have shown that inhibiting nitric oxide system activation using various nitric oxide synthase inhibitors could exert an antidepressant-like effect in the rodent tail suspension test (Yildiz et al., 2000).

The assumption that the L-arginine–nitric oxide–cyclic guanosine monophosphate pathway is involved in the reduction in the immobility time elicited by bis selenide in the tail suspension test is reinforced by the finding that subeffective doses of bis selenide and 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one (a nitric oxide-sensitive inhibitor of soluble guanylate cyclase) produced a synergistic antidepressant-like effect.

Accordingly, Heiberg et al. (2002) have reported that 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one significantly decreased the immobility time in the rat forced swimming test, an effect that was reversed by L-arginine (a nitric oxide precursor). In fact, Kaster et al. (2005) have demonstrated that 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one, depending on its concentration, caused a reduction in the immobility time in the mouse forced swimming test. These results are in accordance with the reported reduction in the immobility time in the forced swimming test by 7-nitroindazole (Yildiz et al., 2000; Volke et al., 2003), methylene blue (Eroglu and Caglayan, 1997) and 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one (Heiberg et al., 2002; Kaster et al., 2005; Ergün and Ergün, 2007). In addition, the antidepressant-like effect of adenosine (Kaster et al., 2005), memantine (Almeida et al., 2006) and venlafaxine (Dhir and Kulkarni, 2007) was also potentiated by the pretreatment with 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one and/or N^G-nitro-L-arginine and prevented by sildenafil and L-arginine, suggesting that the L-arginine–nitric oxide–cyclic guanosine monophosphate route has an important role in the mediation of their behavioural effects (Kaster et al., 2005; Almeida et al., 2006; Kulkarni and Dhir, 2007; Savegnago et al., 2008; Jesse et al., 2008b).

Under a similar experimental condition, bis selenide was 20-fold more potent (inhibition of the immobility time at 0.5 mg/kg, Table 2) than fluoxetine and bupropion (inhibition of the immobility time at 10 mg/kg, Table 3) in the mouse tail suspension test, but it demonstrated similar

Table 3
Effect of classical antidepressants, fluoxetine, paroxetine, desipramine, imipramine and bupropion, on immobility time in the mouse tail suspension test (adapted from Cunha et al., 2008).

Compound	Dose (mg/kg)	Immobility time (s)
Vehicle	–	201.1 ± 6.9
Fluoxetine	5	222.4 ± 15.8
Fluoxetine	10	160.7 ± 7.9[a]
Vehicle	–	253.0 ± 5.2
Paroxetine	0.1	217.0 ± 10.3
Paroxetine	1	160.5 ± 12.8[a]
Vehicle	–	230.5 ± 8.6
Imipramine	0.1	190.3 ± 15.8
Imipramine	1	176.9 ± 12.3[a]
Vehicle	–	230.6 ± 9.9
Desipramine	0.1	240.0 ± 10.1
Desipramine	1	170.3 ± 14.7[a]
Vehicle	–	215.8 ± 17.8
Bupropion	1	181.0 ± 8.2
Bupropion	10	156.6 ± 15.9[a]

Results are expressed as mean + S.E.M. of 6–8 animals.
[a] $P<0.05$ compared to the vehicle treated control.

potency when compared to paroxetine, imipramine and desipramine (inhibition of the immobility time at 1 mg/kg, Table 3) (Cunha et al., 2008). In the mouse forced swimming test, bis selenide was 32- and 64-fold more potent (inhibition of the immobility time at 0.5 mg/kg, Table 2) than desipramine (inhibition of the immobility time at 16 mg/kg, Table 4) and bupropion (inhibition of the immobility time at 32 mg/kg, Table 4), respectively (Bourin et al., 2009).

Some studies have demonstrated cases of intoxication and several side effects in people that use antidepressants (White et al., 2008; Tournier et al., 2009). Moderate overdoses of fluoxetine (up to 30 times the common daily dose) are associated with minor or no symptoms, while ingestions of greater amounts typically result in drowsiness, tremor, nausea, and vomiting. At very high doses of fluoxetine (>75 times the common daily dose), more serious adverse events, including seizures, electrocardiogram changes, and decreased consciousness may occur (Barbey and Roose 1998). Jabbari et al. (1985) have reported that depressed patients taking the tetracyclic drug maprotiline are at risk for developing epileptic seizures. Moreover, cardiovascular effects are common in suicidal tricyclic antidepressant overdoses, including a high frequency of tachycardia, conduction disturbances, dysrhythmia, and hypotension. Neurological effects (coma, lethargy and seizures) were also prominent (White et al., 2008). Regarding toxicological side effects, bis selenide (up to 100 times the effective dose in tests predictive of depression) did not change plasma aspartate and alanine aminotransferase activities, urea and creatinine levels as well as body weight, food intake and histological analysis of target tissues. Moreover, these toxicological parameters were not altered in mice daily treated with bis selenide at dose range of 5–20 mg/kg for 21 days. In addition, acute or sub-chronic treatment with bis selenide neither induces seizures or death nor affects locomotor activity in the rota-rod and open field tests. Based on the above reported we can infer that bis selenide is a promise antidepressant drug without toxic effects at doses in which it has pharmacological properties.

Table 4
Effect of classical antidepressants, desipramine and bupropion, in the mouse forced swimming test (adapted from Bourin et al., 2009).

Treatment	Dose mg/kg	Immobility time (s)
Vehicle		238.8 ± 0.7
Desipramine	2	236.1 ± 2.3
	4	218.9 ± 4.3
	16	189.7 ± 7.8[b]
Vehicle		238.2 ± 0.8
Bupropion	4	232.5 ± 3.5
	8	232.0 ± 1.8
	32	204.0 ± 6.3[a]

Results are expressed mean immobility time (±SEM) ($n=10$). [a]$P<0.01$ and [b]$P<0.001$ when compared to vehicle treated group.

In the current study, the antidepressant-like effect elicited by bis selenide was prevented by L-arginine, sildenafil and S-nitroso-N-acetyl-penicillamine (drugs that enhance the levels of cyclic guanosine monophosphate and nitric oxide) and potentiated by N^G-nitro-L-arginine (an inhibitor of nitric oxide synthase), 7-nitroindazole (a specific neuronal nitric oxide synthase inhibitor), methylene blue (an inhibitor of nitric oxide synthase and soluble guanylate cyclase) and 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one (a sensitive inhibitor of soluble guanylate cyclase). In addition, we demonstrate that bis selenide, at an effective dose in the tail suspension test, caused a significant decrease in cerebral nitrate/nitrite levels. Thus, based on the considerations above, our findings clearly indicate that the acute administration of bis selenide elicited the antidepressant-like effect in the mouse tail suspension test by a mechanism that involves the inhibition of the L-arginine–nitric oxide–cyclic guanosine monophosphate pathway.

Acknowledgements

The financial support by UFSM, FAPERGS, CAPES and CNPq is gratefully acknowledged.

References

Almeida, R.C., Felisbino, C.S., López, M.G., Rodrigues, A.L.S., Gabilan, N.H., 2006. Evidence for the involvement of L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effect of memantine in mice. Behav. Brain Res. 168, 318–322.
Barbey, J.T., Roose, S.P., 1998. SSRI: safety in overdose. J. Clin. Psychiatry 59, 42–59.
Beavo, J.A., 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol. Rev. 75, 725–748.
Bourin, M., Chenu, F., Ripoll, N., David, D.J.P., 2005. A proposal of decision tree to screen putative antidepressants using forced swim and tail suspensions tests. Behav. Brain Res. 164, 266–269.
Bourin, M., Chenu, F., Prica, C., Hascoët, M., 2009. Augmentation effect of combination therapy of aripiprazole and antidepressants of forced swimming test in mice. Psychopharmacology 206, 97–107.
Brocardo, P.S., Budni, J., Lobato, K.R., Kaster, M.P., Rodrigues, A.L.S., 2008. Antidepressant-like effect of folic acid: involvement of NMDA receptors and l-arginine–nitric oxide–cyclic guanosine monophosphate pathway. Eur. J. Pharmacol. 598, 37–42.
Cryan, J.F., Markou, A., Lucki, I., 2002. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol. Sci. 23, 238–245.
Cunha, M.P., Machado, D.G., Bettio, L.E.B., Capra, J.C., Rodrigues, A.L.S., 2008. Interaction of zinc with antidepressants in the tail suspension test. Prog. Neuropsychopharmacol. Biol. Psychiatry 32, 1913–1920.
Da Silva, G.D., Matteussi, A.S., Santos, A.R.S., Calixto, J.B., Rodrigues, A.L.S., 2000. Evidence for dual effects of nitric oxide in the forced swimming test and in the tail suspension test in mice. Neuroreport 11, 3699–3702.
D'Aquila, P.S., Collu, M., Gessa, G.L., Serra, G., 2000. The role of dopamine in the mechanism of action of antidepressant drugs. Eur. J. Pharmacol. 405, 365–373.
Dhir, A., Kulkarni, S.K., 2007. Involvement of L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effect of venlafaxine in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 921–925.
Elhwuegi, A.S., 2004. Central monoamines and their role in major depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 28, 435–451.
Ergün, Y., Ergün, U.G., 2007. Prevention of pro-depressant effect of l-arginine in the forced swim test by NG-nitro-l-arginine an [1H-[1, 2, 4]Oxadiazole[4, 3-a] quinoxalin-1-one]. Eur. J. Pharmacol. 554, 150–154.
Eroglu, L., Caglayan, B., 1997. Anxiolytic and antidepressant properties of methylene blue in animal models. Pharmacol. Res. 36, 381–385.
Harkin, A.J., Connor, T.J., Burns, M.P., Kelly, J.P., 2004. Nitric oxide synthase inhibitors augment the effects of serotonin reuptake inhibitors in the forced swimming test. Eur. Neuropsychopharmacol. 14, 274–281.
Hawkes, W.C., Hornbostel, L., 1996. Effects of dietary selenium on mood in healthy men living in a metabolic research unit. Biol. Psychiatry 39, 121–128.
Heiberg, I.L., Wegener, G., Rosenberg, R., 2002. Reduction of cGMP and nitric oxide has antidepressant-like effects in the forced swimming test in rats. Behav. Brain Res. 134, 479–484.
Jabbari, B., Bryan, G.E., Marsh, E.E., Gunderson, C.H., 1985. Incidence of seizures with tricyclic and tetracyclic antidepressants. Arch. Neurol. 42, 480–481.
Jesse, C.R., Savegnago, L., Nogueira, C.W., 2007. Role of nitric oxide/cyclic GMP/K+ channel pathways in the antinociceptive effect caused by 2, 3-bis(mesitylseleno) propenol. Life Sci. 81, 1694–1702.
Jesse, C.R., Savegnago, L., Nogueira, C.W., 2008a. Spinal mechanisms of antinociceptive effect caused by oral administration of bis selenide in mice. Brain Res. 1231, 25–33.

Jesse, C.R., Bortolatto, C.F., Savegnago, L., Rocha, J.B.T., Nogueira, C.W., 2008b. Involvement of L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effect of tramadol in the rat forced swimming test. Prog. Neuropsychopharmacol. Biol. Psychiatry 32, 1838–1843.
Kaehler, S.T., Singewald, N., Sinner, C., Philippu, A., 1999. Nitric oxide modulates the release of serotonin in the rat hypothalamus. Brain Res. 835, 346–349.
Kaster, M.P., Rosa, A.O., Santos, A.R.S., Rodrigues, A.L.S., 2005. Involvement of nitric oxide–cGMP pathway in the antidepressant-like effects of adenosine in the forced swimming test. Int. J. Neuropsychopharmacol. 8, 601–606.
Kulkarni, S.K., Dhir, A., 2007. Possible involvement of L-arginine–nitric oxide (NO)–cyclic guanosine monophosphate (cGMP) signaling pathway in the antidepressant activity of berberine chloride. Eur. J. Pharmacol. 569, 77–83.
Lorrain, D.S., Hull, E.M., 1993. Nitric oxide increases dopamine and serotonin release in the medial preoptic area. Neuroreport 5, 87–89.
Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5, 62–71.
Moro, A.V., Nogueira, C.W., Barbosa, N.B.V., Menezes, P.H., Rocha, J.B.T., Zeni, G., 2005. Highly stereoselective one-pot producers to prepare bis- and tris chalcogenide alkenes via addition of disulfides and diselenides to terminal alkynes. J. Org. Chem. 70, 5257–5268.
Murray, C.J., Lopez, A.D., 1997. Global mortality, disability, and the contribution of risk factors: global burden of disease study. Lancet 349, 1436–1442.
Palucha, A., Pilc, A., 2002. On the role of metabotropic glutamate receptors in the mechanisms of action of antidepressant. Pol. J. Pharmacol. 54, 581–586.
Papakostas, G.I., 2006. Dopaminergic-based pharmacotherapies for depression. Eur. Neuropsychopharmacol. 16, 391–402.
Porsolt, R.D., Le Pichon, M., Jalfre, M., 1977. Depression: a new animal model sensitive to antidepressant treatments. Nature 266, 730–732.
Posser, T., Kaster, M.P., Baraúna, S.C., Rocha, J.B.T., Rodrigues, A.L.S., Leal, R.B., 2009. Antidepressant-like effect of the organoselenium compound ebselen in mice: evidence for the involvement of the monoaminergic system. Eur. J. Pharmacol. 602, 85–91.
Rayman, M.P., 2000. The importance of selenium to human health. Lancet 356, 23–41.
Rodrigues, A.L.S., Da Silva, G.L., Mateussi, A.S., Fernandes, E.S., Miguel, O.G., Yunes, R.A., 2002. Involvement of monoaminergic system in the antidepressant-like effect of the hydroalcoholic extract of *Siphocampylus verticillatus*. Life Sci. 70, 1347–1358.
Savegnago, L., Jesse, C.R., Moro, A.V., Borges, V.C., Santos, F.W., Rocha, J.B.T., Nogueira, C.W., 2006. Bis selenide alkene derivatives: a class of potential antioxidant and antinociceptive agents. Pharmacol. Biochem. Behav. 83, 221–229.
Savegnago, L., Jesse, C.R., Pinto, L.G., Rocha, J.B.T., Nogueira, C.W., Zeni, G., 2007. Monoaminergic agents modulate antidepressant-like effect caused by diphenyl diselenide in rats. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 1261–1269.
Savegnago, L., Jesse, C.R., Pinto, L.G., Rocha, J.B.T., Barancelli, D.A., Nogueira, C.W., Zeni, G., 2008. Diphenyl diselenide exerts antidepressant-like and anxiolytic-like effects in mice: involvement of 1-arginine–nitric oxide–soluble guanylate cyclase pathway in its antidepressent-like action. Pharmacol. Biochem. Behav. 88, 418–426.
Smith, J.C., Whitton, P.S., 2000. Nitric oxide modulates N-methyl-daspartate-evoked serotonin release in the raphe nuclei and frontal cortex of the freely moving rat. Neurosci. Lett. 291, 5–8.
Snyder, S.H., 1992. Biosensors. NO release for good measure. Nature 358, 623.
Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370.
Tournier, M., Grolleau, A., Cougnard, A., Molimard, M., Verdoux, H., 2009. Factors associated with choice of psychotropic drugs used for intentional drug overdose. Eur. Arch. Psychiatry Clin. Neurosci. 259, 86–91.
Volke, V., Wegener, G., Bourin, M., Vasar, E., 2003. Antidepressant- and anxiolytic-like effects of selective neuronal NOS inhibitor 1-(2-trifluoromethylphenyl)-imidazole in mice. Behav. Brain Res. 140, 141–147.
White, N., Litovitz, T., Clancy, C., 2008. Suicidal antidepressant overdoses: a comparative analysis of antidepressant type. J. Med. Toxicol. 4, 238–250.
Yildiz, F., Erden, B.F., Ulak, G., Utkan, T., Gacar, N., 2000. Antidepressant-like effect of 7-nitroindazole in the forced swimming test in rats. Psychopharmacology 149, 41–44.

4.3. Papel dos diferentes canais de potássio e dos receptores ativados por proliferadores de peroxissoma γ na atividade do tipo antidepressiva do bis seleneto no teste da suspensão da cauda em camundongos

4.3.1. Artigo 3

Papel dos diferentes canais de potássio e dos receptores ativados por proliferadores de peroxissoma γ na atividade do tipo antidepressiva do bis seleneto no teste da suspensão da cauda em camundongos

Cristiano R. Jesse, Ethel A. Wilhelm, Cristiani F. Bortolatto, Cristina W. Nogueira

Neuroscience Letters 490 (2011) 205–208

Neuroscience Letters 490 (2011) 205–208

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Role of different types of potassium channels and peroxisome proliferator-activated receptors γ in the antidepressant-like activity of bis selenide in the mouse tail suspension test

Cristiano R. Jesse [1], Ethel A. Wilhelm [1], Cristiani F. Bortolatto [1], Cristina W. Nogueira *

Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

ARTICLE INFO

Article history:
Received 9 August 2010
Received in revised form
20 December 2010
Accepted 22 December 2010

Keywords:
Selenium
Tail suspension test
Antidepressant
Potassium channels
Peroxisome proliferator-activated receptor gamma

ABSTRACT

In the present study we investigated the role of potassium (K^+) channels and peroxisome proliferator-activated receptor gamma (PPARγ) in the antidepressant-like effect of bis selenide in the mouse tail suspension test (TST). Intracerebroventricular (i.c.v.) pretreatment with tetraethyl ammonium (TEA, a non-specific inhibitor of K^+ channels, 25 pg/site), glibenclamide (an ATP-sensitive K^+ channel inhibitor, 0.5 pg/site), charybdotoxin (a large and intermediate conductance calcium-activated K^+ channel inhibitor, 25 pg/site) or apamin (a small-conductance calcium-activated K^+ channel inhibitor, 10 pg/site) produced a synergistic action with a sub effective dose of bis selenide (0.1 mg/kg, per oral – p.o.). Picrotoxin (1 mg/kg, intraperitoneally – i.p.) pretreatment did not prevent the reduction in immobility time elicited by bis selenide (1 mg/kg, p.o.) in the TST. The reduction in the immobility time elicited by an effective dose of bis selenide (1 mg/kg, p.o.) was prevented by the pretreatment of mice with cromakalim, minoxidil (K^+ channel openers, 10 μg/site, i.c.v.) and GW 9662 (a PPARγ antagonist, 10 μg/site, i.c.v.). The findings clearly suggest that an acute oral dose of bis selenide produced an antidepressant-like effect in the mouse TST by a mechanism that involves the K^+ channels and PPARγ receptors.

Bis selenide has antioxidant [27], antinociceptive and anti-inflammatory properties [10,11] and modulates N-methyl-D-aspartate (NMDA) receptors [11] and nitric oxide synthase (NOS) in the brain [13]. Other organoselenium compounds (diphenyl diselenide and ebselen) have been also reported as potential antidepressant drugs [21,28]. We have previously demonstrated that bis selenide elicits an antidepressant-like action in the mouse TST by a mechanism that involves the inhibition of the L-arginine–nitric oxide (NO)–guanosine cyclic monophosphate (cGMP) pathway and the modulation of the serotonergic system [14]. It has been reported that not only NO [8,31] but also NO donors [5,35] are capable of activating different types of potassium (K^+) channels. We have previously demonstrated that bis selenide elicits an antidepressant-like action in the mouse TST by a mechanism that involves the inhibition of the L-arginine–nitric oxide (NO)–guanosine cyclic monophosphate (cGMP) pathway and the modulation of the serotonergic system [14]. This assumption was further reinforced by a previous study that demonstrated that the antidepressant-like effects of K^+ channel inhibitors in the FST were prevented by the pretreatment of mice with the NO precursors, L-arginine and cGMP [16]. Different types of K^+ channel inhibitors such as tetraethyl ammonium (TEA), apamin, charybdotoxin, gliquidone or glibenclamide were able to produce an antidepressant-like effect in the mouse FST [6,16], whereas K^+ channel openers such as minoxidil or cromakalim increased the immobility time, evidencing the induction of a depressant-like effect [12,17].

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor super family and form heterodimers with the retinoid X receptor before binding to PPAR response elements in the promoter region of their target genes. Three PPAR isoforms have been identified (alpha, delta/beta and gamma). PPARγ might be important for mood modulation since it is expressed in a plethora of brain areas in rats [24] including some areas known to be involved in depression such as hippocampus, basal ganglia, frontal cortex, hypothalamus and pituitary [1,4,7,18].

Additional mechanisms of action could be involved in the antidepressant-like effect of bis selenide. Therefore, we investigated the role of different types of K^+ channels and PPARγ receptors

Abbreviations: FST, forced swimming test; cGMP, guanosine cyclic monophosphate; i.c.v., intracerebroventricular; i.p., intraperitoneal; LSN, lateral septal nucleus; NOS, nitric oxide synthase; NO, nitric oxide; NMDA, N-methyl-D-aspartate; OFT, open field test; p.o., per oral; PPARγ, peroxisome proliferator-activated receptor gamma; K^+, potassium; TST, tail suspension test; TEA, tetraethylammonium.

* Corresponding author. Tel.: +55 55 32209611; fax: +55 55 32208978.
E-mail address: criswn@quimica.ufsm.br (C.W. Nogueira).

[1] These authors have contributed equally to this work.

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.12.053

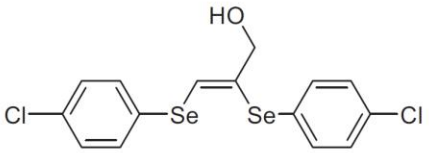


Fig. 1. Chemical structure of bis selenide.

in the antidepressant-like effect of bis selenide in the mouse TST.

Bis selenide [(*Z*)-2,3-bis(4-chlorophenylselanyl)prop-2-en-1-ol] was prepared and characterized in our laboratory by the method previously described [20] (Fig. 1). The doses of bis selenide used in this study (0.1 mg/kg, a sub effective dose and 1 mg/kg, an effective dose) were chosen based on previously published studies [13,14]. The following drugs were used: tetraethyl ammonium (TEA), 2-chloro-5-nitro-N-phenylbenzamide (GW 9662), minoxidil, apamin (Sigma Chemical Co., USA), charybdotoxin, cromakalim and glibenclamide (Tocris Cookson, Ballwin, MO, USA). Cromakalim was dissolved in saline with 10% Tween 80, whereas all other drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use. Drugs (K^+ channel inhibitors and openers and GW 9662) were administered by intracerebroventricular (i.c.v.) route, in a volume of 5 μl per mouse. All other chemicals were of analytical grade and obtained from standard commercial suppliers. The behavioral experiments were conducted using male adults Swiss mice (25–35 g) maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle, with lights on at 07:00 a.m. 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. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

To test the hypothesis that the antidepressant-like effect of bis selenide is mediated through the inhibition of K^+ channels, distinct groups of animals were treated with different classes of drugs. For this purpose animals were pretreated by i.c.v. route with sub effective doses of TEA (a non-specific inhibitor of K^+ channels, 25 pg/site), glibenclamide (an ATP-sensitive K^+ channel inhibitor, 0.5 pg/site), charybdotoxin (a large- and intermediate-conductance calcium-activated K^+ channel inhibitor, 25 pg/site) or apamin (a small-conductance calcium-activated K^+ channel inhibitor, 10 pg/site) [12,14]. Fifteen minutes later the animals received a single oral administration of bis selenide (0.1 mg/kg, p.o, a sub effective dose) or vehicle (canola oil, 10 ml/kg, p.o.) and a further 1 h was allowed to elapse before the animals were tested in the TST. In order to rule out any stimulant effect of the interaction of K^+ channel inhibitors and bis selenide, mice received the same treatment used in the TST. Then, after 1 h the open field test (OFT) was carried out.

To address the role played by K^+ channels in the antidepressant-like effect caused by bis selenide in the TST, distinct groups of animals were treated with cromakalim (a K^+ channel opener, 10 μg/site, i.c.v.) or minoxidil (a K^+ channel opener, 10 μg/site, i.c.v.) [16] 15 min before bis selenide (1 mg/kg, an effective dose) administration, and 1 h later the TST or OFT was carried out.

To investigate the role of voltage dependent Ca^{2+}, K^+ and chloride channels in the antidepressant-like effect of bis selenide, picrotoxin, a nonselective ligand-gated chloride channel blocker, was administered intraperitoneally at the dose of 1 mg/kg [25]. After 30 min, bis selenide (1 mg/kg, p.o.) or vehicle (canola oil) was administrated, and 1 h later the TST and the OFT was carried out.

In order to investigate the involvement of PPARγ in the antidepressant-like effect of bis selenide, mice were pretreated with GW 9662, a PPARγ antagonist (10 μg/site, i.c.v., a dose that produces no effect in the TST) [26]. After 15 min, bis selenide (1 mg/kg, i.p, an effective dose) or vehicle (canola oil) was injected, and 1 h later the TST and the OFT was carried out.

The TST have some drawbacks represented by the possibility of obtaining false positives or negatives. Drugs enhancing locomotor activity can evoke a 'false' positive effect in these tests, whereas drugs decreasing locomotion may give a 'false' negative result [16,23,28,29]. Thus, to investigate whether administration of bis selenide with other drugs impairs motor abilities in mice, the same treatment and drugs were used in the TST and OFT.

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. [33]. Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period.

The open-field was made of plywood and surrounded by walls 30 cm in height. The floor of the open field, 45 cm in length and 45 cm in width, was divided by masking tape markers into 09 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 6 min to record the locomotor and exploratory activities (number of segments crossed with the four paws) [28,29].

i.c.v. administration was performed under anesthesia [16]. Briefly, a 0.4 mm external diameter hypodermic needle attached to a cannula, was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse. A volume of 5 μl was then administered. The injection site was 1 mm to the right or left from the mid-point on a line drawn through to the anterior base of the ears. To ascertain that the drugs were administered exactly into the cerebral ventricle, the brains were dissected and examined macroscopically after the test.

All experimental results are given as the mean (s) ± S.E.M. Comparisons between experimental and control groups were performed by two-way ANOVA (inhibitors of K^+ channels, openers, GW 9662 and/or a K^+ channel inhibitors, openers and GW 9662 X bis selenide) followed by Newman–Keuls test for post hoc comparison when appropriate. A value of $P < 0.05$ was considered to be significant. Main effects of first order interactions are presented only when interaction was not significant.

Results depicted in Fig. 2 show that TEA (25 pg/site, i.c.v.) produced a synergistic effect with a sub effective dose of bis selenide (0.1 mg/kg, p.o.) in the mouse TST ($F(1,24) = 21.37$, $P < 0.001$). No significant effects were observed on the number of crossings ($F(1,24) = 0.02$, $P = 0.879$) in the OFT in mice treated with TEA and bis selenide (data not shown).

Fig. 2 demonstrates that glibenclamide (0.5 pg/site, i.c.v.) caused a synergistic action with bis selenide (0.1 mg/kg, p.o.) on the mouse TST ($F(1,24) = 16.03$, $P < 0.005$). The number of crossings ($F(1,24) = 0.02$, $P = 0.963$) in the OFT was unmodified by glibenclamide and bis selenide administration in mice (data not shown).

Fig. 2 shows a synergistic effect of a sub effective dose of bis selenide (0.1 mg/kg, p.o.) and charybdotoxin (25 pg/site, i.c.v.) ($F(1,24) = 30.70$, $P < 0.001$) in the mouse TST. Bis selenide and charybdotoxin did not modify the number of crossings ($F(1,24) = 0.11$, $P = 0.740$) of mice in the OFT (data not shown).

Fig. 2 demonstrates that apamin (10 pg/site, i.c.v.) was able to produce a synergistic action with a sub effective dose of bis selenide (0.1 mg/kg, p.o.) in the mouse TST ($F(1,24) = 9.99$, $P < 0.004$). The combined administration of bis selenide and apamin to mice did

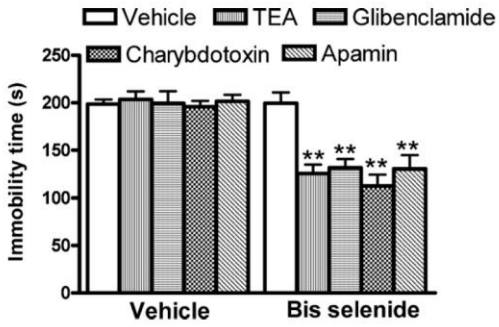


Fig. 2. Effect of TEA (25 pg/site, i.c.v.), glibenclamide (0.5 pg/site, i.c.v.), charybdotoxin (25 pg/site, i.c.v.) or apamin (10 pg/site, i.c.v.) in potentiating the action of a sub effective dose of bis selenide (0.1 mg/kg, p.o.) in the TST. Values are expressed as mean ± S.E.M. (n = 7 mice/group). Data were analyzed by two way analysis of variance (ANOVA) followed by Newman–Keuls test. $^{**}P < 0.01$ compared to the vehicle group pretreated with K^+ channel inhibitors.

not alter the number of crossings in the OFT ($F(1,24) = 0.05$, $P = 0.82$) (data not shown).

Fig. 3 shows the pretreatment of mice with cromakalim (10 μg/site, i.c.v.) in the antidepressant-like effect of bis selenide (1 mg/kg, p.o.) in the TST. Cromakalim reversed the reduction of the immobility time produced by bis selenide (1 mg/kg, p.o.) ($F(1,24) = 31.51$, $P < 0.001$). No significant differences were observed in the number of crossings in the OFT in mice treated with cromakalim and bis selenide ($F(1,24) = 0.30$, $P = 0.588$).

Results depicted in Fig. 3 demonstrate that the pretreatment of mice with minoxidil (a K^+ channel opener, 10 μg/site, i.c.v.) also reversed the antidepressant-like effect of bis selenide (1 mg/kg, p.o.) in the TST ($F(1,24) = 24.69$, $P < 0.001$). The combined administration of bis selenide and minoxidil to mice did not alter the number of crossings ($F(1,24) = 0.11$, $P = 0.746$) in the OFT (data not shown).

Picrotoxin (1 mg/kg, i.p.) did not prevent the reduction in immobility time elicited by bis selenide (1 mg/kg, p.o.) in the TST ($F(1,24) = 0.03$, $P = 0.0816$). No significant effect was observed on the number of crossings ($F(1,24) = 0.19$, $P = 0.671$) in the OFT when mice received bis selenide and picrotoxin (data not shown).

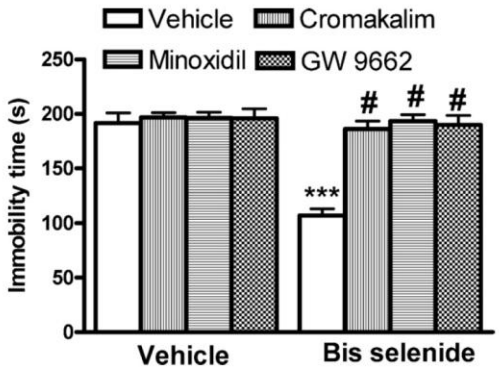


Fig. 3. Effect of pretreatment of mice with cromakalim (10 μg/site, i.c.v.), minoxidil (10 μg/site, i.c.v.) or GW 9662 (10 μg/site, i.c.v.) on the action of an effective dose of bis selenide (1 mg/kg, p.o.) in the immobility time in the TST. Values are expressed as mean ± S.E.M. (n = 7 mice/group). Data were analyzed by two way analysis of variance (ANOVA) followed by Newman–Keuls test. $^{***}P < 0.01$ compared to the vehicle/vehicle group. $^{\#}P < 0.01$ compared to the bis selenide group pretreated with vehicle.

Fig. 3 shows that the pretreatment of mice with GW 9662 (10 μg/site, i.c.v.) blocked the reduction in immobility time elicited by bis selenide (1 mg/kg, p.o.) in the TST ($F(1,24) = 17.69$, $P < 0.003$). No significant effects were observed on the number of crossings ($F(1,24) = 0.06$, $P = 0.803$) in the OFT when mice received bis selenide and GW 9662 (data not shown).

We demonstrated the antidepressant-like effect of an oral administration of bis selenide in the mouse TST, which is possibly related to the modulation of K^+ channels. Our research group has demonstrated that the antidepressant-like effect of bis selenide in the TST is dependent on the inhibition of L-arginine–NO–cGMP pathway [13]. Targets for NO, K^+ channels are being characterized as one of the most important; its activation by NO is mediated by cGMP [32,34] or by NO itself [2]. Kaster et al. [16] have suggested that NO and cGMP are important modulators of some K^+ channels at central level and the inhibition of these channels might represent an important role in the mechanisms involved in major depressive disorder. It was reported that the administration of K^+ channel inhibitors, such as TEA, glibenclamide, apamin and charybdotoxin, produced an antidepressant-like effect in the mouse FST, probably by inhibiting the hyperpolarization and leading to an increased excitatory response. This effect is prevented by the pretreatment of mice with L-arginine (a precursor of NO) or sildenafil (a specific phosphodiesterase type 5 inhibitor), indicating that antidepressant-like effect of these compounds is dependent on the L-arginine–NO–cGMP pathway [16].

In this study, pretreatment of mice with pharmacological drugs able to block different types of K^+ channels, such as TEA, glibenclamide, charybdotoxin and apamim, was able to potentiate a sub effective dose of bis selenide. Our results showed that the effect of K^+ channel inhibitors in potentiating the action of a sub effective dose of bis selenide in the TST cannot be attributed to a stimulant effect of these drugs, since they were devoid of effects on locomotor activity in the OFT. TEA is a quaternary ammonium compound that blocks different types of K^+ channels in neurons including Ca^{2+} activated and voltage dependent K^+ channels. Apamin and charybdotoxin were reported to block specifically currents through the Ca^{2+}-activated K^+ channels. Large- conductance Ca^{2+}-activated K^+ channels are sensitive to inhibition by charybdotoxin [21], whereas small-conductance Ca^{2+}-activated K^+ channels are sensitive to extracellular apamin [32]. Pretreatment of mice with drugs able to activate K^+ channels, such as cromakalim or minoxidil, prevented the anti-immobility effect of an effective dose of bis selenide in the mouse TST, without affecting the locomotor activity. These results support the hypothesis that K^+ channels inhibition is involved in the antidepressant-like effect of bis selenide in mice.

Picrotoxin was administered to mice to investigate the role of voltage dependent Ca^{2+}, K^+ and chloride channels in the antidepressant-like effect of bis selenide. Some reports suggest that voltage dependent calcium (Ca^{2+}) channels are essentially involved in picrotoxin-induced epileptic activity and in antidepressant-like effects [3,22]. There are some reports that indicate that K^+ (ATP) channels in brain are likely related to enhance seizure susceptibility induced by picrotoxin in rats [9,15]. The results of the present study showed that voltage dependent Ca^2, K^+ and chloride channels are not involved in the antidepressant-like effect of bis selenide in mice.

Since TRAM-34 is a highly selective blocker of intermediate conductance Ca^{2+}-activated K^+ channels (IKCa channels) [30] and the antidepressant-like effect of bis selenide is dependent of different types of Ca^{2+} channels (ATP-sensitive K^+ channel; large-conductance calcium-activated K^+ channel and small-conductance calcium-activated K^+ channel), we speculate that the use of TRAM-34 could block the antidepressant-like effect of bis selenide.

PPARγ might be important for mood modulation since it was shown to be expressed in a plethora of brain areas in rats [19]

including some areas known to be involved in depression such as hippocampus, basal ganglia, frontal cortex, hypothalamus and pituitary [1,4,7,18]. There is evidence in the literature that PPARγ receptors could exert an antidepressant-like effect in the FST or TST [26]. Furthermore, we showed that GW-9662 reversed the effects of bis selenide in the mouse TST. It seems that the antidepressant-like effect of bis selenide involves the modulation of PPARγ receptors.

In conclusion, the results indicate that an acute oral dose of bis selenide produced an antidepressant-like action in the mouse TST by a mechanism that involves the K^+ channels and PPARγ receptors.

Acknowledgements

The financial support by UFSM, FAPERGS, CAPES and CNPq is gratefully acknowledged. This study was supported in part by the FINEP research grant "Rede Instituto Brasileiro de Neurociência (IBNNet)" #01.06.0842-00 and FAPERGS/CNPq (PRONEX) research grant # 10/0005-1.

References

[1] M. Aihara, I. Ida, N. Yuuki, A. Oshima, H. Kumano, K. Takahashi, HPA axis dysfunction in unmedicated major depressive disorder and its normalization by pharmacotherapy correlates with alteration of neural activity in prefrontal cortex and limbic/paralimbic regions, Psychiatry Res. 155 (2007) 245–256.
[2] V.M. Bolotina, S. Najibi, J.J. Palacino, P.J. Pagano, R.A. Cohen, Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle, Nature 368 (1994) 850–853.
[3] C. Cannizzaro, E. Cannizzaro, M. Gagliano, N. Mangiapane, Behavioural responsiveness to picrotoxin and desipramine in adult rats prenatally exposed to different benzodiazepine receptor agonists, Eur. Neuropsychopharmacol. 5 (1995) 523–526.
[4] A. Cimini, E. Benedetti, L. Cristiano, P. Sebastiani, M.A. D'Amico, B. D'Angelo, Expression of peroxisome proliferator-activated receptors (PPARs) and retinoic acid receptors (RXRs) in rat cortical neurons, Neuroscience 130 (2005) 325–337.
[5] K. Fujino, S. Nakaya, T. Wakatsuki, Y. Miyoshi, Y. Nakaya, H. Mori, I. Inoue, Effects of nitroglycerin on ATP-induced Ca(++)-mobilization, Ca(++)-activated K channels and contraction of cultured smooth muscle cells of porcine coronary artery, J. Pharmacol. Exp. Ther. 256 (1991) 371–377.
[6] N. Galeotti, C. Ghelardini, B. Caldari, A. Bartolini, Effect of potassium channel modulators in mouse forced swimming test, Br. J. Pharmacol. 126 (1999) 1653–1659.
[7] N.C. Inestrosa, J.A. Godoy, R.A. Quintanilla, C.S. Koenig, M. Bronfman, Peroxisome proliferator-activated receptor g is expressed in hippocampal neurons and its activation prevents b-amyloid neurodegeneration: role of Wnt signaling, Exp. Cell. Res. 304 (2005) 91–104.
[8] S.Y. Jeong, T.S. Ha, C.S. Park, D.Y. Uhm, S. Chung, Nitric oxide directly activates large conductance Ca^{2+}-activated K^+ channels (rSlo), Mol. Cells 12 (2001) 97–102.
[9] K. Jiang, Z. Yu, Q. Shui, The pattern of ATP-sensitive K^+ channel subunits, Kir6. 2 and SUR1 mRNA expressions in DG region is different from those in CA1-3 regions of chronic epilepsy induced by picrotoxin in rats, Neuropathology 27 (2007) 531–538.
[10] C.R. Jesse, L. Savegnago, C.W. Nogueira, Role of nitric oxide/cyclic GMP/K^+ channel pathways in the antinociceptive effect caused by 2,3-bis(mesitylseleno)propenol, Life Sci. 81 (2007) 1694–1702.
[11] C.R. Jesse, L. Savegnago, C.W. Nogueira, Spinal mechanisms of antinociceptive effect caused by oral administration of bis selenide in mice, Brain Res. 1231 (2008) 25–33.
[12] C.R. Jesse, E.A. Wilhelm, N.B. Barbosa, C.W. Nogueira, Involvement of different types of potassium channels in the antidepressant-like effects of tramadol in the mouse forced swimming test, Eur. J. Pharmacol. 613 (2009) 74–78.
[13] C.R. Jesse, E.A. Wilhelm, C.F. Bortolatto, C.W. Nogueira, Involvement of L-arginine–NO–cGMP pathway in the antidepressant-like effect of bis selenide on the mouse tail suspension test, Eur. J. Pharmacol. 635 (2010) 135–141.
[14] C.R. Jesse, E.A. Wilhelm, C.F. Bortolatto, C.W. Nogueira, Evidence for the involvement of serotonergic 5-$HT_{2A/C}$ and 5-HT_3 receptors in the antidepressant-like effect caused by oral administration of bis selenide in mice, Prog. Neuropsychopharmacol. Biol. Psychiatry 34 (2010) 294–302.
[15] R. Karanjia, L.M. García-Hernández, M. Miranda-Morales, N. Somani, R. Espinosa-Luna, L.M. Montaño, C. Barajas-López, Cross-inhibitory interactions between GABAA and P2X channels in myenteric neurones, Eur. J. Neurosci. 23 (2006) 3259–3269.
[16] M.P. Kaster, P.K. Ferreira, A.R. Santos, A.L. Rodrigues, Effects of potassium channel inhibitors in the forced swimming test: possible involvement of L-arginine–nitric oxide–soluble guanylate cyclase pathway, Behav. Brain Res. 165 (2005) 204–209.
[17] M.P. Kaster, J. Budni, R.W. Binfare, A.R. Santos, A.L. Rodrigues, The inhibition of different types of potassium channels underlies the antidepressant-like effect of adenosine in the mouse forced swimming test, Prog. Neuropsychopharmacol. Biol. Psychiatry 31 (2007) 690–696.
[18] R.S. Konarski, M. Sidney, H. Kennedya, S. Rafi-Tarib, J.K. Soczynskaa, T.E. Ketter, Volumetric neuroimaging investigations in mood disorders: bipolar disorder versus major depressive disorder, Bipolar Disord. 10 (2008) 1–37.
[19] S. Moreno, S. Farioli-Vecchio, M.P. Ceru, Immunolocalization of peroxisome proliferator activated receptors and retinoic × receptors in the adult rat CNS, Neuroscience 123 (2004) 131–145.
[20] A.V. Moro, C.W. Nogueira, N.B.V. Barbosa, P.H. Menezes, J.B.T. Rocha, G. Zeni, Highly stereoselective one-pot producers to prepare bis- and tris chalcogenide alkenes via addition of disulfides and diselenides to terminal alkynes, J. Org. Chem. 70 (2005) 5257–5268.
[21] M.T. Nelson, J.M. Quayle, Physiological roles and properties of potassium channels in arterial smooth muscle, Am. J. Physiol. 268 (1995) 799–822.
[22] S. Otoom, Z. Hasan, Nifedipine inhibits picrotoxin-induced seizures activity: further evidence on the involvement of L-type calcium channel blockers in epilepsy, Fundam. Clin. Pharmacol. 20 (2006) 115–119.
[23] R.D. Porsolt, M. Le Pichon, M. Jalfre, Depression: a new animal model sensitive to antidepressant treatments, Nature 266 (1977) 730–732.
[24] T. Posser, M.P. Kaster, S.C. Baraúna, J.B.T. Rocha, A.L.S. Rodrigues, R.B. Leal, Antidepressant-like effect of the organoselenium compound ebselen in mice: evidence for the involvement of the monoaminergic system, Eur. J. Pharmacol. 602 (2009) 85–91.
[25] J.F. Rodrìgues-Landa, C.M. Contreras, B. Bernal-Morales, A.G. Gutièrrez-Garcìa, M. Saavedra, Allopregnanolone reduces immobility in the forced swimming test and increases the firing rate of lateral septal neurons through actions on the $GABA_A$ receptor in the rat, J. Psychopharmacol. 21 (2007) 76–84.
[26] A.O. Rosa, M.P. Kaster, R.W. Binfaré, S. Morales, E. Martin-Aparicio, M.L. Navarro-Rico, A. Martinez, M. Medina, A.G. Garcia, M.G. Lopes, A.L. Rodrigues, Antidepressant-like effect of the novel thiadiazolidinone NP031115 in mice, Prog. Neuropsychopharmacol. Biol. Psychiatry 32 (2008) 1549–1556.
[27] L. Savegnago, C.R. Jesse, A.V. Moro, V.C. Borges, F.W. Santos, J.B.T. Rocha, C.W. Nogueira, Bis selenide alkene derivatives: a class of potential antioxidant and antinociceptive agents, Pharmacol. Biochem. Behav. 83 (2006) 221–229.
[28] L. Savegnago, C.R. Jesse, L.G. Pinto, J.B.T. Rocha, C.W. Nogueira, G. Zeni, Monoaminergic agents modulate antidepressant-like effect caused by diphenyl diselenide in rats, Prog. Neuropsychopharmacol. Biol. Psychiatry 31 (2007) 1261–1269.
[29] L. Savegnago, C.R. Jesse, L.G. Pinto, J.B.T. Rocha, D.A. Barancelli, C.W. Nogueira, G. Zeni, Diphenyl diselenide exerts antidepressant-like and anxiolytic-like effects in mice: involvement of L-arginine–nitric oxide–soluble guanylate cyclase pathway in its antidepressent-like action, Pharmacol. Biochem. Behav. 88 (2008) 418–426.
[30] J.Z. Sheng, A.P. Braun, A.P. Small-, intermediate-conductance Ca^{2+}-activated K^+ channels directly control agonist-evoked nitric oxide synthesis in human vascular endothelial cells, Am. J. Physiol. Cell. Physiol. 293 (2007) 458–567.
[31] J.H. Shin, S. Chung, E.J. Park, D.Y. Uhm, S.K. Suh, Nitric oxide directly activates calcium-activated potassium channels from rat brain reconstituted into planar lipid bilayer, FEBS Lett. 415 (1997) 299–302.
[32] M. Stocker, Ca^{2+}-activated K^+ channels: molecular determinants and function of the SK family, Nat. Rev. Neurosci. 5 (2004) 758–770.
[33] L. Steru, R. Chermat, B. Thierry, P. Simon, The tail suspension test: a new method for screening antidepressants in mice, Psychopharmacology 85 (1985) 367–370.
[34] J. Taniguchi, K.I. Furukawa, M. Shigekawa, K^+ channels are stimulated by cyclic guanosine monophosphate-dependent protein kinase in canine coronary artery smooth muscle cells, Pflugers Arch. 423 (1993) 167–172.
[35] D.L. Williams, G.M. Katz, L. Roy-Contancin, J.P. Reuben, Guanosine 5′-monophosphate modulates gating of high-conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle cells, Proc. Natl. Acad. Sci. U.S.A. 85 (1985) 9360–9634.

4.4. O comportamento do tipo depressivo e a alodínia mecânica são reduzidos pelo tratamento com bis seleneto em camundongos com injúria por constrição crônica: uma comparação com fluoxetina, amitriptilina e bupropiona

4.4.1. Artigo 4

O comportamento do tipo depressivo e a alodínia mecânica são reduzidos pelo tratamento com bis seleneto em camundongos com injúria por constrição crônica: uma comparação com fluoxetina, amitriptilina e bupropiona

Cristiano R. Jesse, Ethel A. Wilhelm, Cristina W. Nogueira

Psychopharmacology 212 (2010) 513-522

Psychopharmacology (2010) 212:513–522
DOI 10.1007/s00213-010-1977-6

ORIGINAL INVESTIGATION

Depression-like behavior and mechanical allodynia are reduced by bis selenide treatment in mice with chronic constriction injury: a comparison with fluoxetine, amitriptyline, and bupropion

Cristiano R. Jesse · Ethel A. Wilhelm · Cristina W. Nogueira

Received: 23 January 2010 / Accepted: 25 July 2010 / Published online: 6 August 2010

Abstract
Rationale Neuropathic pain is associated with significant co-morbidities, including depression, which impact considerably on the overall patient experience. Pain co-morbidity symptoms are rarely assessed in animal models of neuropathic pain. Neuropathic pain is characterized by hyperexcitability within nociceptive pathways and remains difficult to treat with standard analgesics.
Objectives The present study determined the effect of bis selenide and conventional antidepressants (fluoxetine, amitriptyline, and bupropion) on neuropathic pain using mechanical allodynic and on depressive-like behavior.
Methods Male mice were subjected to chronic constriction injury (CCI) or sham surgery and were assessed on day 14 after operation. Mice received oral treatment with bis selenide (1–5 mg/kg), fluoxetine, amitriptyline, or bupropion (10–30 mg/kg). The response frequency to mechanical allodynia in mice was measured with von Frey hairs. Mice were evaluated in the forced swimming test (FST) test for depression-like behavior.
Results The CCI procedure produced mechanical allodynia and increased depressive-like behavior in the FST. All of the drugs produced antiallodynic effects in CCI mice and produced antidepressant effects in control mice without altering locomotor activity. In CCI animals, however, only the amitriptyline and bis selenide treatments significantly reduced immobility in the FST.
Conclusion These data demonstrate an important dissociation between the antiallodynic and antidepressant effects in mice when tested in a model of neuropathic pain. Depressive behavior in CCI mice was reversed by bis selenide and amitriptyline but not by the conventional antidepressants fluoxetine and buproprion. Bis selenide was more potent than the other drugs tested for antidepressant-like and antiallodynic effects in mice.

Keywords Selenium · Bis selenide · Antidepressant · Neuropathic pain · Chronic constriction injury

C. R. Jesse · E. A. Wilhelm · C. W. Nogueira (✉)
Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, CEP 97105-900 Santa Maria, RS, Brazil
e-mail: criswn@quimica.ufsm.br

Introduction

Animal models of chronic pain have demonstrated the importance of serotonergic, dopaminergic, and noradrenergic systems in regulating pain (Fishbain 2000; Millan 2002). Descending pathways serve to inhibit input from the gut and muscle skeletal system to a greater or lesser extent depending on the level of stress and the input from painful stimuli (Millan 2002; Benarroch 2008). A dysfunction of these descending pathways can engender a heightened sensitivity to pain and even a painful reaction to normally non-noxious stimuli. Serotonin (5-HT), dopamine (DA), and noradrenaline (NA) are implicated in enhancing endogenous analgesic mechanisms via the descending inhibitory pain pathways in the brain and spinal cord (Fishbain 2000).

Compounds enhancing 5-HT, DA, and NA neurotransmission would be expected to be effective in the control of

chronic pain. Clinical guidelines rank antidepressants, together with anticonvulsants, as first-line drugs for neuropathic pain treatment (Moulin et al. 2007). Neuropathic pain is generally a chronic condition, severe, and resistant to most analgesics (Attal et al. 2006). These antidepressant drugs increase extracellular concentrations of 5-HT, DA, and NA by blocking reuptake transporters (Berton and Nestler 2006). It has been clinically and preclinically demonstrated that specific serotonin reuptake inhibitors (SSRIs) (fluoxetine, paroxetine, and reboxetine), tricylic antidepressants (TCAs) (imipramine, desipramine, and amitriptyline), and atypical antidepressants (AA) (bupropion, trazodone, and venlafaxine) alleviate neuropathic pain (Mico et al. 2006; Benbouzid et al. 2008).

Rodent models are valuable in identifying pathophysiological mechanisms and therapeutic targets for emotional (Cryan and Holmes 2005) and pain disorders (Seltzer et al. 1990). However, recently, a few studies have investigated the potential effects of neuropathic pain on measure of depression-related behavior in rodents (Wang and Wang 2003). The current study examined whether the chronic constriction injury (CCI) model of neuropathic pain causes depression-like behavior in animals and whether this behavior could be reversed by antidepressant drugs. The CCI model (Bennett and Xie 1988) was selected because, compared with other peripheral nerve injury models, a robust mechanical hypersensitivity has been reported (Dowdall et al. 2005; Roeska et al. 2008). Moreover, in different models of traumatic peripheral nerve injury, the measure in the forced swimming test (FST) reflects a depression-like behavior. Since the FST evaluates motivated behavior, it provides possible correlates of the affective-motivational component of ongoing pain in animals.

Low selenium status (low selenium diet contains 32–36 μg/day) has been associated with a significant increased incidence of depression, anxiety, confusion, and hostility (Rayman 2000). Besides, some studies have reported the role of selenium in the management of nociception (Nogueira et al. 2004; Savegnago et al. 2007a, b, 2008). In this context, bis selenide, an organoselenium compound with antinociceptive, anti-inflammatory, and antidepressant-like properties (Savegnago et al. 2006; Jesse et al. 2008, 2009, 2010), could be an attractive target for the treatment of depression and chronic pain.

The first objective of the present study was to examine if rodents display depressive-like behavior induced by nerve injury to better understand the relationship between chronic pain and depression. The second objective of this study was to compare the effect of bis selenide with conventional antidepressants (fluoxetine, amitriptyline, and bupropion) in a mechanical allodynia, with von Frey hair filaments, and depressive-like behavior, in the FST, in mice with neuropathic pain.

Materials and methods

Animals

The behavioral experiments were conducted using male adult Swiss mice (25–35 g) maintained at 22–25°C with free access to water and food, under a 12:12 h light/dark cycle, with lights on at 6:00 a.m. All manipulations were carried out between 8:00 a.m. and 4:00 p.m. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil. Experiments were conducted in accordance with the current guidelines for the care of laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals suggested by Zimmerman (1983). The number of animals and intensities of noxious stimuli used were minimum necessary to demonstrate the consistent effects of the drug treatments. At the end of the experimental procedure, the mice were killed by decapitation.

Animal surgery

CCI was performed based on the original description by Bennett and Xie (1988) under pentobarbital sodium (60 mg/kg, intraperitoneal) anesthesia. Briefly, the left sciatic nerve was exposed after the incision of skin and blunt separation of the muscle. The sciatic nerve was freed of the adhering tissue gently for about 7 mm, and four ligatures (4/0 Ethicon GmbH, Norderstedt, Germany) were made loosely with 1.0–1.5 mm interval between each. Great care was taken to tie the ligatures so that the diameter of the nerve was just barely constricted. Sham operation was performed by exposing sciatic nerve except for nerve ligation. Operated mice were routinely tested for the presence of pain-like behavior for up to 14 days after surgery, according to previously described methods (Blackburn-Munro et al. 2004; Bomholt et al. 2005). Mice with CCI model of neuropathic pain did not present paw drooping or autotomy.

Experimental procedure

CCI-injured and sham-operated animals were repeatedly tested for mechanical allodynia with von Frey hairs (VFH). The response frequency to VFH stimulation (percent) was determined before nerve injury (baseline) to obtain data purely derived from CCI-induced allodynia. In order to determine the basal response frequency, all groups were submitted to pre-surgical evaluation. The response frequency was recorded immediately before (0) and after (1, 2, 4, 8, and 12 h) treatment. Same groups of mice were repeatedly scored in different times for mechanical allodynia.

Other groups of mice were utilized to examine the locomotor activity and the depression-like behavior in the mouse OFT and FST, respectively. For these experiments, CCI-injured and sham-operated animals were examined in behavioral tests at one of six time points, 0, 1, 2, 4, 8, and 12 h after treatment with bis selenide or antidepressants (n= 7 per group, i.e., separate groups of animals were used at each time point). The locomotor activity was evaluated in the open field test (OFT), and immediately after that, the same animals were assessed in the FST.

Chemicals and drug treatment

Bis selenide [(*Z*)-2,3-bis(4-chlorophenylselanyl)*prop*-2-*en*-1-*ol*] was prepared and characterized in our laboratory by the method previously described (Moro et al. 2005). Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of bis selenide (99.9%) was determined by GC/HPLC. The following drugs were used: fluoxetine, amitriptyline, and bupropion (Sigma Chemical Co, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers. Bis selenide was dissolved in canola oil. Antidepressants were dissolved in saline solution (0.9% NaCl). Mice received all drugs in a constant volume of 10 ml/kg body weight.

Treatments with doses of bis selenide (1 and 5 mg/kg, p.o.), fluoxetine, amitriptyline, bupropion (10 or 30 mg/kg, p.o.), or vehicle were carried out 2 weeks after the surgical procedure in mice (groups: operated and sham operated). Mice were evaluated 1, 2, 4, 8, and 12 h subsequent to oral administration. Doses and the schedule of administration were chosen on the basis of experiments previously performed (Pedersen et al. 2005; Jesse et al. 2008, 2009, 2010; Sawynok et al. 2008).

Mechanical allodynia induced by CCI injury

The response frequency was measured after ten applications (duration of 1–2 s each) of VFH (Stoelting, Chicago, IL). To this end, mice were further habituated in individual clear Plexiglas boxes (9×7×11 cm) on an elevated wire mesh platform to allow access to the ventral surface of the hind paws. A previous study indicated that 0.6 g VFH produced a mean withdrawal frequency of approximately 15%, which is considered to be an adequate value for the measurement of mechanical allodynia (Savegnago et al. 2007a). Therefore, 0.6 g VFH alone was used in these experiments. The filament was applied for a period of 1–2 s. Both the ipsilateral (right hind paw) and the contralateral hind paw were tested in order to evaluate the presence of "mirror pain", described elsewhere as present in neuropathic pain pathologies (Tal and Bennett 1994). In order to obtain data purely derived from the CCI model-induced allodynia, maximal inhibition (MI) values were represented as the difference between the basal values of vehicle- or drug-treated animals and respective controls.

Open-field test

The OFT was carried out to exclude the possibility that the lesion of the CCI model affects the ambulation of the animals. The open field was made of plywood and surrounded by walls 30 cm in height. The floor of the open field, 45 cm in length and 45 cm in width, was divided by masking tape markers into 9 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 6 min to record the locomotor (number of segments crossed with the four paws) (Walsh and Cummins 1976).

Motor coordination for swimming—motivational test

This test was performed to further exclude the possibility that the lesion of the CCI model affects locomotion of the animals, since the motor coordination for swimming is different of those necessary for the open field test. The motor coordination for swimming was carried out in an iron pool (86×17×37 cm) filled with clear water to a depth of 20 cm. Water temperature was maintained at 20±0.5°C. At the end of the pool, there was a red platform on which the mice could climb. The location of the platform was made visible by a blue-colored picture mounted above the platform. During the test, the mouse was put into the water at the start point and swam to the end of the pool to climb onto the platform. The swimming time was recorded (Li et al. 2001).

Depressive-like behavior—forced swimming test)

The test was performed as in the original method described elsewhere (Porsolt et al. 1977, 1978). Briefly, mice were individually forced to swim in open cylinders (25 cm height×10 cm diameter) containing 19 cm of water at 25± 1°C. The duration of immobility was scored during the 6-min test period as described previously. The immobility time was determined when no additional activity was observed other than the movements necessary to keep the mice head above the water.

Statistical analysis

All experimental results are given as the mean ± S.E.M. The withdrawal response frequency, number of crossings and depression-like behavior in sham and CCI animals was done by analyzing Newman–Keuls test for post hoc

comparison when appropriate. Comparisons between experimental and control groups were analyzed with two-way ANOVA, including the factors surgery (sham and CCI), treatment (bis selenide, fluoxetine, amitriptyline, and bupropion), and interaction. A value of $P<0.05$ was considered to be significant. MI values were represented as the difference between the basal values of vehicle- or drug-treated animals and respective controls.

Results

Mechanical allodynia

Mechanical sensitivity of the animal hind paws was evaluated by VHF. CCI model produced a marked and long-lasting development of mechanical allodynia in the ipsilateral (injured) side in mice (Fig. 1a–d), but not in the contralateral side (results not shown). Before surgery, no significant difference was observed between operated and sham-operated groups for the baseline of response frequency. Sham surgery did not influence the mechanical threshold in mice (Fig. 1a–d).

Animals that received bis selenide (1 and 5 mg/kg, p.o.) showed a significant reduction in the response frequency induced by CCI model (Fig. 1a). This reduction started 1 h after bis selenide (1 and 5 mg/kg) administration (MI was 82±5%). CCI-injured mice that received 1 and 5 mg/kg of bis selenide demonstrated a significant reduction in the response frequency, which was maintained for up to 4 and 8 h, respectively (Fig. 1a).

Acute treatment with fluoxetine (10 and 30 mg/kg, p.o.) significantly decreased the response frequency in the ipsilateral side 1 h after antidepressant administration (MI

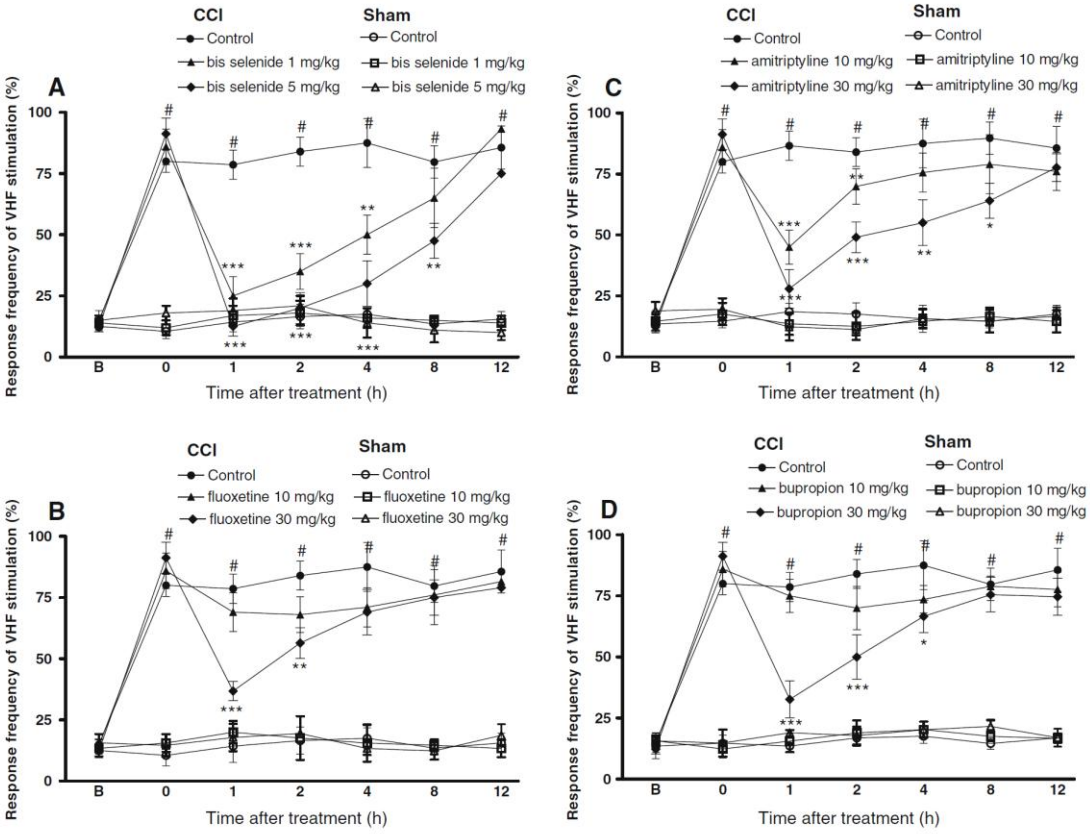


Fig. 1 Effect of oral treatment with bis selenide (1 and 5 mg/kg) (**a**), fluoxetine (10 and 30 mg/kg) (**b**), amitriptyline (10 and 30 mg/kg) (**c**), and bupropion (10 and 30 mg/kg) (**d**) on the response frequency to VFH stimulation (%) in ipsilateral paw in CCI (*closed points*) and sham-operated mice (*open points*). *Each point* represents the mean of seven animals and the *error bars* indicate the S.E.M. *$P<0.05$, **$P<0.01$, and ***$P<0.001$ when compared to the CCI control, and $^{\#}P<0.001$ versus baseline (*B*) values

values were 35±2% and 51±2%). This antiallodynic effect was kept for 2 h after fluoxetine treatment (Fig. 1b).

Treatment with amitriptyline at doses of 10 and 30 mg/kg (p.o.) was markedly effective in reducing the response frequency induced by CCI model in mice. The reduction in the response frequency started 1 h after amitriptyline administration (30 mg/kg), and the MI was 62±5%. The significant decrease in the response frequency was maintained for up to 2 and 8 h when CCI-injured mice received 10 and 30 mg/kg of amitriptyline, respectively (Fig. 1c).

CCI-injured mice treated orally with bupropion (30 mg/kg, p.o.) showed a significant reduction in the response frequency. Antiallodynic effect was observed 1, 2, and 4 h after bupropion administration (30 mg/kg) in CCl-injured mice (MI was 78±5% at 1 h; Fig. 1d).

Acute treatment with bis selenide and conventional antidepressants had no influence in the response frequency in sham-operated mice (Fig. 1a–d).

Depression-like behavior

CCI-injured mice showed a marked prolongation of the immobility time compared to sham-operated mice in the FST (Fig. 2a–d), which indicates that CCI animals displayed depression-like behavior.

Bis selenide administration (1 and 5 mg/kg, p.o.) showed a reduction in depression-like behavior in CCI-injured mice. This effect started 1 h after bis selenide administration (MI was 35±3% at 5 mg/kg). The significant reduction in the immobility time was maintained for up to 2 and 8 h when CCI-injured mice received 1 and 5 mg/kg of bis

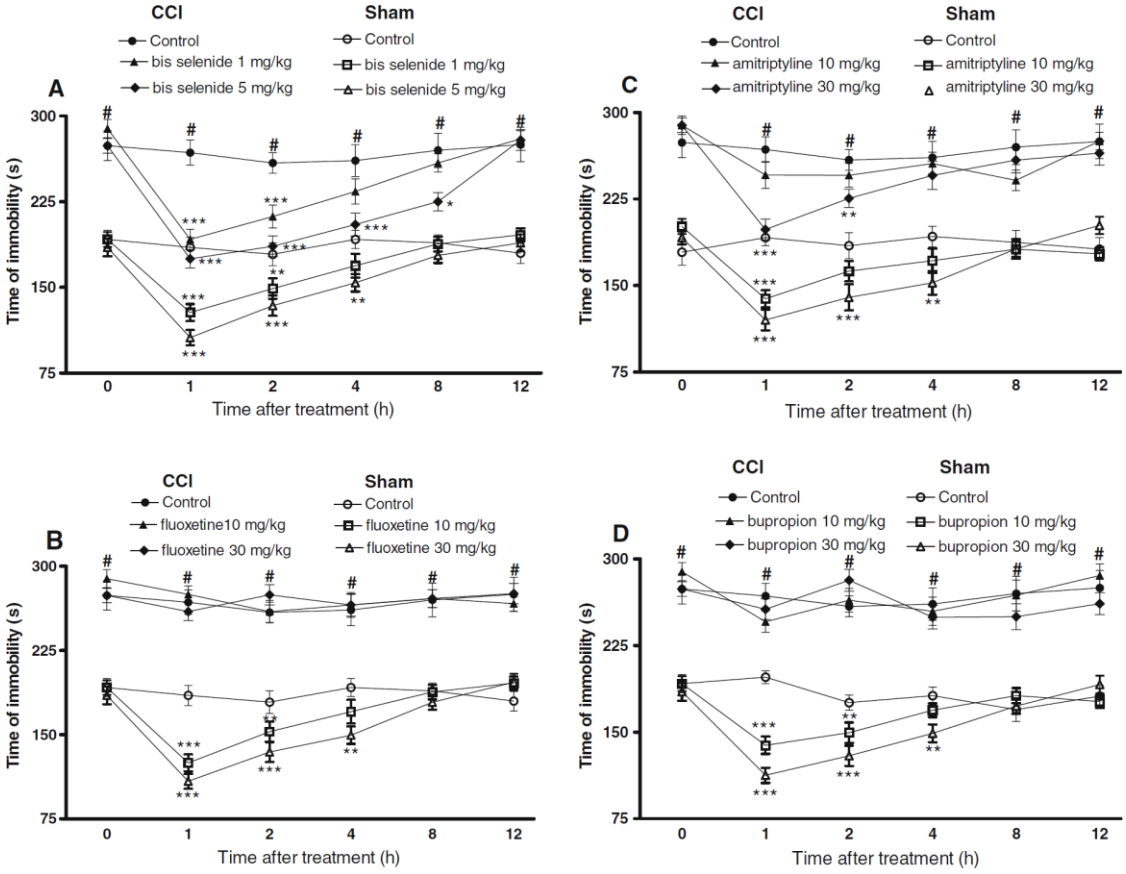


Fig. 2 Effect of oral treatment with bis selenide (1 and 5 mg/kg) (**a**), fluoxetine (10 and 30 mg/kg) (**b**), amitriptyline (10 and 30 mg/kg) (**c**), and bupropion (10 and 30 mg/kg) (**d**) on immobility time (s) in the FST in CCI (*closed points*) and sham-operated (*open points*) mice. *Each point* represents the mean of seven animals and the *error bars* indicate the S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ when compared to the CCI or sham-operated group, and $^{\#}P<0.001$ versus sham-operated control group

selenide, respectively (Fig. 2a). Bis selenide administration (1 and 5 mg/kg, p.o.) caused a reduction in the immobility time in sham-operated mice. The antidepressant-like effect of bis selenide (5 mg/kg) reached its peak at 1 h (MI was 43±3%) and remained significant up to 4 h after administration in sham-operated mice (Fig. 2a).

Fluoxetine, at doses of 10 and 30 mg/kg, did not attenuate the depression-like behavior in CCI-injured mice at all time points. It can be seen that fluoxetine, given by oral route at the doses of 10 and 30 mg/kg, decreased the immobility time in the FST in sham-operated mice (Fig. 2b). The anti-immobility effect started 1 h after fluoxetine (30 mg/kg) administration in sham-operated mice (MI was 40±2%). The significant reduction in the immobility time was maintained for up to 2 and 4 h when mice received 10 and 30 mg/kg of fluoxetine, respectively (Fig. 2b).

Treatment with amitriptyline, at the dose of 30 mg/kg (p.o.), significantly reduced the depression-like behavior in CCI-injured mice (Fig. 2c). The anti-immobility effect started 1 h after amitriptyline (30 mg/kg) administration, and the MI observed was 34±4%. The significant decrease in the immobility time induced by amitriptyline (30 mg/kg) was maintained for up to 2 h in CCI-injured mice. Amitriptyline administration (10 and 30 mg/kg, p.o.) produced a reduction in the immobility time in sham-operated mice. The antidepressant-like effect of amitriptyline, at the dose of 30 mg/kg, reached its peak at 1 h (MI was 33±3%) and remained significant up to 4 h after p.o. administration (Fig. 2c).

The effects of bupropion administered orally in mice are illustrated in Fig. 2d. Acute treatment with bupropion (10 and 30 mg/kg) did not modify the depression-like behavior in CCI-injured mice at any time point. The anti-immobility effect was observed 1, 2, and 4 h after bupropion administration (30 mg/kg) in sham-operated mice, with a peak effect in 1 h (MI was 29±5%) (Fig. 2d).

Locomotor activity

There was no significant difference between sham-operated and CCI animals in the number of crossings in the OFT. Bis

Table 1 Effect of oral treatment of mice with bis selenide, fluoxetine, amitriptyline, or bupropion on the number of crossings in the OFT

Groups	Dose (mg/kg)	Time after treatment with bis selenide or antidepressants					
		0	1	2	4	8	12
Sham operated	–	75.2±2.32	64.5±2.31	72.3±1.59	63.4±2.24	64.5±2.31	75.6±3.55
+Bis selenide	1	69.2±2.81	67.8±3.13	70.2±1.68	64.5±3.12	68.9±3.02	72.5±3.65
+Bis selenide	5	71.2±3.01	68.4±1.85	71.2±2.55	67.8±2.45	65.6±3.89	71.2±3.54
CCl	–	70.2±2.52	64.9±2.14	68.9±2.58	66.8±3.08	68.9±3.20	69.8±2.55
+Bis selenide	1	65.2±2.01	68.8±2.45	66.8±3.10	69.8±3.26	70.4±2.89	67.8±3.68
+Bis selenide	5	66.7±2.67	63.8±1.22	67.1±1.98	70.1±2.65	66.4±3.61	70.3±2.55
Sham operated	–	77.1±3.11	63.1±1.87	68.9±2.12	64.5±1.29	64.5±4.01	74.6±2.54
+Fluoxetine	10	71.2±2.95	64.5±2.03	65.6±3.25	65.5±2.31	68.9±3.25	72.3±3.54
+Fluoxetine	30	72.2±2.45	69.8±2.44	64.5±3.31	67.4±2.42	67.5±3.54	71.2±3.02
CCl	–	75.6±2.22	69.4±2.56	69.4±3.41	68.9±3.02	66.5±2.75	68.7±2.35
+Fluoxetine	10	71.2±1.98	68.8±2.49	70.2±2.98	68.9±2.45	68.7±3.56	67.9±3.05
+Fluoxetine	30	68.9±1.85	70.2±2.19	71.3±2.87	62.9±3.48	67.8±3.24	69.8±2.31
Sham operated	–	68.8±1.74	69.5±2.48	67.8±2.74	71.2±2.48	71.2±2.59	70.3±3.55
+Amitriptyline	10	69.5±2.15	66.5±2.10	69.8±2.84	70.2±3.05	74.5±3.55	65.8±4.02
+Amitriptyline	30	72.3±1.77	65.5±2.31	70.3±3.08	72.5±4.01	73.2±3.41	66.8±3.87
CCl	–	67.5±1.69	68.7±3.10	72.5±3.45	73.0±3.54	70.5±2.54	72.6±3.55
+Amitriptyline	10	71.2±2.31	64.5±2.91	73.2±3.51	70.9±2.59	67.3±3.01	73.5±2.95
+Amitriptyline	30	70.2±1.55	64.9±1.85	68.9±2.48	68.8±3.56	68.9±2.55	67.9±2.55
Sham operated	–	74.5±2.13	68.7±1.22	65.8±3.25	68.7±2.59	71.2±3.12	68.9±3.05
+Bupropion	10	71.2±1.96	71.2±2.58	64.5±1.29	64.5±2.55	70.1±2.65	70.1±4.02
+Bupropion	30	68.9±2.88	70.1±2.71	69.4±2.85	67.5±3.02	67.8±3.85	66.5±2.65
CCl	–	65.5±1.23	70.1±1.85	71.2±2.91	72.3±3.15	66.5±3.02	73.5±3.22
+Bupropion	10	70.5±2.31	67.8±2.31	63.9±1.89	73.2±2.85	72.5±3.26	72.3±2.85
+Bupropion	30	71.2±3.11	66.5±3.15	65.2±2.31	74.5±2.99	73.1±2.58	70.2±2.65

Results are expressed as mean + S.E.M. of seven animals

selenide and antidepressants had no significant effect on the number of crossings in the OFT, at any time point monitored (0, 1, 2, 4, 8, and 12 h), when compared to respective controls (Table 1).

There was no significant difference in the latency for swimming in the motor coordination test between sham-operated and CCI groups. Treatment with bis selenide, at the dose of 5 mg/kg (p.o.), did not influence the motor function (Fig. 3).

Discussion

The present study demonstrated that mice with CCI of the sciatic nerve developed depression-like behavior as reflected in an increase in the time of immobility in the FST and mechanical allodynia demonstrated by the increase of baseline values in the response frequency to VFH. The main original pharmacological finding of the present study was that bis selenide diminished both the mechanical hypersensitivity and depressive-like behavior in CCI animals, without having an effect on locomotor activity.

The CCI model (Bennett and Xie 1988) was selected for this study due to a significant mechanical hypersensitivity in comparison with other animal neuropathic pain models (Dowdall et al. 2005; Roeska et al. 2008). We used a pharmacologically validated test for depression-like behavior, the FST (Porsolt et al. 1977, 1978). This test is frequently used to assess depression-like behavior and is validated by its sensitivity to clinically effective antidepressants that cause in mice to actively and persistently engage in escape-directed behavior compared with non-treated controls. Moreover, the fact that animals with neuropathic pain exhibit depression-like behavior is in agreement with the clinical evidence reporting a relationship between chronic pain and depression (Attal et al. 2006; Mico et al. 2006).

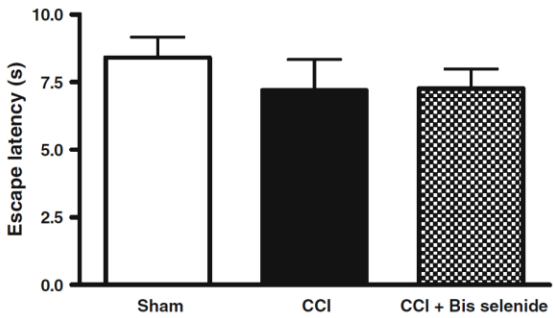


Fig. 3 Effect of sham-operated, CCI-injured, and CCI-injured+bis selenide animals on motor coordination for mouse swimming. Bis selenide was administered at the dose of 5 mg/kg (p.o.) 30 min before the test. *Each point* represents the mean ± S.E. of seven mice

The current study also demonstrated that fluoxetine, at the dose of 30 mg/kg, generated an antiallodynic effect. Additionally, fluoxetine reduced the immobility time in sham animals but did not modify the depressive-like behavior in CCI animals in the FST. It has been reported that fluoxetine, commonly used to treat major depression in humans (Holtzheimer and Nemeroff 2006), is modestly active in a test of tactile allodynia and nociceptive tests (Sindrup et al. 1992; Pedersen et al. 2005). The SSRI employed in this study, fluoxetine, displays distinct selectivity for 5-HT neuronal uptake sites (Hyttel 1994). Thus, fluoxetine produces its pharmacological action via the blockade of 5-HT reuptake, enhancing the efficacy of the 5-HT transmission (Weiss et al. 1986). Taken together, these findings indicated that fluoxetine treatment may cause the attenuation of pain but did not modify the depression-like behavior induced by CCI.

In this study, treatment with amitriptyline produced a significant antiallodynia effect, which is in accordance with other reports (Bomholt et al. 2005; Vissers et al. 2006). However, to the best of our knowledge, this is the first time that the anti-immobility effect of amitriptyline in a CCI neuropathic pain-like state was demonstrated. Treatment with amitriptyline also showed antidepressant-like effect in sham-operated mice. These results were corroborated by Hu et al. (2009) who demonstrated that desipramine (20 mg/kg) reduced the depression-like behavior in rats with mononeuropathy. In a number of neuropathic pain conditions, their analgesic action has been demonstrated to be independent of mood alteration (Max et al. 1987; Wolfe and Trivedi 2004; Sindrup et al. 2005). Acute treatment with amitriptyline has been shown to decrease thermal and chemical hyperalgesia (Casas et al. 1995). Amitriptyline is well known as a dual NA and 5-HT reuptake inhibitor, and its ability to inhibit the uptake of both amines can be implicated in its action demonstrated in this study. In a previous study, it was reported that chronic treatment with the TCAs, nortriptyline, and amitriptyline, but not with the SSRI, fluoxetine, alleviated cuff-induced mechanical allodynia (Benbouzid et al. 2008). This suggests that chronic inhibition of the 5-HT reuptake site alone does not relieve allodynia and that the inhibition of NA uptake plays a critical role in the management of allodynia. A variety of mechanisms have been suggested to explain amitriptyline antinociceptive actions, including block of uptake of NA and 5-HT, engagement of opioid mechanisms, inhibition of ion channel activity, block of *N*-methyl-D-aspartic acid receptors, increased γ-aminobutyric acid receptor activity, and modulation of immune function (Mico et al. 2006; Dick et al. 2007). Adenosine mechanisms are also implicated in antinociception produced by systemic (Ulugol et al. 2002) and peripherally administered amitriptyline (Sawynok et al. 1999; Ulugol et al. 2002) in models of chronic pain. Adenosine A1 receptors (A1Rs) mediate suppression of

nociception, and methylxanthines block adenosine analog effects on pain mediated via A1Rs (Sawynok and Liu 2003). Amitriptyline has been demonstrated to inhibit uptake of adenosine (Phillis and Wu 1982).

Bupropion administration produced an antidepressant-like effect in sham-operated mice, while causing an antiallodynic action in the CCI model in mice. The antiallodynic action of bupropion has been reported (Pedersen et al. 2005). Acute administration of bupropion, an AA, decreases the reuptake of DA and NA into rat and mouse synaptosomes, reduces the firing rate of central NA- and DA-containing neurons, and increases extracellular DA levels (Cooper et al. 1994). Thus, the ability of bupropion to reduce mechanical allodynia suggests that inhibition of NA and DA reuptake accounted for this action. Different from sham-operated mice, bupropion did not alleviate the depressive-like behavior in the FST in CCI animals.

Although, bupropion is a dopamine reuptake inhibitor and increases dopamine levels in the brain, it had no significant effect on the locomotor activity. In this context, Pedersen et al. (2005) demonstrated that bupropion (3–30 mg/kg, i.p.) administered in rats had no significant effect on motor performance at any of the doses tested at 30 or up to 120 min after administration when compared with corresponding vehicle treatment. Although some studies showed that there was no change in locomotor activity, other studies reported alterations (Redolat et al. 2005; Mitchell et al. 2006). However, most of the changes in locomotor activity have been observed when the route of administration is intraperitoneal (Sekihashi et al. 2001). Thus, it is possible that the results we found with bupropion could be explained by the administration route used, the oral route.

In the current study, we showed that the depression-like behavior was diminished by bis selenide in both sham and CCI animals. These data further support the interpretation that the increased time of immobility of CCI mice measured in the FST reflects a depression-like behavior. Bis selenide was more potent than amitriptyline, the only antidepressant that demonstrated anti-immobility effect in CCI mice. In addition, bis selenide was also more potent than fluoxetine, amitriptyline, and bupropion when the immobility time in sham-operated mice was compared. The present study demonstrated the efficacy of bis selenide in the model of CCI neuropathic pain and a rank order of potency was bis selenide>bupropion>amitriptyline>fluoxetine.

Taking together the results, all of the drugs studied reduced immobility in the sham condition, yet only bis selenide and amitriptyline reduced immobility after nerve injury. Bis selenide seems to have a comparable action under both conditions, but amitriptyline has less effect after nerve injury as only the highest dose is active. It seems that the FST reflects presumptive antidepressant activity in the sham state, but the presence of nerve injury (and chronic pain) decreases their ability to produce an antidepressant effect. The suppression of the immobility after nerve injury by amitriptyline could be explained by the dual inhibition of NA and 5-HT reuptake (Benarroch 2008). Conversely, the absence of fluoxetine and bupropion effects on the immobility response after nerve injury in mice could be attributed to the fact that fluoxetine has a distinct selectivity for 5-HT neuronal uptake sites (Hyttel 1994), and bupropion modulates central NA- and DA-containing neurons and increases extracellular DA levels (Cooper et al. 1994).

It has previously been shown that bis selenide elicits antinociceptive, anti-inflammatory, and antidepressant-like effects in mice. The mechanisms through which this organoselenium compound exerts its action involve an interaction with ATP-sensitive and voltage-gated K^+ channels, kainate, *trans*-ACDP, serotonergic (5-HT_{2A} and 5-HT_3), and histamine H_2 receptors (Jesse et al. 2008, 2009, 2010). Thus, we postulate that the efficacy of amitriptyline and bis selenide in a neuropathic pain model and in the depressive-like behavior can be attributed to the modulation of channels and receptors previously mentioned. Based on the results, one could speculate that 5-HT and DA systems are less contributory to the profile observed.

It is important to point out that the therapeutic latency is much shorter in treating neuropathic pain compared to depression (Benbouzid et al. 2008). There has been report that the neuropathic allodynia was suppressed by antidepressants at doses two to four times lower than the ones classically used on mice in models predictive of depression. Moreover, this analgesic effect was only seen after 10–14 days of chronic treatment. Given that, it is possible that the analgesic effect seen acutely with the antidepressants in the CCI model neither reflects an effect on the emotional aspect of pain nor contributes to the alleviation of the comorbid pain depression.

The results demonstrated in this study confirmed previous data (Gonçalves et al. 2008; Hu et al. 2009) in which rodents display depressive-like behavior induced by nerve injury. The data demonstrate an important dissociation between the antiallodynic and antidepressant effects in mice when tested in a model of neuropathic pain. Depressive behavior in CCI mice was reversed only by bis selenide and amitriptyline but not by the conventional antidepressants fluoxetine and buproprion. Bis selenide was more potent than the other drugs tested for antidepressant-like and antiallodynic effects in mice.

References

Attal N, Cruccu G, Haanpää M, Hansson P, Jensen TS, Nurmikko T, Sampaio C, Sindrup S, Wiffen P (2006) EFNS guidelines on pharmacological treatment of neuropathic pain. Eur J Neurol 13:1153–1169

Benarroch EE (2008) Descending monoaminergic pain modulation: bidirectional control and clinical relevance. Neurology 71:217–221
Benbouzid M, Choucair-Jaafar N, YalcinI WE, Muller A, Freund-Mercier MJ, Barrot M (2008) Chronic, but not acute, tricyclic antidepressant treatment alleviates neuropathic allodynia after sciatic nerve cuffing in mice. Eur J Pain 12:1008–1017
Bennett GJ, Xie YK (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33:87–107
Berton O, Nestler EJ (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 7:137–151
Blackburn-Munro G, Bomholt SF, Erichsen HK (2004) Behavioural effects of the novel AMPA/GluR5 selective receptor antagonist NS1209 after systemic administration in animal models of experimental pain. Neuropharmacology 47:351–362
Bomholt SF, Mikkelsen JD, Blackburn-Munro G (2005) Antinociceptive effects of the antidepressants amitriptyline, duloxetine, mirtazapine and citalopram in animals models of acute, persistent and neuropathic pain. Neuropharmacology 48:252–263
Casas J, Gibert-Rahola J, Chover AJ, Mico JA (1995) Test-dependent relationship of the antidepressant and analgesic effects of amitriptyline. Methods Find Exp Clin Pharmacol 17:583–588
Cooper BR, Wang CM, Cox RF, Norton R, Shea V, Ferris RM (1994) Evidence that the acute behavioral and electrophysiological effects of bupropion (Wellbutrin) are mediated by a noradrenergic mechanism. Neuropsychopharmacology 11:133–141
Cryan JF, Holmes A (2005) Model organisms: the ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 4:775–790
Dick IE, Brochu RM, Purohit Y, Kaczorowski GJ, Martin WJ, Priest BT (2007) Sodium channel blockade may contribute to the analgesic efficacy of antidepressants. J Pain 8:315–324
Dowdall T, Robinson I, Meert TF (2005) Comparison of five different rat models of peripheral nerve injury. Pharmacol Biochem Behav 80:93–108
Fishbain D (2000) Evidence-based data on pain relief with antidepressants. Ann Med 32:305–316
Gonçalves L, Silva R, Pinto-Riberio F, Pêgo F, Bessa JM, Pertovaara A, Sousa N, Almeida A (2008) Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat. Exp Neurol 213:48–56
Holtzheimer PE, Nemeroff CB (2006) Advances in the treatment of depression. J NeuroRx 3:42–56
Hyttel J (1994) Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs). Int Clin Psychopharmacol 1:19–26
Hu B, Doods H, Treede RD, Ceci A (2009) Depression-like behaviour in rats with mononeuropathy is reduced by the CB_2-selecetive agonist GW405833. Pain 143:206–212
Jesse CR, Savegnago L, Nogueira CW (2008) Spinal mechanisms of antinociceptive effect caused by oral administration of bis-selenide in mice. Brain Res 1231:25–33
Jesse CR, Savegnago L, Nogueira CW (2009) Mechanisms involved in the antinociceptive and anti-inflammatory effects of bis selenide in mice. J Pharm Pharmacol 61:623–630
Jesse CR, Wilhelm EA, Bortolatto CF, Nogueira CW (2010) Evidence for the involvement of the serotonergic $5\text{-}HT_{2A/C}$ and $5\text{-}HT_3$ receptors in the antidepressant-like effect caused by oral administration of bis selenide in mice. Prog Neuropsychopharmacol Biol Psychiatry 34:294–302
Li Z, Wua CF, Peib G, Xua NJ (2001) Reversal of morphine-induced memory impairment in mice by withdrawal in Morris water maze. Possible involvement of cholinergic system. Pharmacol Biochem Behav 68:507–513
Max MB, Culnane M, Schafer SC, Gracely RH, Walther DJ, Smoller B, Dunbar R (1987) Amitriptyline relieves diabetic neuropathy pain in patients with normal or depressed mood. Neurology 37:589–596
Mico JA, Ardid D, Berrocoso E, Eschalier A (2006) Antidepressants and pain. Trends Pharmacol Sci 7:348–354
Millan MJ (2002) Descending control of pain. Prog Neurobiol 66:355–474
Mitchell HA, Ahern TH, Liles LC, Javors MA, Weinshenker D (2006) The effects of norepinephrine transporter inactivation on locomotor activity in mice. Biol Psychiatry 60:1046–1052
Moro AV, Nogueira CW, Barbosa NBV, Menezes PH, Rocha JBT, Zeni G (2005) Highly stereoselective one-pot producers to prepare bis- and tris-chalcogenide alkenes via addition of disulfides and diselenides to terminal alkynes. J Org Chem 70:5257–5268
Moulin DE, Clark AJ, Gilron I, Ware MA, Watson CP, Sessle BJ, Coderre T, Morley-Forster PK, Stinson J, Boulanger A, Peng P, Finley GA, Taenzer P, Squire P, Dion D, Cholkan A, Gilani A, Gordon A, Henry J, Jovey R, Lynch M, Mailis-Gagnon A, Panju A, Rollman GB, Velly A (2007) Pharmacological management of chronic neuropathic pain—consensus statement and guidelines from the Canadian Pain Society. Pain Res Manag 12:13–21
Nogueira CW, Zeni G, Rocha JB (2004) Organoselenium and organotellurim compounds: toxicology and pharmacology. Chem Rev 104:6255–6285
Pedersen LH, Nielsen AN, Blackburn-Munro G (2005) Anti-nociception is selectively enhanced by parallel inhibition of multiple systems of monoamine transporters in rat models of persistent and neuropathic pain. Psychopharmacology 182:551–556
Phillis J, Wu PH (1982) The effect of various centrally active drugs on adenosine uptake by the central nervous system. Comp Biochem Pharmacol 72:179–187
Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. Nature 266:730–732
Porsolt RD, Anton G, Blavet N, Jalfre M (1978) Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 47:379–391
Rayman MP (2000) The importance of selenium to human health. Lancet 356:23–41
Redolat R, Vidal J, Gomez MC, Carrasco MC (2005) Effects of acute bupropion administration on locomotor activity in adolescent and adult mice. Behav Pharmacol 16:59–62
Roeska K, Doods H, Arndt K, Treede RD, Ceci A (2008) Anxiety-like behaviour in rats with mononeuropathy is reduced by the analgesic drugs morphine and gabapentin. Pain 139:349–357
Savegnago L, Jesse CR, Moro AV, Borges VC, Santos FW, Rocha JB, Nogueira CW (2006) Bis selenide alkene derivatives. A class of potential antioxidant and antinociceptive agents. Pharmacol Biochem Behav 83:221–229
Savegnago L, Jesse CR, Pinto LG, Rocha JB, Nogueira CW (2007a) Diphenyl diselenide attenuates acute thermal hyperalgesia and persistent inflammatory and neuropathic pain behavior in mice. Brain Res 1175:54–59
Savegnago L, Pinto LG, Jesse CR, Alves D, Rocha JB, Nogueira CW, Zeni G (2007b) Antinociceptive properties of diphenyl diselenide: evidences for the mechanisms of action. Eur J Pharmacol 555:129–138
Savegnago L, Jesse CR, Nogueira CW (2008) Caffeine and a selective adenosine $A_{(2B)}$ receptor antagonist but not imidazoline receptor antagonists modulate antinociception induced by diphenyl diselenide in mice. Neurosci Lett 436:120–123
Sawynok J, Liu XJ (2003) Adenosine in the spinal cord and periphery, release and regulation of pain. Prog Neurobiol 69:313–340
Sawynok J, Reid AR, Esser MJ (1999) Peripheral antinociceptive action of amitriptyline in the rat formalin test: involvement of adenosine. Pain 80:45–55
Sawynok J, Reid AR, Fredholm BB (2008) Caffeine reverses antinociception by amitriptyline in wild type mice but not in those lacking adenosine A1 receptors. Neurosci Lett 440:181–184

Sekihashi K, Sasaki T, Yamamoto A, Kawamura K, Ikka T, Tsuda S, Sasaki YF (2001) A comparison of intraperitoneal and oral gavage administration in comet assay in mouse eight organs. Mutat Res 493:39–54

Seltzer Z, Dubner R, Shir Y (1990) A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain 43:205–218

Sindrup SH, Brøsen K, Gram LF (1992) Antidepressants in pain treatment: antidepressant or analgesic effect. Clin Neuropharmacol 15(Suppl 1 Pt A):636A–637A

Sindrup SH, Otto M, Finnerup NB, Jensen TS (2005) Antidepressants in the treatment of neuropathic pain. Basic Clin Pharmacol Toxicol 96:399–409

Tal M, Bennett GJ (1994) Extra-territorial pain in rats with a peripheral mononeuropathy: mechano-hyperalgesia and mechano-allodynia in the territory of an uninjured nerve. Pain 57:375–382

Ulugol A, Karadag HC, Tamer M, Firat Z, Aslantas A, Dokmeci I (2002) Involvement of adenosine in the anti-allodynic effect of amitriptyline in streptozotocin-induced diabetic rats. Neurosci Lett 328:129–132

Vissers KCP, Geenen F, Biermans R, Meert TH (2006) Pharmacological correlation between the formalin test and the neuropathic pain behavior in different species with chronic constriction injury. Pharmacol Biochem Behav 84:479–486

Wang LX, Wang ZJ (2003) Animal and cellular models of chronic pain. Adv Drug Deliv Rev 55:949–965

Walsh RN, Cummins RA (1976) The open-field test: a critical review. Psychol Bull 83:482–504

Weiss GF, Papadakos P, Knudson K, Leibowitz SF (1986) Medial hypothalamic serotonin: effects on deprivation and norepinephrine-induced eating. Pharmacol Biochem Behav 25:1223–1230

Wolfe GI, Trivedi JR (2004) Painful peripheral neuropathy and its nonsurgical treatment. Muscle Nerve 30:3–19

Zimmerman M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16:109–110

4.5. Propriedades anti-hiperalgésicas e anti-edematogênicas do bis seleneto em modelos inflamatórios e neuropáticos em camundongos

4.5.1. Artigo 5 (na forma de manuscrito)

Propriedades anti-hiperalgésicas e anti-edematogênicas do bis seleneto em modelos inflamatórios e neuropáticos em camundongos

Cristiano R. Jesse, Ethel A. Wilhelm, Cristiani F. Bortolatto, Cristina W. Nogueira

Anti-hyperalgesic and anti-oedematogenic properties of bis selenide in inflammatory and neuropathic models in mice

Cristiano R. Jesse[1]*, Ethel A. Wilhelm[1], Cristiani F. Bortolatto[1], Cristina W. Nogueira[1]

[1]Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil.

Short running title:

Anti-hyperalgesic properties of bis selenide

*Correspondence should be sent to:

Cristiano R. Jesse

Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil.

Phone: 55-55-32208140

FAX: 55-55-32208978

E-mail: cristianoricardojesse@yahoo.com.br

Abbreviations

BPA brachial plexus avulsion

CFA Complete Freund's Adjuvant

CG carrageenan

i.p. intraperitoneally

i.pl. intraplantar

IL-1β interleukin-1β

IL-6 Interleukin-6

NO nitric oxide

p.o. per oral

PGE_2prostaglandin E_2

TNF- α tumor necrosis factor-α

VFH Von Frey Hair

Abstract

The purpose of the present study was to investigate the effects of bis selenide upon neuropathic and inflammatory models of nociception in mice. Acute oral treatment with bis selenide (5 and 10 mg/kg) demonstrated a significant reduction in the mechanical hyperalgesic in inflammatory models induced by Complete Freund's Adjuvant (CFA), carrageenan (CG) and prostaglandin E_2 (PGE_2) injections. Bis selenide at the dose of 10 mg/kg decreased the paw oedema formation induced by CFA. In CG and PGE_2 models the effective doses of bis selenide were doses equal and up to 5 mg/kg. The long-term postreatment with bis selenide twice a day decreased markedly the mechanical hyperalgesia (5 and 10 mg/kg) and the paw oedema (10 mg/kg) induced by CFA in ipsilateral and contralateral sides. Bis selenide, given at doses of 5 and 10 mg/kg, in both acute and chronic

schedules of postreatment, consistently reduced the mechanical hyperalgesia induced in the brachial plexus avulsion model of neuropathy. Pretreatment with bis selenide (5 and 10 mg/kg) and vincristine (a chemotherapeutic agent that causes peripheral neuropathy), during 7 days, significantly attenuated the mechanical hyperalgesia from days 3 to 28 in mice. In conclusion, these results indicate that bis selenide produces pronounced anti- hyperalgesic and anti-oedematogenic effects on models of chronic nociception in mice. It also indicates indicated that bis selenide might be potentially interesting in the development of new clinically relevant drugs for the management of this disorder.

Keywords:

Bis selenide, selenium, hyperalgesia, inflammatory chronic nociception, mice

Introduction

Pain is one the classical symptoms of the inflammatory process (Ji and Stricharstz 2004). It is now accepted that sensitization of primary nociceptive neurons is the common denominator of inflammatory pain that leads to states known as hyperalgesia/allodynia in humans or hyperalgesia in animal models (Cunha et al. 2005; Verri et al. 2006). Hyperalgesia is induced by the action of inflammatory mediators, such as prostaglandins and sympathetic amines, on peripheral nociceptors. These directacting hyperalgesic mediators are ultimately released in the inflamed tissue following a cascade of cytokines such as tumor necrosis factor-α (TNF- α), interleukin-1β (IL-1β) and chemokines released by the resident and migratory cells (Cunha et al. 2005; Verri et al. 2006).

Currently available drugs which provide relief of neuropathic and inflammatory pain are effective only in a fraction of such patients. In general, these drugs present low efficacy and numerous side effects (Woolf and Mannion 1999; Mendel and Sahenk 2003). Inflammatory pain is characterized by tissue damage that promotes the liberation of several

chemical mediators that in turn induce the activation or sensitization of nociceptors, eliciting pain at the site of injury (Julius and Basbaum 2001). Because no universally efficacious therapy for it exists, neuropathic pain research has been explored with different animal models where intentional damage is done to the sciatic nerve, branches of spinal nerves or in the spinal cord (Seltzer et al. 1990; Malmber and Basbaum 1998; Zimmermann 2001; Ji and Stricharstz 2004).

Some types of cancer chemotherapy result in peripheral neuropathy associated with loss of sensation and numbness in the feet, hands, and legs accompanied by painful tingling or burning sensations (Visovsky 2003; Thibault et al. 2008).

In this context, drugs that decrease the inflammatory condition could be successful applied in certain chronic pain states. Taking this into account, several studies have described antinociceptive and anti-inflammatory activities of bis selenide in rodents (Savegnago et al. 2006; Jesse et al. 2007, 2008, 2009). Recently, we have reported that bis selenide administration elicits significant antinociception when assessed in acetic acid, capsaicin, tail immersion, hot plate and formalin tests (Savegnago et al. 2006; Jesse et al. 2007).

In this study, we investigated the properties of bis selenide in the mechanical hyperalgesia and paw oedema in inflammatory models of nociception in mice induced by Complete Freund's Adjuvant (CFA), carrageenan (CG) and prostaglandin E_2 (PGE_2). Furthermore, we also evaluated the effect of bis selenide on mechanical hyperalgesia induced by neuropathic models in mice (brachial plexus avulsion- BPA and vincristine administration).

Experimental procedures

Animals

The behavioural experiments were conducted using Swiss male mice (25 - 35g) from our own breeding colony. The mice were kept in a separate animal room, on a 12 h light/dark cycle, at room temperature (22 ± 1°C), with free access to food and water. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann 1983). The number of animals and intensities of noxious stimuli used were minimum necessary to demonstrate the consistent effects of the drugs treatments.

Drugs

Bis selenide [(*Z*)-2,3-Bis(4-chlorophenylselanyl)prop-2-en-1-ol] was prepared and characterized in our laboratory by the method previously described (Moro et al. 2005). Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of bis selenide (99.9%) was determined by gas chromatography/high-performance liquid chromatography. Bis selenide was dissolved in canola oil and administered by oral route (p.o.). The mice received bis selenide in a constant volume of 10 ml/kg body weight. CFA, CG and PGE_2 were purchased from Sigma-Aldrich (St. Louis, MO). Vincristine sulfate was purchased from Oncovin; Nippon Kayaku, Tokyo, Japan. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Mechanical hyperalgesia and paw oedema induced by CFA

To produce a persistent inflammatory response, mice received a 20 µl intraplantar (i.pl.) injection of CFA (1 mg/ml heat-killed and dried Mycobacterium tuberculosis; each ml

of vehicle contained 0.85 ml paraffin oil plus 0.15 ml mannide monooleate) into the right hindpaw (Quintão et al. 2005). To assess the effect of the acute treatment on mechanical hyperalgesia and paw oedema, animals received a single oral (p.o.) dose of bis selenide (1, 5 and 10 mg/kg) or vehicle (canola oil) 24 h after CFA injection. The mechanical hyperalgesia was assessed by Von Frey Hair (VFH) filaments and paw oedema was evaluated by micrometer.

To investigate the effect of the long-term treatment, bis selenide (5 and 10 mg/kg, p.o.) or vehicle was administered in mice, twice a day (every 12 h). The mechanical hyperalgesia and paw oedema were evaluated only 4 h after treatment. Treatment was extended until the maximum reduction effect was achieved, and it was then interrupted. Next, the treatment was re-initiated to assess the development of tolerance. Developments of mechanical hyperalgesia and paw oedema formation were evaluated in CFA-injected animals for up to 12 h or 18 days in acute and chronic treatments, respectively.

Mechanical hyperalgesia and paw oedema induced by CG

For the induction of inflammatory nociception, mice received an i.pl. injection of 50 µl of CG (300 µg/paw) under the surface of the right hindpaw (Quintão et al. 2005). To assess the acute effect of drug treatment, mice received bis selenide (1, 5 and 10 mg/kg, p.o.) or vehicle 30 min before CG injection. The mechanical hyperalgesia was assessed by VHF filaments. Paw oedema was evaluated by micrometer for up to 96 h after CG administration.

Mechanical hyperalgesia and paw oedema induced by PGE_2

To evaluate mechanical hyperalgesia and paw oedema, mice were treated with bis selenide (1, 5 and 10 mg/kg, p.o.) or vehicle 30 min before the i.pl. injection of PGE_2 (0.1 nmol/paw) (Kassuya et al. 2007). The mechanical hyperalgesia was measured with VHF filaments and paw oedema was evaluated with micrometer, as described below, at different time-points after the injection of the algogenic mediator. Development of mechanical hyperalgesia and oedema formation were evaluated in PGE_2-injected animals for up to 48 h.

Neuropathic model of nociception induced by BPA

The BPA model for rats (Rodrigues-Filho et al. 2003) and it was adapted for mice (Quintão et al. 2006). Briefly, animals were anaesthetized with 7% chloral hydrate (8 ml/kg; i.p.). The right brachial plexus was approached through a longitudinal incision parallel to the clavicle, running from the sternum to the axillary region (1 cm approximately). The subclavian vessels were located and the lower trunk of brachial plexus was dissected from them. In one group of mice, the lower trunk was grasped and extorted by traction using forceps. In the sham-operated group, the brachial plexus was exposed and dissected without any lesion to the nerve. The tissue layers were then brought together and the skin closed with a 4.0 silk suture string (Ethicon, Edinburgh). To assess the effects of the acute treatment, animals received a single dose of bis selenide (1, 5 and 10 mg/kg, p.o.) or vehicle following 4 days after the BPA.

To investigate the effect of the long-term treatment, bis selenide (5 and 10 mg/kg, p.o.) or vehicle was administered in mice, twice a day (every 12 h). Mechanical hyperalgesia response was evaluated only 4 h after treatment. Treatment was extended until the maximum reduction effect was achieved, and it was then interrupted. Next, the treatment was re-initiated to assess the development of tolerance.

Neuropathic model of nociception induced by vincristine

Vincristine sulfate was administered intraperitoneally (i.p.) in a dose of 0.1 mg/kg, once per day for 7 consecutive days in mice. The dose of vincristine was decided based on previous report (Kamei et al. 2006; Kiguchi et al. 2008). The time course of the mechanical hyperalgesia produced by this dose and dosing schedule was examined over the course of 28 days with comparison to a vehicle-injected control group. Mice received bis selenide administration at doses of 1, 5 and 10 mg/kg (p.o.) or vehicle once every 7 days (i.e., days 1-7) 30 minutes before the injection of vincristine. The mechanical hyperalgesia of all groups were assessed by means of VHF filaments for up to 28 days after vincristine administration.

Hindpaw withdrawal response evaluated by VHF filaments and paw oedema assessed by micrometer

To assess the mechanical hyperalgesia induced by CFA, CG, PGE_2 and BPA models, mice were placed individually in clear Plexiglas boxes (9 x 7 x 11 cm) on elevated wire mesh platforms to allow access to the ventral surface of the right and left hindpaws. Animals were acclimatized for 30 min prior to behavioral testing. The withdrawal response frequency was measured following 10 applications (duration of 1-2 s each) of VHF filament (Stoelting, Chicago, USA). Stimuli were delivered from below to the plantar surface of the right hindpaw. 0.6 g VHF produces a mean withdrawal frequency of about 15%, which is considered to be an adequate value for the measurement of mechanical hyperalgesia (Quintão et al. 2005, 2006). Therefore, 0.6 g VHF was used throughout this study. In order to determine the basal mechanical thresholds, all the groups were submitted to pre-surgical or pre-injection evaluation.

In the mechanical hypernociception induced by vincristine, VHF filaments were inserted through the mesh floor bottom and were applied to the middle of planter surface of

each hind paw 10 times with the weight of 0.4 g (days 0–10) or 0.6 g (days 14–28). The two kinds of filaments (0.4 g and 0.6 g) were used, because the withdrawal response is different according to growth of mice, ie, the % response to the stimulation by filament of 0.4 g on day 14 was extremely lower than the day 7 in control mice (Kiguchi et al. 2008).

To evaluate the oedema induced by algogenic mediators, paw thickness was measured after mechanical tests. Here, mice were gently placed in a piece of cloth and, after settling, a calibrated micrometer was used to measure the maximal dorso-ventral thickness of the paw.

Statistical analysis

Results are presented as the mean ± S.E.M. Each point of graphs represents the mean of 8 animals. Maximum reduction values were calculated in the effective dose used in the acute treatments. Percentage of reduction was calculated in chronic models and this reduction represented the difference between the values of vehicle and inflammatory/neuropathic groups. The statistical significance of differences between groups was performed by Repeated Measures ANOVA followed by Newman-Keuls test. Probability values less than 0.05 ($P<0.05$) were considered as statistically significant.

Results

Effects of bis selenide on mechanical hyperalgesia and paw oedema induced by CFA injection

The i.pl. injection of CFA induced a marked mechanical hyperalgesia (Fig. 1A) and paw oedema (Fig. 1B) on the ipsilateral side, but not on the contralateral side (results not shown) for up to 12h. The injection of CFA produced a profound and long-lasting mechanical hyperalgesia and paw oedema on the ipsilateral side, and to a lesser extent on the contralateral side for up to 18 days (Fig. 2A, 2B, 3A and 3B). Postreatment with bis selenide (5 and 10 mg/kg, p.o.) demonstrated a significant reduction on the mechanical hyperalgesia induced by

CFA injection (Fig. 1A). This reduction started 1 h after bis selenide administration at dose of 5 mg/kg (maximum reduction was 43±3%) and 10 mg/kg (maximum reduction was 61±5%). The significant reduction in the mechanical hyperalgesia was maintained for up to 4h and 8 h when mice received 5 and 10 mg/kg of bis selenide, respectively (Fig. 1A). Acute postreatment with bis selenide at the dose of 10 mg/kg (p.o.) significantly decreased the paw oedema formation induced by CFA injection from 2 h (maximum reduction was 26±2%) to 12 h (maximum reduction was 34±3%) (Fig. 1B).

The long-term postreatment with bis selenide twice a day (every 12 h) decreased markedly the mechanical hyperalgesia in the ipsilateral side (percentage of reduction was 55±3% at 5 mg/kg and 68±7% at 10 mg/kg) (Fig. 2A). This effect was evident from days 2 to 10 and 14 to 16, when occurred the repeated treatment with bis selenide (Fig. 2A). When analyzed on the contralateral side, the long-term oral treatment with bis selenide produced a reduction of the mechanical hyperalgesia induced by CFA injection (percentage of reduction was 33±3% at 5 mg/kg and 37±5% at 10 mg/kg) (Fig. 2B). This effect was evident from days 4 to 10 and 14 to 16, when occurred the repeated treatment with bis selenide (Fig. 2B). When the treatment was suspended (from days 11 to 13; 17 to 18), the mechanical hyperalgesia induced by bis selenide was not observed on ipsilateral (Fig. 2A) and contralateral (Fig. 2B) sides. When bis selenide treatment was re-initiated on day 14, the same anti- hyperalgesic response was detected, showing that bis selenide did not induce tolerance.

As observed in Fig. 3A, the chronic postreatment of mice with bis selenide (10 mg/kg, p.o.) produced a significant and long-lasting reduction of the paw oedema formation induced by CFA from days 2 to 18 on ipsilateral side (percentage of reduction was 34±5%). Curiously, when the treatment was suspended (from days 11 to 13; days 17 and 18), the reduction in the paw oedema induced by bis selenide was observed in ipsilateral side (Fig. 3A). When analyzed on the contralateral side (Fig. 3B), the long-term oral treatment with bis selenide at

the dose of 10 mg/kg significantly diminished the paw oedema induced by CFA from days 6 to 16 (percentage of reduction was 34±5%).

Effects of bis selenide on mechanical hyperalgesia and paw oedema induced by CG injection

The i.pl. injection of CG induced a marked mechanical h hyperalgesia and paw oedema on the ipsilateral side (Fig. 4A and 4B), but not on the contralateral side (results not shown). Oral pretreatment of mice with bis selenide was capable of reducing the mechanical hyperalgesia induced by CG, an effect observed for up to 1 h at 1mg/kg, 8 h at 5 mg/kg and 24 h at 10 m/kg (Fig. 4A). The maximum reduction observed at 1 h at doses of 1, 5 and 10 mg/kg of bis selenide was 33±3%, 46±4% and 69±4%, respectively. As described for mice, pretreatment with bis selenide produced a significant reduction of the paw oedema induced by CG (Fig. 4B) for up to 8 h at 5 mg/kg and 48 h at 10 mg/kg. The maximum reduction observed at 1 h at doses of 5 and 10 mg/kg of bis selenide was 36±4% and 54±4%, respectively.

Effects of bis selenide on mechanical hyperalgesia and paw oedema induced by PGE_2 injection

The i.pl. injection of PGE_2 induced a marked mechanical hyperalgesia and paw oedema on the ipsilateral side (Fig. 5A and 5B), respectively, but not on the contralateral side (results not shown). Bis selenide pretreatment (5 and 10 mg/kg) was found to be effective in significantly preventing the mechanical hyperalgesia caused by PGE_2 in mice (Fig. 5A) for up to 4 h. The maximum reduction observed at 1 h was 28±1% at 5 mg/kg and 40±2% at 10 mg/kg. Pretreatment with bis selenide at doses of 5 and 10 mg/kg produced also a significant reduction of the paw oedema evoked by PGE_2 in mice (Fig. 5B) for up to 4 h. The maximum reduction observed at 1 h was 29±2% at 5 mg/kg and 34±2% at 10 mg/kg.

Effects of of bis selenide on the mechanical hyperalgesia induced by BPA

BPA produced a marked and long-lasting development of allodynia on the ipsilateral (injured) side (Fig. 6A and 6B), but not on the contralateral side (results not shown). Postreatment with bis selenide at all doses did not affect the mechanical hyperalgesia of sham-operated animals (data not shown). Animals that received bis selenide (5 and 10 mg/kg, p.o.) demonstrated a significant reduction on the mechanical hyperalgesia induced by BPA model (Fig. 6A). This reduction started 1 h after bis selenide administration at doses of 5 mg/kg (maximum reduction was 59±5%) and 10 mg/kg (maximum reduction was 82±5%). The significant reduction in the mechanical hyperalgesia was maintained for up to 4 h and 8 h when BPA-injured mice received 5 and 10 mg/kg of bis selenide, respectively (Fig. 6A). The long-term postreatment with bis selenide twice a day (every 12 h) decreased markedly the mechanical hyperalgesia on the ipsilateral side on BPA-injured mice (percentage of reduction: 42±5 at 5 mg/kg and 62±4% at 10 mg/kg) (Fig. 6B). This effect was evident from day 4 and persisted until 10 days when the treatment was interrupted. The suspension of treatment with bis selenide at days 11-13 and 17-18 abolished its anti- hyperalgesic effect. The continuation of long-term treatment, with bis selenide starting at day 14, produced essentially the same anti- hyperalgesic effect, which remained stable until the treatment was suspended again (day 17). Results in Fig. 6B show that there is no evidence for the development of tolerance.

Effects of of bis selenide on the mechanical hyperalgesia induced by vincristine

The withdrawal responses by mechanical stimulation applied to each hind paw using VFH filaments from day 0 to day 28 in mice was evaluated. In vehicle-treated mice, the percentage of response was constant (approximately 15%) during days 0–28. On the other hand, in mice given vincristine (0.1 mg/kg) for 7 days, the percentage of response

significantly increased on day 3 and the maximal % response was observed on day 7 (Fig. 7). The mechanical hyperalgesia was maintained after the cessation of vincristine administration (from day 8 to 28). Pretreatment with bis selenide and vincristine during 7 days significantly attenuated the mechanical hyperalgesia from days 3 to 28 in comparison with vincristine-treated mice (percentage of reduction:42±4% at 5 mg/kg and 49±3% at 10 mg/kg) (Fig. 7).

Discussion

This study shows that bis selenide orally administered attenuated mechanical hyperalgesia induced by neuropathic and inflammatory models of nociception: CFA, CG, PGE_2, BPA and vincristine.

The present results show a consistent anti- hyperalgesic and anti-oedematogenic effects for bis selenide in the CFA model, when postreatred orally, in acute and repeated schedules of treatment. Importantly, the effect of the repeated postreatment with bis selenide did not induce tolerance, whereas the interruption of treatment abolished the anti-hyperalgesic property. CFA produces long-term inflammatory and nociceptive responses (Chan et al. 2000), which are probably associated with significant changes in neurons of the central nervous system, culminating in the sensitization of the contralateral paw (Sluka 2002). Furthermore, CFA effects have been also associated to a significant increase of multiple inflammatory and nociceptive mediators (TNF-α, IL-1β, PGE_2, glutamate, histamine and nitric oxide-NO) production in the inflamed paw, which might contribute to the initialization of the inflammation (Woolf et al. 1997).Mechanisms involved in formation of persistent oedema in the contralateral paw after CFA injection is mediated by local nociceptor sensitisation and systemic neuronal (such as central sensitisation) and immune (such as increase in cytokine serum level) mechanisms (Samad et al. 2001). Mechanisms of action of bis selenide in the CFA model could be due the interaction with prostanoid, glutamatergic

(kainate and trans-ACDP receptors), histaminergic (H_2 receptors) receptors, NO-soluble guanylate cyclase pathways and pro-inflammatory cytokines (TNF-α and IL-1β), since there are evidences of these mechanisms of action in the antinociceptive and anti-inflammatory effects (Jesse et al. 2007, 2008, 2009).

Pretreatment of mice with bis selenide produced anti- hyperalgesic effect for up to 24 h in the CG acute model of nocicepton. Interestingly, administration of bis selenide markedly decreased CG-induced paw oedema (for up to 48 h). The model of hyperalgesia and paw oedema induced by the injection of CG has been widely employed in order to assess the effects of new anti-inflammatory drugs (Henriques et al. 1987; Ianaro et al. 1994; Posadas et al. 2004; Quintão et al. 2005). The injection of CG is followed by an inflammatory response, accompanied by cell migration and release of inflammatory mediators (such as prostaglandins, histamine, glutamate and substance P). It is generally accepted that neutrophils, through the release of cytotoxic enzymes and reactive oxygen species with consequent exacerbation of inflammatory conditions (Koennecke et al. 1999). Cunha et al (2005) have suggested that release of primary mediators responsible for CG-induced mechanical hyperalgesia is preceded by cytokine production. Conversely, it has been shown that NO (derived from both the endothelial and inducible nitric oxide synthase isoforms) is apparently implicated in the two phases of CG-evoked mouse paw oedema (Posadas et al. 2004; Bucci et al. 2005). The role for glutamatergic (kainate and trans-ACDP receptors), prostanoid, histaminergic (H_2 receptors) and neuropeptide (NK_1 receptors) receptors and NO-soluble guanylate cyclase pathways and and pro-inflammatory cytokines (TNF-α an IL-1β) in the anti- hyperalgesic and anti-oedematogenic effects of bis selenide can be involved, since these mechanisms contributed to the antinociceptive effect of this compound (Jesse et al. 2007, 2008, 2009). In addition, bis selenide is an antioxidant agent as a result its action could

involve the reduction of reactive oxygen species with consequent decrease of inflammatory conditions (Savegnago et al. 2006).

The results demonstrated that pretreatment with bis selenide reduced the mechanical hyperalgesia and paw oedema induced by PGE_2. It is worth mentioning that several anti-inflammatory drugs used clinically to treat different inflammatory states modulated proinflammatory cytokines and prostaglandins effects, such as cyclooxygenases inhibitors or glucocorticoids (Santini et al. 2001; Chivers et al. 2004). During an inflammatory event, pain generation is a consequence of complex interactions between a number of inflammatory mediators, including prostaglandins, some of which (notably PGE_2) are known to have a critical role in the generation and maintenance of the nociceptive response (Samad et al. 2001). Some studies have demonstrated that the mechanical hyperalgesia caused by peripheral PGE_2 injection in rodents is mediated by cAMP–protein kinase A, protein kinase C and mitogen-activaged protein kinases pathways (Ferreira and Nakamura 1979; Kassuya et al. 2007). Bis selenide modulated protein kinases A and C signaling pathways and anti-hypernociceptive and anti-oedematogenic effects in the PGE_2 model could be due the interaction with these signaling pathways (Jesse et al. 2009).

Bis selenide postreatment was able to acutely and long-lastingly prevent the mechanical hypernociception induced by BPA, when given systemically. In this models of neuropathic nociception, the hyperalgesic starts on day 4 following surgery and BPA is restricted to the ipsilateral side. Importantly, the effect of the chronic treatment with bis selenide had a long-lasting profile of duration and the anti- hyperalgesic effect was detected when the treatment re-initiated. Thus, we considered that chronic treatment with bis selenide did not induce tolerance. Neuropathic models such as BPA causes persistent nociception, which leads to the release of multiple inflammatory and nociceptive mediators (Quintão et al. 2006, 2008a, 2008b). These mediators increase long-lasting discharge of primary sensory

fibers that modifies neuronal, neuro-glial and neuro-immune cell phenotype and function in the central nervous system (Rodrigues-Filho et al. 2003). These alterations can occur at translational or post-translational levels and achieve receptors, ion channels, soluble mediators and molecules played in cell signaling (Woolf and Mannion 1999; Ji and Stricharstz, 2004). Mechanical anti- hyperalgesic action of bis selenide, first reported here, could be related to these different neurotransmitter systems as glutamatergic, prostanoid, serotonergic, histaminergic and neuropeptidergic, since these systems contributed to the antinociceptive effect of this compound (Jesse et al. 2008, 2009).

The mechanical hyperalgesia was significantly attenuated by bis selenide pretreatment during vincristine administration (from days 0 to 6), and the attenuation of the mechanical hyperalgesia persisted until day 28. Experimental models of vincristine-induced peripheral neuropathic pain have been established in rodents (Kamei et al. 2006; Kiguchi et al. 2008). Some reports demonstrate that inflammatory and immune responses in the injured peripheral nervous system play an important role in the development and maintenance of neuropathic nociception following peripheral nerve injury (Cui et al. 2000; Scholz and Woolf 2007). When the peripheral nerve is damaged, immune modulating cells, such as macrophages and lymphocytes, invade the injured nerve fiber and dorsal root ganglion, and express various pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) and neuropeptide expression (Hu and McLachlan 2002; Ma and Quirion 2005). The cellular changes could lead to increased activity and hypersensitivity of nociceptive neurons, which may contribute to the spontaneous pain and pain hypersensitivity that are characteristic of neuropathic pain (Woolf and Mannion 1999; Zimmermann 2001). The interaction of bis selenide with neuropeptide (NK_1) receptors and pro-inflammatory cytokines could be involved in the bis selenide action, since these mechanisms modulated the antinociceptive effect of this compound (Jesse et al. 2008).

Together these results indicate that bis selenide produces pronounced anti-hyperalgesic and anti-oedematogenic effects on models of chronic nociception in mice. Further functional, electrophysiological and molecular studies are necessary to clarify the precise mechanisms through which bis selenide exerts its anti- hyperalgesic action in persistent models of pain. Considering that few drugs are currently available for the treatment of persistent pain, especially of neuropathic origin, the present results may have clinical relevance and open the possibility of the development of new drug.

Acknowledgements

Financial support by UFSM, CAPES, CNPq and FAPERGS is acknowledged. The authors are also thankful to the FINEP research grant ‘‘Rede Instituto Brasileiro de Neurociência (IBN-Net)” # 01.06.0842-00, INCT for Excitotoxicity and Neuroprotection-CNPq and FAPERGS/CNPq (PRONEX) research grant # 10/0005-1.

References

Bucci M, Roviezzo F, Posadas I, Yu J, Parente L, Sessa WC, Ignarro LJ, Cirino G (2005) Endothelial nitric oxide syntahse activation is critical for vascular leakage during acute inflammation in vivo. Proc Natl Acad Sci USA 102:904-908

Chan CF, Sun WZ, Lin JK, Lin-Shiau SY (2000) Activation of transcription factors of nuclear factor kappa B, activator protein-q and octamer factors in hyperalgesia. Eur J Pharmacol 420:61-68

Chivers JE, Cambrigde LM, Catley MC, Mak JC, Donnelly LE, Barnes PJ, Newton R (2004) Differential effects of RU486 reveal distinct mechanisms for glucorticoid repression of prostalglandin E release. Eur J Biochem 271:4042-4052

Cui JG, Holmin S, Mathiesen T, Meyerson BA, Linderoth B (2000) Possible role of inflammatory mediators in tactile hypersensitivity in rat models of mononeuropathic. Pain 88:239-248

Cunha TM, Verri WAJr, Silva JS, Poole S, Cunha FQ, Ferreira SH (2005) A cascade of cytokines mediates mechanical inflammatory hyperalgesia in mice. Proc Natl Acad Sci USA 102:1755-1760

Ferreira SH, Nakamura MI (1979) Prostaglandin hyperalgesia, a cAMP/Ca^{2+} dependent process. Prostaglandins 18:179-190

Henriques MG, Silva PM, Martins MA, Flores CA, Cunha FQ, Assreuy-Filho J, Cordeiro RS (1987) Mouse paw oedema: a new model for inflammation. Braz J Med Biol Res 20:243-249

Hu P, McLachlan EM (2002) Macrophage and lymphocyte invasion of dorsal root ganglia after peripheral nerve lesions in the rat. Neuroscience 112:23-28

Ianaro A, O'Donnell CA, Di Rosa M, Liew FY (1994) A nitric oxide inhibitor reduces inflammation, down-regulates infammatory cytokines and enhances interleukin-10 production in carrageenin-induced oedema in mice. Immunology 82:370-375

Jesse CR, Savegnago L, Nogueira CW (2007) Role of nitric oxide/cyclic GMP/K+ channel pathways in the antinociceptive effect caused by 2,3-bis(mesitylseleno)propenol. Life Sci 81:1694-1702

Jesse CR, Savegnago L, Nogueira CW (2008) Spinal mechanisms of antinociceptive effect caused by oral administration of bis selenide in mice. Brain Res 1231:25-33

Jesse CR, Savegnago L, Nogueira CW (2009) Mechanisms involved in the antinociceptive and anti-inflammatory effects of bis selenide in mice. J Pharm Pharmacol 61:623-630

Ji RR, Stricharstz G (2004) Cell signaling and the genesis of neuropathic pain. Science 252:1-19

Julius D, Basbaum AI (2001) Molecular mechanisms of nociception. Nature 413:203-210

Kamei J, Nozaki C, Saitoh A (2006) Effect of mexiletine on vincristine-induced painful neuropathy in mice. Eur J Pharmacol 536:23-127

Kassuya CA, Ferreira J, Claudino RF, Calixto JB (2007) Intraplantar PGE_2 causes nociceptive behaviour and mechanical allodynia: the role of prostanoid E receptors and protein kinases. Br J Pharmacol 150:727-737

Kiguchi N, Maeda T, Kobayashi Y, Kondo T, Ozaki M, Kishioka S (2008) The critical role of invading peripheral macrophage-derived interleukin-6 in vincristine-induced mechanical allodynia in mice. Eur J Pharmacol 592:87-92

Koennecke LA, Zito MA, Proescholdt MG, van Rooijen N, Heyes MP (1999) Depletion of systemic macrophages by liposome-encapsulated clodronate attenuates increases in brain quinolinic acid during CNS- localized and systemic immune activation. J Neurochem 73:770-779

Ma W, Quirion R (2005) The ERK/MAPK pathways, as a target for the treatment of neuropathic pain. Expert Opin Ther Targets 9:699-713

Malmber AB, Basbaum AI (1998) Partial sciatic nerve injury in the mouse as a model of neuropathic pain: behavioral and neuroanatomical correlates. Pain 76:215-222

Mendell JR, Sahenk A (2003) Painful sensory neuropathy. N Engl J Med 348, 1243-1255

Moro AV, Nogueira CW, Barbosa NBV, Menezes PH, Rocha JBT, Zeni G (2005) Highly stereoselective one-pot producers to prepare bis- and tris chalcogenide alkenes via addition of disulfides and diselenides to terminal alkynes. J Org Chem 70:5257-5268

Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, Cirino G (2004) Carrageenan-induced mouse paw oedema in biphasic, age-weight dependent and diplays differential nitric oxide cyclooxygenase-2 expression. Br J Pharmacol 142:331-338

Quintão NL, Medeiros R, Santos AR, Campos MM, Calixto JB (2005) The effects of diacerhein on mechanical allodynia in inflammatory and neuropathic models of nociception in mice. Anesth Analg 101:1763-1769

Quintão NL, Balz D, Santos AR, Campos MM, Calixto JB (2006) Long-lasting neuropathic induced by brachial plexus injury in mice: Role triggered by the pro-inflammatory cytokine, tumor necrosis factor alpha. Neuropharmacology 50:614-620

Quintão NL, Passos GF, Medeiros R, Paszcuk AF, Motta FL, Pesquero JB, Campos MM, Calixto J.B (2008a) Neuropathic pain-like behavior after brachial plexus avulsion in mice: the relevance of kinin B_1 and B_2 receptors. J Neurosci 28:2856-2863

Quintão NL, Santos AR, Campos MM, Calixto JB (2008b) The role of neurotrophic factors in genenis and maintenance of mechanical hypernociception after brachial plexus avulsion in mice. Pain 136:125-133

Rodrigues-Filho R, Santos AR, Bertelli JA, Calixto JB (2003) Avulsion injury of the rat brachial plexus triggers hyperalgesia and allodynia in the hindpaws: a new model for the study of neuropathic pain. Brain Res 29:186-194

Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S, Bonventre JV, Woolf CJ (2001) Interleukin-1 beta-mediated induction of COX-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 410:471-475

Santini G, Patrignani P, Sciulli MG, Seta F, Tacconelli S, Panara MR, Ricciotti E, Capone ML, Patrono C (2001) The human pharmacology of monocyte cyclooxygenase 2 inhibition by cortisol and synthetic glucocorticoids. Clin Pharmacol Ther 70:475-483

Savegnago L, Jesse CR, Moro AV, Borges VC, Santos FW, Rocha JB, Nogueira CW (2006) Bis selenide alkene derivatives: A class of potential antioxidant and antinociceptive agents. Pharmacol Biochem Behav 83:221-229

Scholz J, Woolf CJ (2007) The neuropathic pain triad: neurons, immune cells and glia. Nat Neurosci 10:1361-1368

Seltzer Z, Dubner R, Shir Y (1990) A novel behavioural model of neuropathic pain disorders produced in rats by partial nerve injury. Pain 43: 205-218

Sluka KA (2002) Stimulation of deep somatic tissue with capsaicin produced long-lasting mechanical allodynia and heat hypoalgesia that depends on early activation of the cAMP pathway. J Neurosci 22:5687-5693

Thibault K, Elisabeth B, Sophie D, Claude FZM, Bernard R, Bernard C (2008) Antinociceptive and anti-allodynic effects of oral PL37, a complete inhibitor of enkephalin-catabolizing enzymes, in a rat model of peripheral neuropathic pain induced by vincristine. Eur J Pharmacol 600:71-77

Verri WAJr, Cunha TM, Parada CA, Poole S, Cunha FQ, Ferreira SH (2006) Hypernociceptive role of cytokines and chemokines: targets for analgesic drug development? Pharmacol Ther 112:116-138

Visovsky C (2003) Chemotherapy-induced peripheral neuropathy. Cancer Invest 21:439-451

Woolf CJ, Allchorne A, Safieh-Garabedian B, Poole S (1997) Cytokines, nerve growth factor and inflammatory hyperalgesia: the contribution of tumor necrosis factor alpha. Br J Pharmacol 121:417-424

Woolf CJ, Mannion RJ (1999) Neuropathic pain: aetiology, symptoms, mechanisms and management. Pain 353:1959-1964

Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109-110

Zimmermann M (2001) Pathobiology of neuropathic pain. Eur J Pharmacol 429:23-27

Legends

Fig. 1 - Effect of bis selenide on mechanical hyperalgesia (Panel A) and paw oedema (Panel B) induced by CFA injection in mice. The animals received vehicle (canola oil) (●) or bis selenide at doses of 1 (▲), 5 (♦) or 10 (■) mg/kg 24 h after CFA injection. (A) The baseline (□) was recorded before CFA injection and in (B) baseline paw thickness was discounted from total thickness to give the oedema value. Results represent the mean±S.E.M. of eight animals. Symbols denote a significant difference at ** P<0.01 and ***P<0.001 between vehicle treated and bis selenide treated mice and #P< 0.001 versus baseline value.

Fig. 2 - Effect of bis selenide on mechanical hyperalgesia in ipsilateral (Panel A) or contralateral (Panel B) sides induced by CFA injection in mice. Chronically treatment with vehicle (canola oil) (●) or bis selenide at doses 5 (♦) or 10 (■) mg/kg was given to mice. The baseline response (□) was recorded before CFA injection. Results represent the mean±S.E.M. of eight animals. Symbols denote a significant difference at ***P<0.001 between vehicle treated and bis selenide treated mice and #P< 0.001 versus baseline value.

Fig. 3 - Effect of bis selenide on paw oedema in ipsilateral (Panel A) or contralateral (Panel B) sides induced by CFA injection in mice. Chronically treatment with vehicle (canola oil) (●) or bis selenide at doses of 5 (♦) or 10 (■) mg/kg was given to mice. The baseline (□) was recorded before CFA injection. Results represent the mean±S.E.M. of eight animals. The symbols denote a significant difference at *P>0.05, **P<0.01 and ***P<0.001 between vehicle treated and bis selenide treated mice and #P< 0.001 versus baseline value.

Fig. 4 - Effect of bis selenide on mechanical hyperalgesia (Panel A) and paw oedema (Panel B) induced by CG injection in mice. The animals received vehicle (canola oil) (●) or bis

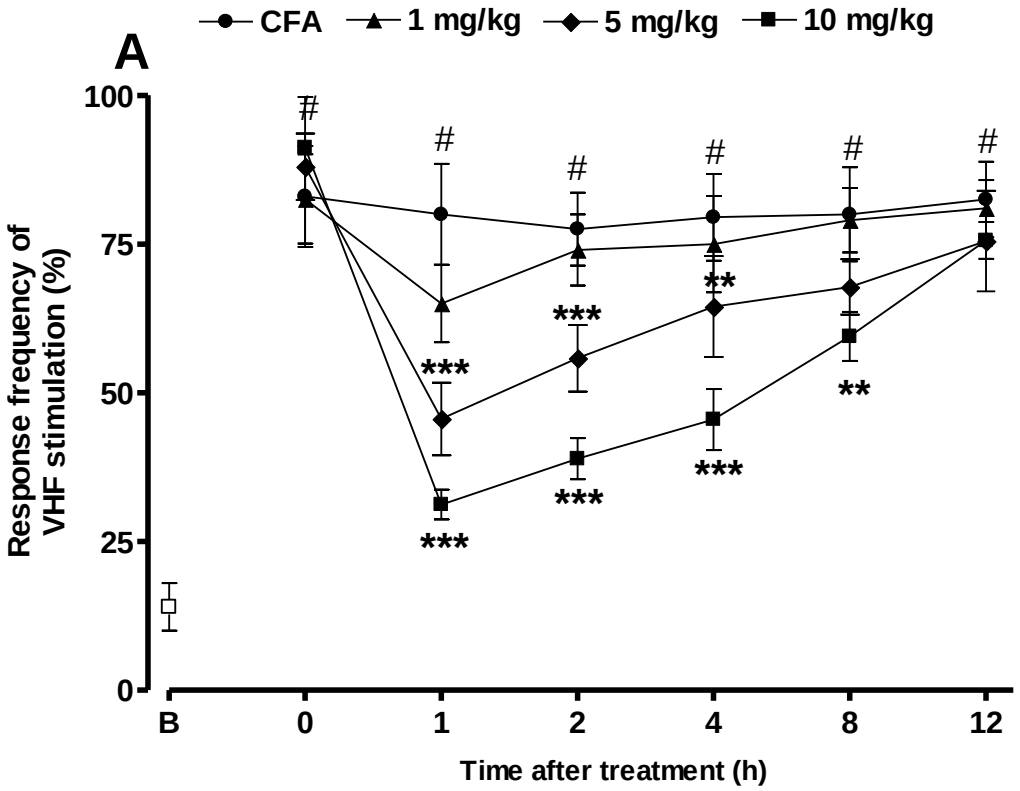
selenide at doses of 1 (▲), 5 (♦) or 10 (■) mg/kg 30 min before CG injection. (A) The baseline (□) was recorded before CG injection and in (B) baseline paw thickness was discounted from total thickness to give the oedema value. Symbols denote a significant difference at **P<0.01 and ***P<0.001 between vehicle treated and bis selenide treated mice and #P< 0.001 versus baseline value.

Fig. 5 - Effect of bis selenide on mechanical hyperalgesia (Panel A) and paw oedema (Panel B) induced by PGE_2 injection in mice. The animals received vehicle (canola oil) (●) or bis selenide at doses of 1 (▲), 5 (♦) or 10 (■) mg/kg 30 min before PGE_2 injection. (A) The baseline (□) was recorded before PGE_2 injection and in (B) baseline paw thickness was discounted from total thickness to give the oedema value. Results represent the mean±S.E.M. of eight animals. Symbols denote a significant difference at *P<0.05, **P<0.01 and ***P<0.001 between vehicle treated and bis selenide treated mice and #P< 0.001 versus baseline value.

Fig. 6 - Effect of bis selenide on BPA-induced mechanical hyperalgesia on acute (Panel A) or chronic (Panel B) treatment. The assessment was carried out in mice sham operated (○). Operated mice were acutely treated with vehicle (canola oil) (●) or bis selenide at doses of 1 (▲), 5 (♦) or 10 (■) mg/kg 4 days after surgery (A). Chronically treatment with vehicle (canola oil) (●) or bis selenide at doses 5 (♦) or 10 (■) mg/kg was given to mice (B). Results represent the mean±S.E.M. of eight animals. Symbols denote a significant difference at * P < 0.05, ** P<0.01 and ***P<0.001 between vehicle treated and bis selenide treated mice and #P< 0.001 versus sham operated group.

Fig. 7 - Effect of chronic treatment with bis selenide on vincristine-induced mechanical hyperalgesia in mice. The assessment was carried out in mice treated with saline (○), vincristine (●) or bis selenide at doses of 1 (▲), 5 (♦) or 10 (■) mg/kg once per day for 7 days. Results represent the mean±S.E.M. of eight animals. Symbols denote a significant difference at ** P<0.01 and ***P<0.001 between vehicle treated and bis selenide treated mice and #P< 0.001 versus vehicle (saline) group.

Figure 1


CFA
1 mg/kg
5 mg/kg
10 mg/kg
A
Response frequency of VHF stimulation (%)
100
75
50
25
0
B
0
1
2
4
8
12
Time after treatment (h)
#
***
**

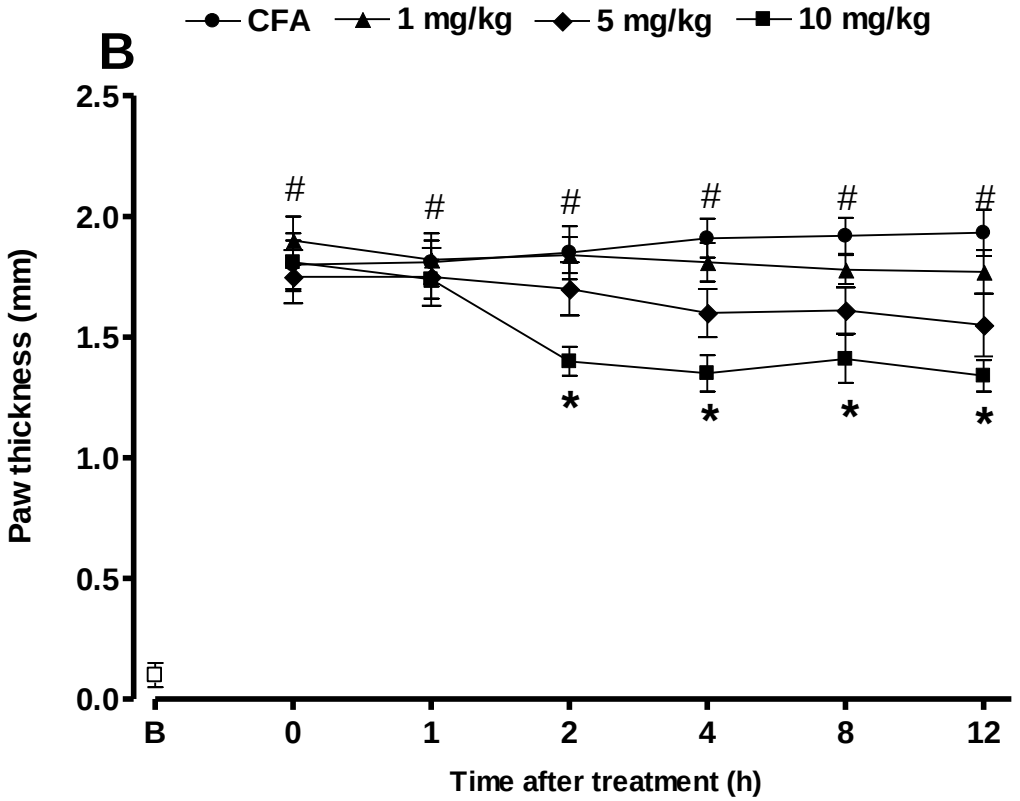

CFA
1 mg/kg
5 mg/kg
10 mg/kg
B
Paw thickness (mm)
2.5
2.0
1.5
1.0
0.5
0.0
B
0
1
2
4
8
12
Time after treatment (h)
#
*

Figure 2

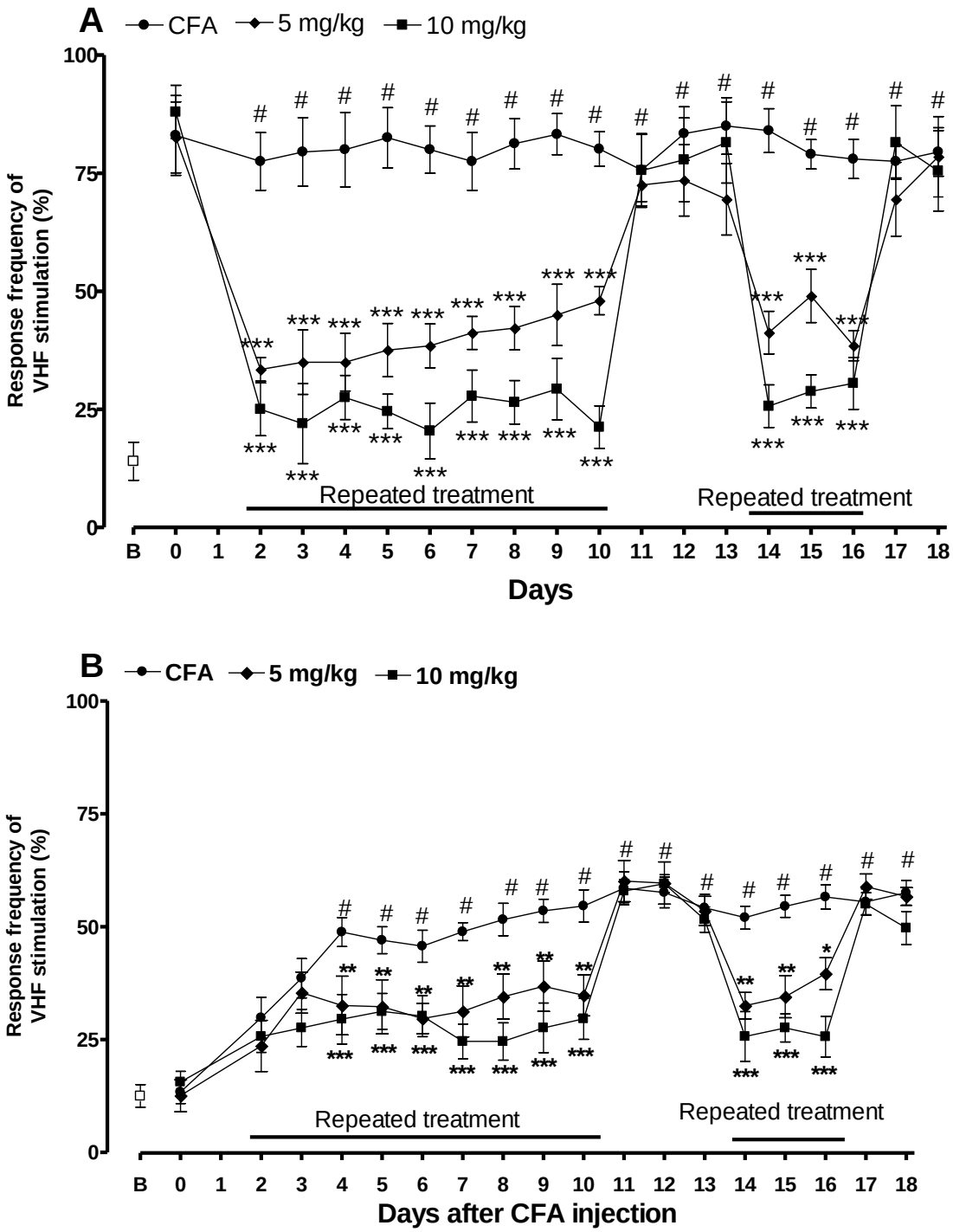
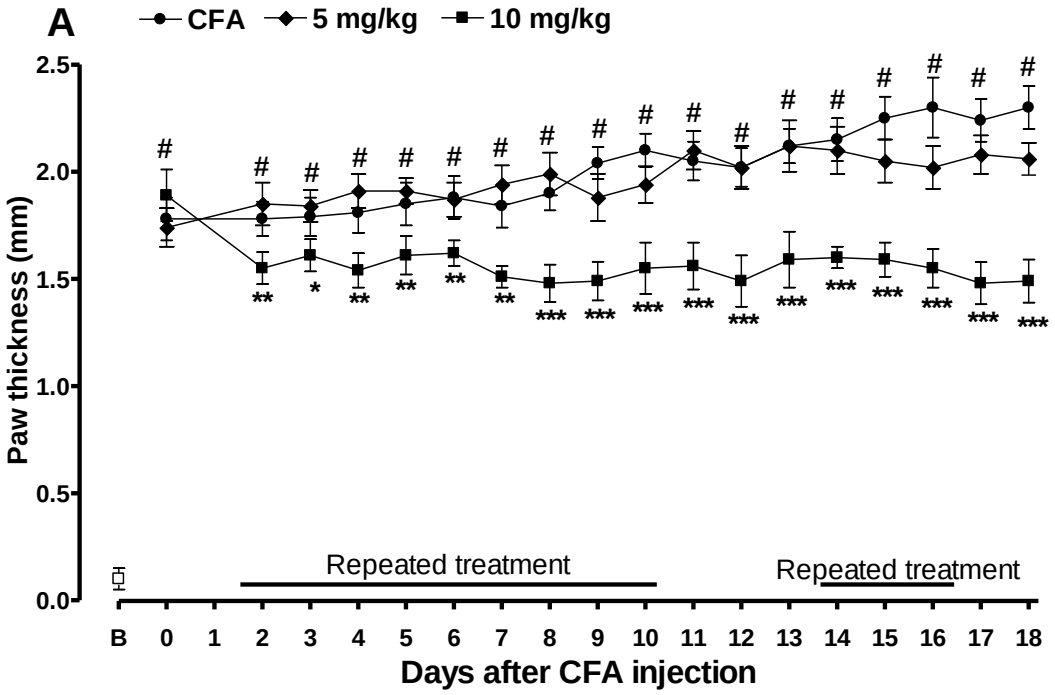

A
CFA
5 mg/kg
10 mg/kg
Response frequency of VHF stimulation (%)
Repeated treatment
Repeated treatment
Days
B
CFA
5 mg/kg
10 mg/kg
Response frequency of VHF stimulation (%)
Repeated treatment
Repeated treatment
Days after CFA injection

Figure 3


A
CFA
5 mg/kg
10 mg/kg
Paw thickness (mm)
2.5
2.0
1.5
1.0
0.5
0.0
Repeated treatment
Repeated treatment
B 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Days after CFA injection

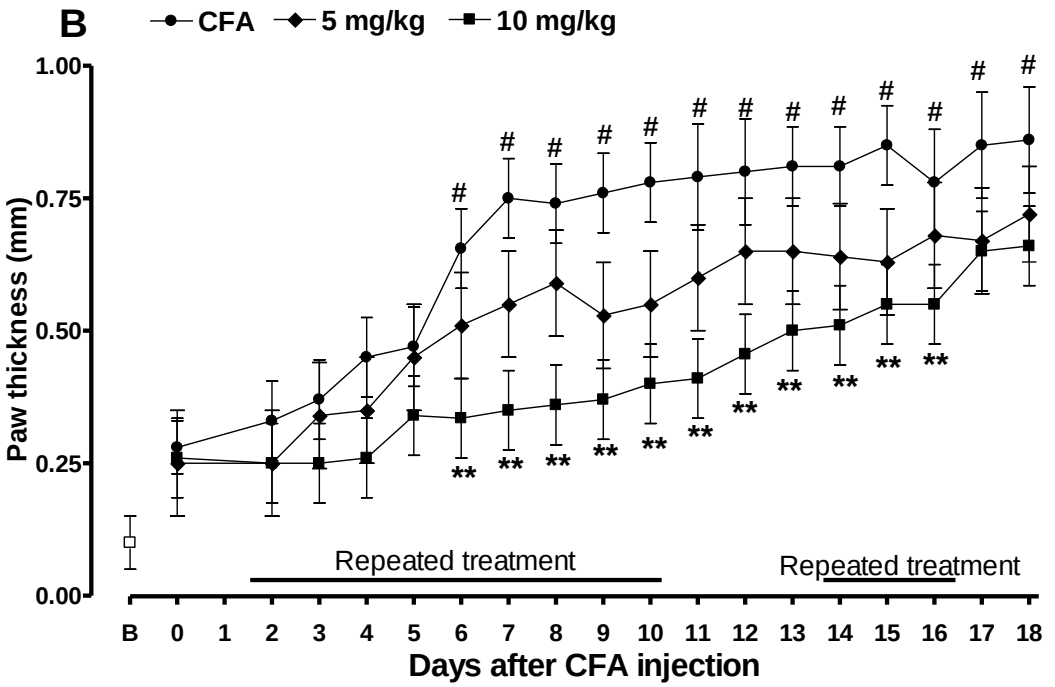

B
CFA
5 mg/kg
10 mg/kg
Paw thickness (mm)
1.00
0.75
0.50
0.25
0.00
Repeated treatment
Repeated treatment
B 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Days after CFA injection

Figure 4

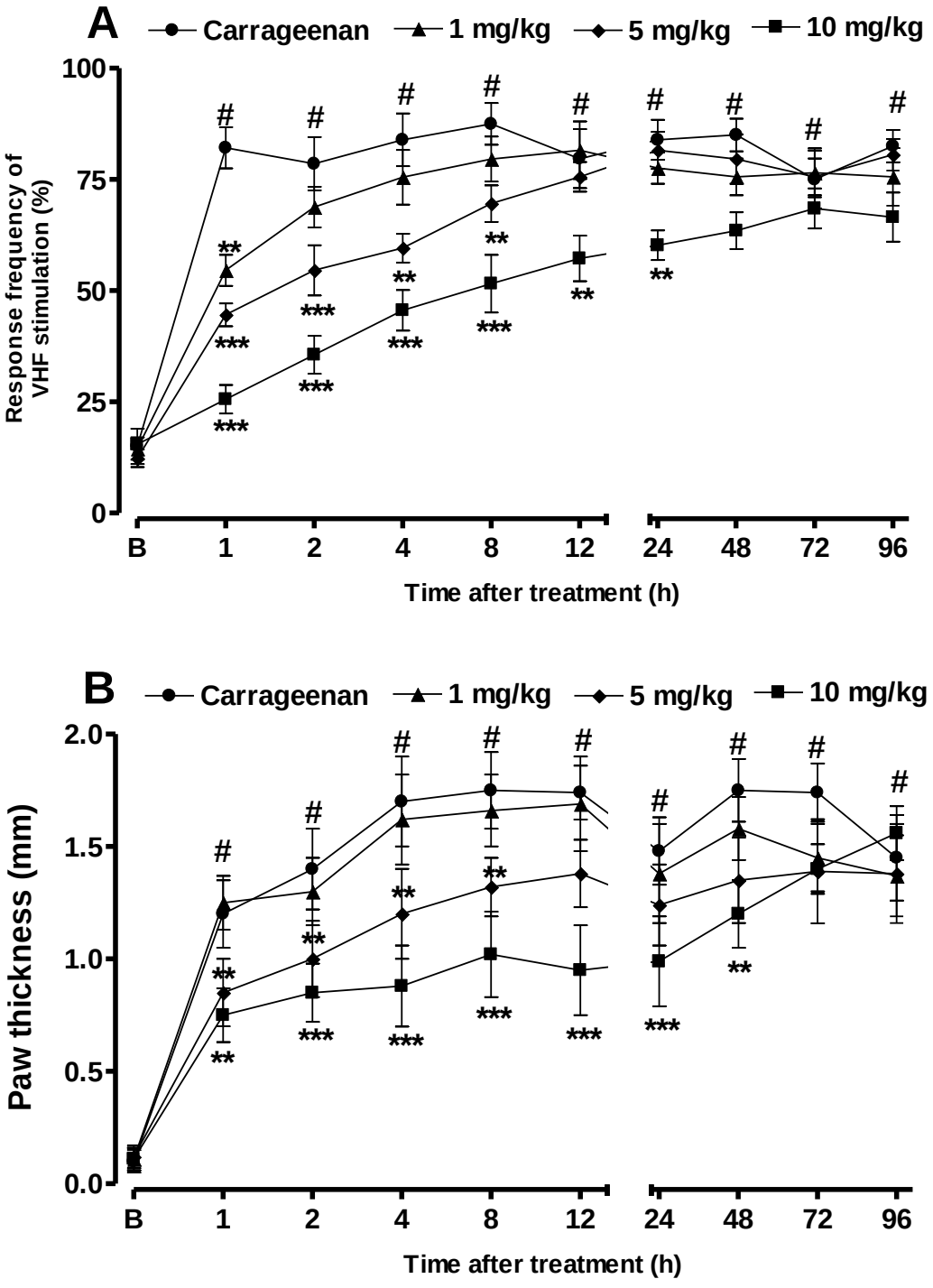

A
Carrageenan
1 mg/kg
5 mg/kg
10 mg/kg
Response frequency of VHF stimulation (%)
100
75
50
25
0
B
1
2
4
8
12
24
48
72
96
Time after treatment (h)
B
Carrageenan
1 mg/kg
5 mg/kg
10 mg/kg
Paw thickness (mm)
2.0
1.5
1.0
0.5
0.0
Time after treatment (h)

Figure 5

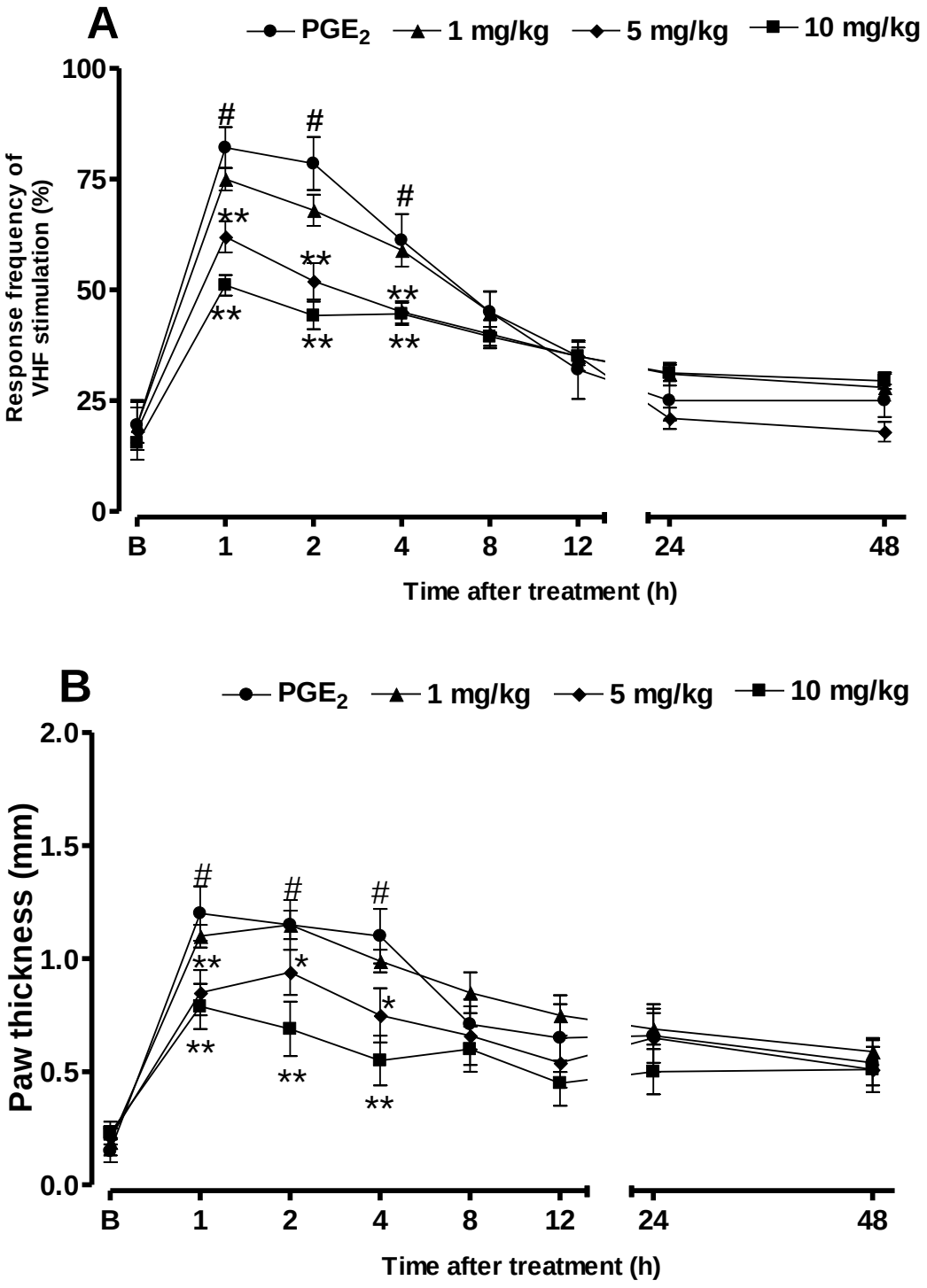

A
PGE2
1 mg/kg
5 mg/kg
10 mg/kg
Response frequency of VHF stimulation (%)
100
75
50
25
0
B
1
2
4
8
12
24
48
Time after treatment (h)
B
PGE2
1 mg/kg
5 mg/kg
10 mg/kg
Paw thickness (mm)
2.0
1.5
1.0
0.5
0.0
B
1
2
4
8
12
24
48
Time after treatment (h)

Figure 6

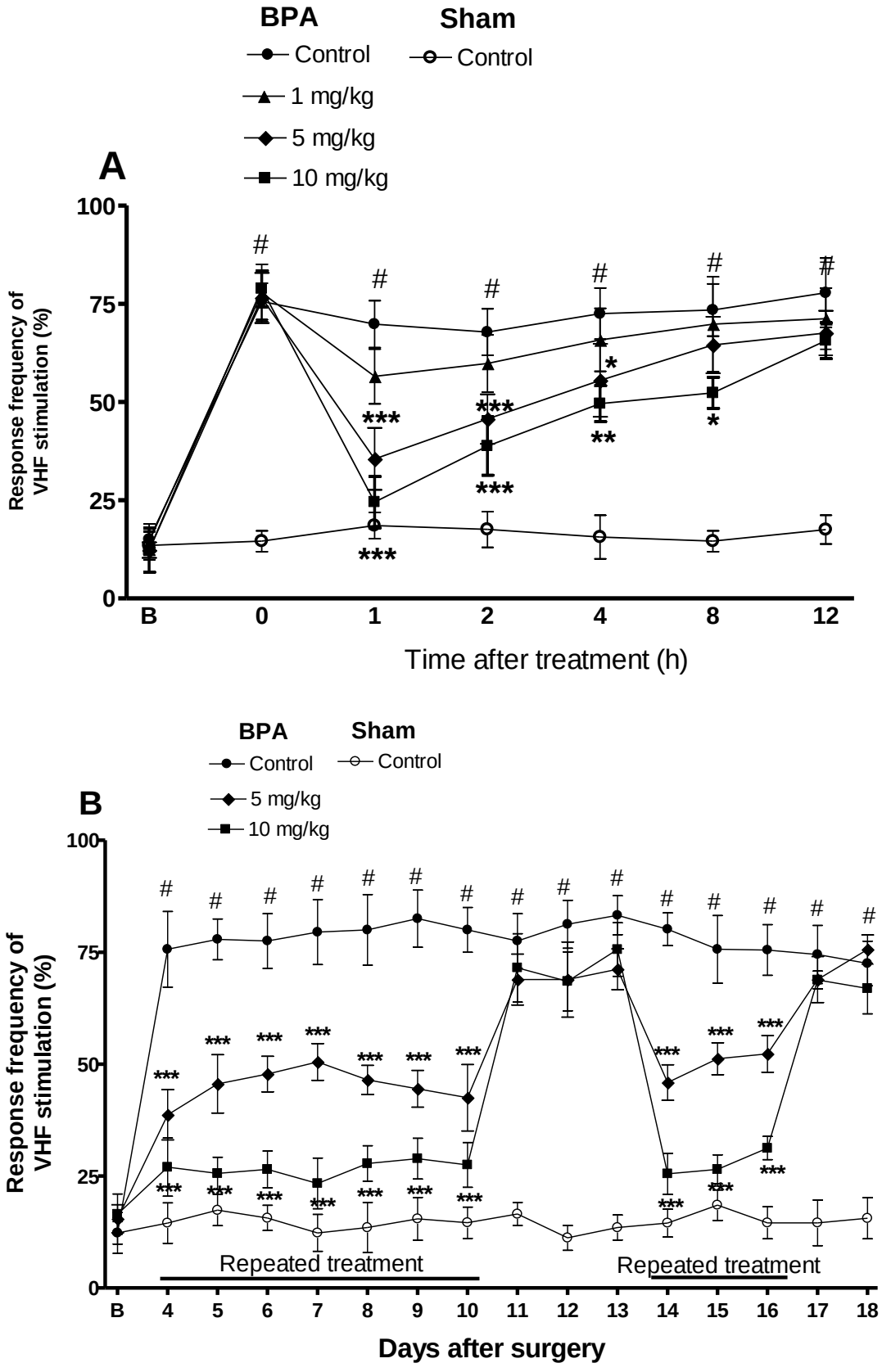
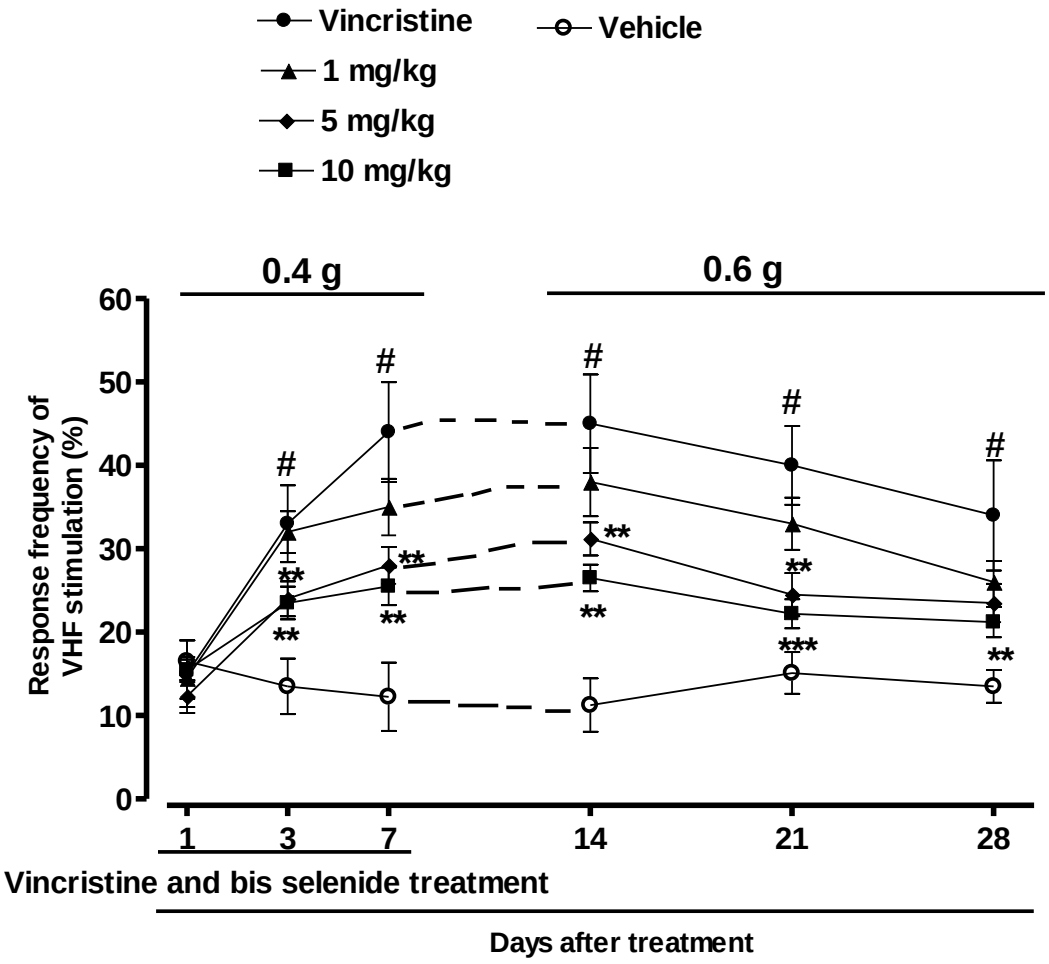

BPA
Sham
Control
Control
1 mg/kg
5 mg/kg
10 mg/kg
A
Response frequency of VHF stimulation (%)
Time after treatment (h)
B
Repeated treatment
Repeated treatment
Days after surgery

Figure 7


Vincristine
Vehicle
1 mg/kg
5 mg/kg
10 mg/kg
0.4 g
0.6 g
Response frequency of VHF stimulation (%)
60
50
40
30
20
10
0
1
3
7
14
21
28
Vincristine and bis selenide treatment
Days after treatment

5. DISCUSSÃO

O elevado número de pacientes com depressão e dores persistentes que são refratários aos medicamentos disponíveis atualmente torna importante a busca por novos fármacos para o tratamento destas doenças. Desta forma, este estudo demonstrou que a adminstração pela via oral do SVBS apresentou efeitos do tipo antidepressivo e anti- hiperalgésico em camundongos e evidenciou alguns mecanismos farmacológicos envolvidos.

A administração aguda do SVBS (doses de 0,5, 1 e 5 mg/kg, p.o.) produziu um efeito do tipo antidepressivo no TNF e no TSC, sem produzir alterações locomotoras no TCA (artigo 1). O TNF é um modelo experimental que tem sido amplamente utilizado na pesquisa de compostos com potencial ação antidepressiva, tendo em vista que a grande maioria das classes de antidepressivos reduz o tempo de imobilidade neste teste (Porsolt et al., 1977). O TSC é um modelo experimental que tem sido amplamente utilizado na pesquisa de fármacos com possível potencial antidepressivo, tendo em vista que a maioria dos antidepressivos, incluindo os tricíclicos, os iMAOs e os atípicos reduzem o tempo de imobilidade neste teste (Steru et al., 1985; Cryan et al., 2005). O efeito do tipo antidepressivo do SVBS foi demonstrado em doses menores quando comparado a outros organocalcogênios, pois o disseleneto de difenila, o ebselen e o 3-(4-fluorofenilselenil)2,5-difenilselenofeno apresentaram efeito em testes preditivos de depressão em camundongos a partir de 5, 10 e 50 mg/kg, respectivamente (Savegnago et al., 2007d; Posser et al., 2009; Gay et al., 2010). Assim, podemos evidenciar que a administração do SVBS produz um efeito antidepressivo em doses menores em comparação a outros organocalcogênios. A ação do tipo antidepressiva do SVBS foi mais prolongada quando comparado ao disseleneto de difenila (2 horas após o tratamento) (Savegnago et al., 2007d) e ao 3-(4-fluorofenilselenil)2,5-difenilselenofeno (30 minutos após o tratamento) (Gay et al., 2010), pois o efeito do tipo antidepressivo do SVBS ocorreu dos 30 minutos até 6 horas após o tratamento.

Estudos demonstrataram que o selênio está associado aos transtornos de humor (Burk, 2002; Sher, 2008). As baixas concentrações de selênio (ingestas entre 32 e 36 μg por dia) estão relacionadas com uma maior incidência de depressão, ansiedade e hostilidade. Ao contrário, dietas ricas neste elemento (226 μg por dia) melhoram o humor das pessoas e diminuem os sintomas depressivos (Rayman, 2000). Desta maneira, o SVBS poderia ser uma solução terapêutica atrativa para o tratamento da depressão, apesar de os mecanismos envolvidos na regulação do humor pelo Se não estarem esclarecidos.

O sistema monoaminérgico é um dos alvos mais importantes na patofisiologia e tratamento da depressão (Millan, 2004), assim investigamos o envolvimento dos sistemas serotoninérgico, noradrenérgico e dopaminérgico no efeito antidepressivo do SVBS no TSC. O sistema serotoninérgico desempenha um papel fundamental no mecanismo de ação de fármacos antidepressivos (Millan, 2004). A 5-HT é um neurotransmissor monoaminérgico que modula funções como comportamento, cognição, sono, apetite, humor e desempenha um importante papel em desordens como ansiedade, depressão, agressão e modulação da dor (Gingrich e Hen, 2001). A concentração de 5-HT sináptica é controlada diretamente pela recaptação nos terminais pré-sinápticos e assim, bloqueadores de 5-HT têm sido utilizados no tratamento da depressão e da dor crônica (Mico et al., 2006).

O envolvimento do sistema serotoninérgico na ação antidepressiva do SVBS no TSC foi investigado utilizando estratégias farmacológicas, como o inibidor da síntese da serotonina, *para*-clorofenilalanina (PCPA) e de antagonistas serotoninérgicos seletivos (artigo 1). Nossos resultados indicam que o sistema serotoninérgico está envolvido no efeito antidepressivo do SVBS, visto que o tratamento prévio dos animais com PCPA e com os antagonistas cetanserina (antagonista 5-$HT_{2A/C}$) e ondasentrona (antagonista 5-HT_3) foi capaz de prevenir completamente o efeito do SVBS no TSC. Além disso, a administração concomitante de doses sub-efetivas de SVBS com fluoxetina (inibidor seletivo da recaptação de 5-HT) produziu um efeito do tipo antidepressivo no TSC em camundongos. Entretanto, quando o SVBS foi adminstrado simultaneamente com a imipramina (inibidor não seletivo da recaptação das monoaminas), não foi observado o efeito do tipo antidepressivo, reforçando a hipótese que o sistema noradrenérgico não está envolvido na atividade do tipo antidepressiva do SVBS.

Muitos antidepressivos são antagonistas de receptores 5-HT_2 e apresentam a capacidade de inibir a ligação da 5-HT aos receptores após administração prolongada (Khisti e Chopde, 2000). Sugere-se que a "down-regulation" de receptores 5-$HT_{2A/c}$ possa mediar as ações a longo prazo dos antidepressivos (Deakin, 1988). No presente estudo, o tratamento prévio dos animais com cetanserina foi capaz de reverter o efeito do tipo antidepressivo do SVBS, evidenciando a participação dos receptores 5-$HT_{2A/C}$ neste efeito. Além disso, foi mostrado que o agonista preferencial de receptores $5HT_{2A}$, DOI, aumenta o efeito antidepressivo da agmatina e da 3α-hidroxi-5α-pregnan-20-ona (Zomkowski et al., 2005; Khisti e Chopde, 2000). Redrobe e Bourin (1977) demonstraram que o bloqueio dos receptores 5-$HT_{2A/C}$ potencializa o efeito da imipramina no TNF. O presente estudo indica que o efeito antidepressivo do SVBS parece ser mediado pela estimulação dos receptores 5-

$HT_{2A/C}$, que são receptores fundamentais no tratamento de desordens de humor. Este sub-tipo de receptor está envolvido com o efeito antinociceptivo do SVBS no teste da formalina em camundongos (Jesse et al., 2009) e com a atividade do tipo antidepressiva do disseleneto de difenila em ratos (Savegnago et al., 2007d) e do 3-(4-fluorofenilselenil)2,5-difenilselenofeno em camundongos (Gay et al., 2010).

Além disso, o tratamento prévio com ondansentrona foi capaz de prevenir o efeito do tipo antidepressivo do SVBS, demonstrando que este efeito é mediado pela interação com este subtipo de receptor serotoninérgico (5-HT_3). Alguns trabalhos demonstram que diferentes classes de antidepressivos agem como antagonistas funcionais de receptores 5-HT_3, indicando que a supressão da atividade do receptor 5-HT_3 pode contribuir para a ação dos antidepressivos (Eisensamer et al., 2003). O receptor 5-HT_3 também está envolvido com o efeito antinociceptivo do SVBS (Jesse et al., 2009) e do disseleneto de difenila (Zasso et al., 2005) em camundongos. Assim, o mecanismo de ação do SVBS em modelos de depressão e nocicepção devem ser similares já que ambos envolvem os receptores 5-HT_3. Este sub-tipo de receptor também é modulado no efeito do tipo antidepressivo do disseleneto de difenila em ratos (Savegnago et al., 2007d) e do 3-(4-fluorofenilselenil)2,5-difenilselenofeno em camundongos (Gay et al., 2010).

O papel da NA na patofisiologia da depressão tem sido estudado, visto que alguns antidepressivos aumentam os níveis de noradrenalina na fenda sináptica e alguns destes fármacos agem diretamente nos receptores noradrenérgicos (Elhwuegi, 2004). Além disso, foi demonstrado que camundongos deficientes de NA não respondem aos antidepressivos, incluindo os ISRS (Cryan et al., 2004). Neste estudo, o tramento prévio com prazosim (antagonista α_1-adrenérgico), ioimbina (antagonista α_2-adrenérgico) e propranolol (antagonista β adrenérgico) não reverteu o efeito do tipo antidepressivo do SVBS nos camundongos. Assim, não há modulação pelos receptores α e β adrenérgicos no efeito causado pelo SVBS no TSC nos camundongos. Já a atividade do tipo antidepressiva de outros organocalcogênios, tais como disseleneto de difenila e ebselen, foi bloqueada pelo tratamento prévio com antagonistas como prazosim e ioimbina (Savegnago et al., 2007d; Posser et al., 2009). Entretanto, não houve participação de receptores β, pois o uso do propranolol também não bloqueou o efeito do tipo antidepressivo do disseleneto de difenila (Savegnago et al., 2007d).

O sistema dopaminérgico também está implicado na regulação do humor (Lin Z, Madras, 2006). Algumas evidências bioquímicas originadas de estudos clinicos mostraram que os níveis plasmáticos do ácido homovanílico e do ácido 3,4-dihidroxifenilacético, dois

metabólitos da DA, estavam significativamente diminuídos em pacientes deprimidos, indicando uma diminuição no "turnover" da DA (Mitani et al., 2006). Existem várias evidências farmacológicas em relação à eficácia dos antidepressivos com efeitos dopaminérgicos no tratamento da depressão (Papakostas, 2006). Dados da literatura indicam que tanto os receptores D_1 como os receptores D_2 desempenham um papel importante na modulação dos estados de humor (Papakostas, 2006). Neste estudo, o tratamento prévio com SCH 23390 (antagonista seletivo dos receptores D_1) e sulpirida (antagonista seletivo dos receptores D_2) não reverteu o efeito do tipo antidepressivo do SVBS no TSC. Em comparação ao disseleneto de difenila e ao ebselen, o SVBS apresentou mecanismos de ação mais seletivos. Isto ocorre porque os demais organocalcogênios possuem o efeito do tipo antidepressivo bloqueado por antagonistas dopaminérgicos enquanto o SVBS não é modulado por estes tipos de receptores. Assim, o mecanismo envolvido no efeito do tipo antidepressivo do SVBS não está ligado a estes receptores dopaminérgicos e o SVBS apresenta modulação seletiva dos receptores monoaminérgicos do tipo 5-$HT_{2A/C}$ e 5-HT_3.

A MAO-A e B são enzimas fundamentais no metabolismo das monoaminas, regulando sua concentração intracelular no cérebro. Assim, a função anormal destas enzimas está associada com muitas desordens psíquicas, como a depressão (Deniker, 1983). Outra enzima importante na homeostase neuronal é a Na^+ K^+ ATPase, que é responsável pelo transporte dos íons Na^+ e K^+ no SNC, mantendo o gradiente necessário para excitabilidade neuronal. A atividade desta enzima está inibida em doenças psíquicas afetivas (EI-Mallakh and Wyatt, 1995). Neste estudo, as atividades das enzimas MAO-A, MAO-B e Na^+ K^+ ATPase no cérebro dos camundongos não foram alteradas com o tratamento com SVBS, sugerindo que estas enzimas não participam no efeito do tipo antidepressivo. Em outro estudo, o composto 3-(4-fluorofenilselenil)2,5-difenilselenofeno, um organocalcogênio que apresenta efeito do tipo antidepressivo em camundongos, também não modificou a atividade das enzimas MAO-A e MAO-B (Gay et al., 2010). Entretanto, o disseleneto de difenila inibe a enzima MAO no cérebro de ratos, porém durante o estudo não foi demonstrado qual isoforma é inibida (Savegnago et al., 2007d).

Este estudo também investigou o envolvimento da via L-arginina-NO no efeito antidepressivo do SVBS (artigo 2). No cérebro, o NO é produzido principalmente em estruturas pós-sinápticas como resposta à ativação de receptores NMDA (Denninger e Marletta, 1999). A inibição da produção de NO exerce efeitos semelhantes aos efeitos dos antagonistas de receptores NMDA e potencializa a ação de antidepressivos (Harkin et al., 2004). Nossos resultados demonstraram que o tratamento prévio com o aminoácido precursor

de NO, L-arginina, ou com S-nitroso-N-acetil-penicilamina (SNAP), um doador de NO, preveniu significativamente o efeito do tipo antidepressivo do SVBS no TSC. Além disso, o SVBS apresentou um efeito sinérgico com L-NNA, azul de metileno e ODQ. O L-NNA é um análogo da L-arginina que age como um inibidor competitivo da enzima NOS, reduzindo assim os níveis de NO. O azul de metileno inibe tanto a NOS quanto a GC, e o ODQ age especificamente inibindo a enzima GC e reduzindo os níveis de GMPc. Estes resultados indicam que o efeito do tipo antidepressivo causados pelo SVBS são dependentes, pelo menos em parte, da inibição da síntese de NO, o que está de acordo com os dados da literatura que mostram que inibidores da NOS têm propriedades antidepressivas em modelos animais (Volke et al., 2003; Harkin et al., 2004). Outro resultado que reforça a modulação do NO pelo SVBS é que a administração deste composto em uma dose antidepressiva causou a diminuição dos níveis de nitrito/nitrato no cérebro dos camundongos. A via L-arginina-NO-GMPc também é modulada pelo composto da classe dos bis selentos (Jesse et al., 2007) no efeito antinociceptivo em ratos. Este estudo reforça o envolvimento desta via na atividade do tipo antidepressiva do SVBS. A via L-arginina-NO-GMPc participa do efeito do tipo antidepressivo do disseleneto de difenila (Savegnago et al., 2008), melatonina (Mantovani et al., 2003), adenosina (Kaster et al., 2005), ácido fólico (Brocardo et al., 2008), escitalopram (Zomkowski et al., 2010), venlafaxina (Kumar et al., 2010), imipramina (Kumar et al., 2009), paroxetina (Ghasemi et al., 2009) e bupropiona (Dhir e Kulkarni, 2007). Desta forma, a via L-arginina-NO-GMPc é modulada por inúmeros compostos para que estes exerçam atividade do tipo antidepressiva em roedores.

Dados da literatura demonstram que inibidores de canais de K^+ apresentam efeito antidepressivo no TNF em camundongos, além de potencializar o efeito de antidepressivos clássicos (Kaster et al., 2005). Por outro lado, agentes que promovem abertura dos canais de K^+ causam efeito depressogênico (Galeotti et al., 1999). No cérebro, a abertura dos canais de K^+ leva a uma hiperpolarização, redução da liberação de neurotransmissores e na excitabilidade neuronal. O efeito antidepressivo causado pelo SVBS no TSC é dependente de uma interação com canais de K^+, uma vez que a administração de doses sub-efetivas dos inibidores dos canais de K^+ (glibenclamida, apamina, charibtoxina e TEA) potencializou o efeito de doses sub-efetivas de SVBS (artigo 3). O uso de drogas que abrem os canais de K^+, como o cromacalim e o minoxidil, bloquearam o efeito do tipo antidepressivo do SVBS. Em outros estudos, foi demonstrado que os canais de K^+ modulam o efeito do tipo antidepressivo da adenosina, fluoxetina (Kaster et al., 2007), agmatina (Budni et al., 2007), venlafaxina (Bortolatto et al., 2010) e disseleneto de difenila (Wilhelm et al., 2010) em camundongos. Os

canais de K^+ também estão envolvidos com o efeito antinociceptivo de um composto da classe dos bis selenetos (Jesse et al., 2007), que possui estrutura análoga ao SVBS.

A via L-arginina-NO-GMPc ativa canais de K^+, pois o tratamento prévio dos camudongos com L-arginina ou sildenafil previne o efeito dos inibidores de canais de K^+ no TNF (Kaster et al., 2005). Desta maneira, o NO produzido em estruturas pós-sinápticas pode atuar ativando canais de K^+, diretamente ou através da produção de GMPc, inibindo assim a excitabilidade neuronal. Além disso, o NO difunde-se e age em canais de K^+ pré-sinápticos, causando uma hiperpolarização e inibição da liberação de diversos neurotransmissores, como a DA e 5-HT (Galeotti et al., 1999).

Os receptores PPARγ podem modular o humor já que estão expressos em áreas relacionadas a esta atividade, incluindo centros nervosos envolvidos com a depressão, como o hipocampo e cortex frontal (Moreno et al., 2004). Há evidências na literatura que os receptores PPARγ podem exercer atividade do tipo antidepressiva no TNF e no TSC em camundongos, pois o GW-9662, um antagonista dos receptores PPARγ, reverteu o efeito do tipo antidepressivo do NP03115, da rosiglitazona (Rosa et al., 2008) e do disseleneto de difenila (Wilhelm et al., 2010). O GW-9662 bloqueou o efeito do tipo antidepressivo do SVBS no TSC em camundongos, demonstrando que os receptores PPARγ estão envolvidos no efeito antidepressivo (artigo 3). Embora os estudos na literatura com o envolvimento dos receptores PPARγ em doenças do humor ainda sejam escassos, estes receptores representam um alvo terapêutico promissor.

Os camundongos submetidos à injúria por constrição crônica (ICC) no nervo ciático demonstraram um comportamento do tipo depressivo refletido no aumento no tempo de imobibilidade no TNF (artigo 4). Esta resposta após a ICC foi diminuída pelo tramento com SVBS (1 e 5 mg/kg, p.o.) sem afetar a atividade locomotora. Este resultado confirma a evidência clínica da ligação entre dor neuropática e depressão (Attal et al. 2006; Mico et al. 2006; Rice and Hill, 2006; Dworkin et al., 2007). O tratamento com SVBS diminui o tempo de imobilidade dos camundongos submetidos à ICC e foi mais potente quando comparado ao antidepressivo clássico (amitriptilina, efetiva na dose de 30 mg/kg). Além disso, o SVBS diminui às respostas à neuropatia de forma mais potente que os antidepressivos clássicos, na ordem SVBS > bupropiona > amitriptilina > fluoxetina. Este resultado é importante porque demonstra que o SVBS possui elevada eficácia em um modelo próximo ao que ocorre em humanos e em baixíssimas doses. Quando o SVBS é comparado ao disselento de difenila no modelo de lesão do nervo ciático em camundongos (Savegnago et al., 2007a), apresentou efeitos significativos em doses menores. A dor e a depressão envolvem mecanismos

bioquímicos similares que podem resultar em menor disponibilidade de neurotransmissores no SNC, incluindo a 5-HT, a NA e a DA (Millan, 2004). A modulação dos receptores serotoninérgicos pelo SVBS, principalmente do tipo 5-$HT_{2A/C}$ e 5-$_{HT3}$, e do sistema L-arginina-NO-GMPc/K^+ participam no efeito do tipo antidepressivo e anti-hiperalgésico nos camundongos submetidos à ICC, pois são mecanismos farmacológicos envolvidos nas atividades do SVBS.

A avulsão ao plexo braquial (APB) e a injeção i.pl. de ACF, Cg e PGE_2 tornaram os animais hipersensíveis e responsivos frente a estímulos inócuos aos animais falso-operados ou não injetados (hiperalgesia) (Cunha et al., 2008). O tratamento com SVBS, nas doses de 5 e 10 mg/kg, após as injúrias nervosas ou a administração dos agentes flogísticos inibiu a hiperalgesia mecânica nos camundongos (artigo 5). Além disso, a resposta anti- hiperalgésica mecânica causada pelo SVBS (5 e 10 mg/kg, p.o.) não foi susceptível a tolerância nos modelos induzidos pela APB e injeção de ACF. Esta conclusão é baseada nos dados que mostram que a interrupção do tratamento com o SVBS foi seguida pelo retorno completo da hiperalgesia e que o tratamento com o SVBS duas vezes por dia produziu efeitos antihiperalgésicos pronunciados. Quando comparado ao efeito do disseleneto de difenila no modelo do ACF, o SVBS mostrou efeito mais potente e ação mais prolongada. Isto porque o efeito anti-hiperalgésico do SVBS ocorre nas doses de 5 e 10 mg/kg até 8 horas enquanto do disselento de difenila apenas em 10 mg/kg até 4 horas após o tratamento (Savegnago et al., 2007a).

A injeção de ACF, Cg e PGE_2 também causou edema na pata injetada. O modelo de edema de pata induzido tem sido amplamente usado para a investigação da atividade antiinflamatória de fármacos. O desenvolvimento do edema é inibido em extensão variável por drogas que inibem a produção e a ação de diferentes mediadores inflamatórios. Entre essas drogas podem ser mencionadas antiinflamatórios não esteroidais (AINEs), antagonistas de receptores de bradicinina, de 5-HT e de histamina. O tratamento com SVBS (doses de 5 e 10 mg/kg, p.o.) diminui o edema nos modelos de ACF, Cg e PGE_2, reforçando o potencial antiinflamatório do composto. O efeito anti-edematogênico foi demonstrado em modelos agudos de nocicepção como a injeção de formalina, glutamato, sertonina, histamina e PGE_2 em camundongos (Jesse et al., 2009). Já está bem estabelecido que alterações na permeabilidade dos vasos acontecem através de processos independentes da adesão, migração de neutrófilos, liberação de mediadores inflamatórios e por mecanismos dependentes da liberação de EROs (Zhu et al., 2005). Estes resultados sustentam a idéia de que o SVBS, um agente antioxidante, diminui os danos causados pelo estresse oxidativo e pela inflamação,

contribuindo com os efeitos contra a hiperalgesia mecânica e o edema nos modelos de injeção de ACF, Cg e PGE_2.

Outro modelo de neuropatia utilizado foi da administração crônica da vincristina. Uma das seqüelas da quimioterapia frequentemente associada a dores crônicas é a neuropatia periférica (Saika et al., 2009). As drogas mais comumente associadas a esta complicação são os alcalóides da vinca, especialmente a viscristina e os taxanos, principalmente o paclitaxel. A neuropatia periférica geralmente é associada à infusão da vincristina ou paclitaxel e caracteriza-se como dor contínua em quimor ou lancinante, muitas vezes associada à alodínia, que pode acompanhar-se de fraqueza, redução ou perda da sensibilidade dos membros (Callizot et al., 2008; Kanbayashi et al., 2010). O tratamento concomitante de vincristina e SVBS (5 e 10 mg/kg, p.o.) durante os primeiros 7 dias diminui a hiperalgesia mecânica até o término da avaliação aos 28 dias (artigo 5). Neste modelo também não foi observado tolerância, pois a interrupção do tratamento com o SVBS foi seguida pelo retorno completo da hiperalgesia e o retorno do tratamento com o SVBS duas vezes por dia produziu efeitos anti-hiperalgésicos pronunciados nos camundongos.

Neste contexto, o efeito causado pelo SVBS na lesão do nervo (modelos de ICC, APB e administração de vincristina) e a inflamação induzida pelo ACF, Cg e PGE_2 estão, provavelmente, associados à capacidade deste composto em interferir com a sinalização celular, particularmente, os canais de K^+ sensíveis à ATP e voltagem-dependentes, receptores glutamatérgicos (tipo cainato e trans-ACPD) 5-$HT_{2A/2C}$, 5-HT_3 e H_2 e as vias relacionadas com PGE_2, PKC e PKA (figura 7). A participação no efeito anti-hiperalgésico do SVBS neste estudo está relacionado ao fato que estes mecanismos contribuem com o efeito antinociceptivo e antiinflamatório do SVBS em estudos realizados previamente (Savegnago et al., 2006; Jesse et al., 2007, 2008, 2009).

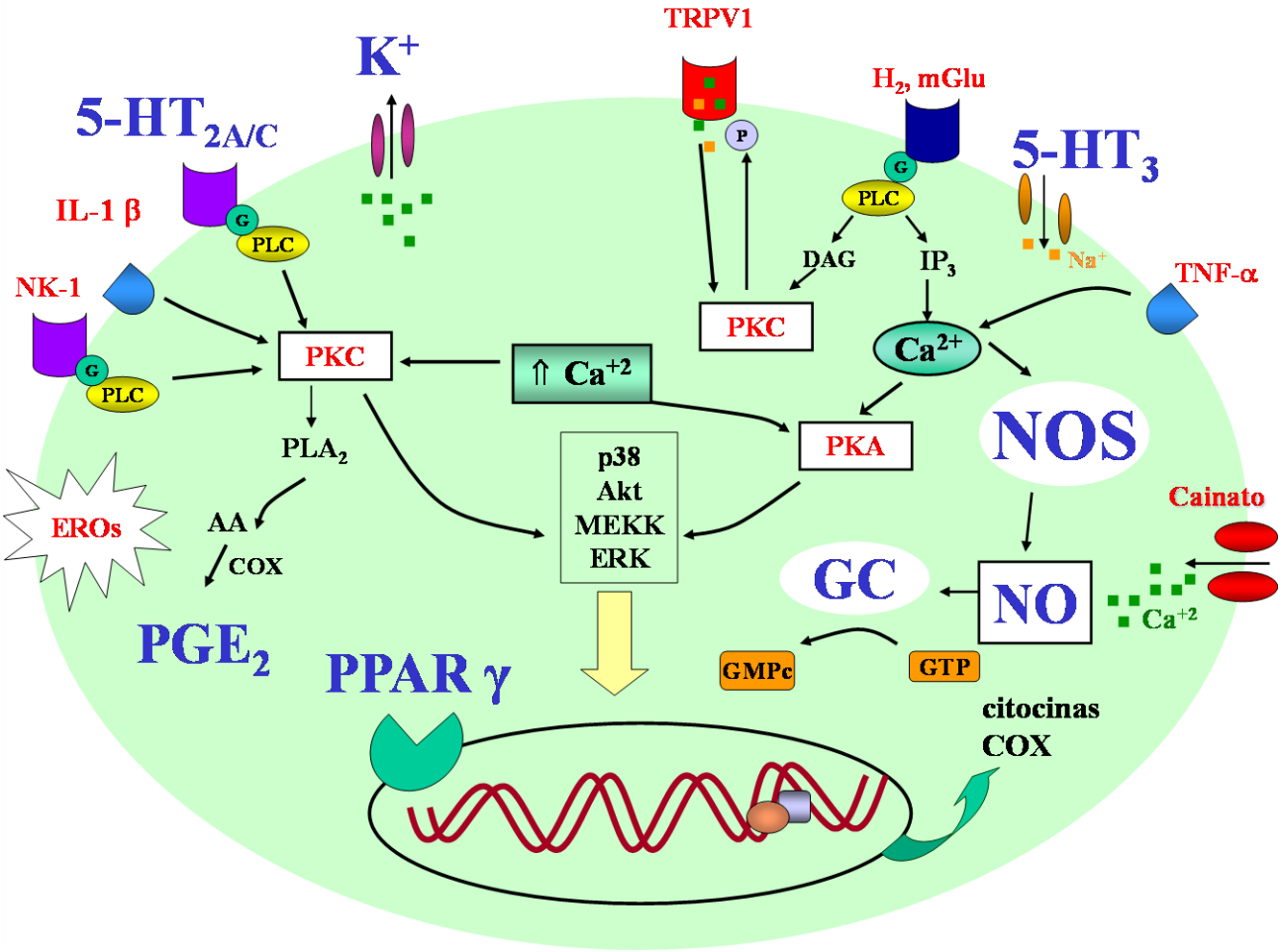


Figura 7: Prováveis sítios de ação do SVBS envolvidos nos efeitos do tipo antidepressivo e anti-hiperalgésico demonstrados na tese (em azul): K^+ (canais de potássio); receptores serotoninérgicos $5\text{-}HT_{2A/C}$ e $5\text{-}HT_3$; receptores PPARγ; PGE_2 (prostaglandina E_2); NOS (óxido nítrico sintase); NO (óxido nítrico) e GC (guanilato ciclase).

Sítios modulados pelo SVBS no efeito antinociceptivo e antiinflamatório demonstrados em artigos anteriores (em vermelho) (Savegnago et al., 2006; Jesse et al., 2008; 2009): IL-1β (receptor para interleucina 1β); TNF-α (receptor para o fator de necrose tumoral α); NK-1 (receptor para SP); TRPV1 (receptor vanilóide); PKC (proteína quinase C); PKA (proteína quinase A); receptores glutamatérgicos do tipo cainato e mGlu (metabotrópico); H_2 (receptores histaminérgicos do tipo H_2) e EROs (espécies reativas de oxigênio).

6. CONCLUSÕES

- A administração aguda do SVBS produziu uma redução no tempo de imobilidade no TNF e TSC, modelos animais preditivos de atividade antidepressiva;

- Os testes farmacológicos sugerem que o efeito do tipo antidepressivo causado pelo SVBS envolve a modulação dos receptores 5-$HT_{2A/2C}$, 5-HT_3, PPAR-γ e a via L-arginine/NO/GMPc/K^+.
- A atividade do tipo antidepressiva do SVBS não está relacionada com o efeito inespecífico sobre a atividade locomotora.
- O aumento no tempo de imobibilidade no TNF causado pela ICC nos camundongos foi diminuída com o tratatamento com SVBS sem alterar a atividade locomotora;
- O efeito do tipo antidepressivo do SVBS foi mais potente que os efeitos dos antidepressivos clássicos (amitriptilina, fluoxetina e bupropiona) no TNF nos camundongos submetidos à ICC;
- A atividade exercida pelo SVBS foi mais potente quando comparada à amitriptilina na hiperalgesia mecânica nos camundongos;
- A administração oral do SVBS diminui o efeito hiperalgésico em modelos inflamatórios induzidos pela injeção i.pl. de ACF, Cg e PGE_2 em camundongos;
- A administração oral do SVBS reduziu a hiperalgesia neuropática causada pela ABP e vincristina em camundongos;

- A resposta anti-hiperalgésica mecânica causada pelo SVBS não foi susceptível a tolerância nos modelos induzidos pela APB, administração de vincristina e injeção i.pl. de ACF em camundongos.

7. REFERÊNCIAS BIBLIOGRÁFICAS

ARTEEL, G.E., SIES, H. The biochemistry of selenium and the glutathione system. **Environ.Toxicol. Pharmacol**. 10, 153-158, 2001.

ATTAL, N., CRUCCU, G., HAANPÄÄ, M., HANSSON, P., JENSEN, T.S., NURMIKKO, T., SAMPAIO, C., SINDRUP, S., WIFFEN, P. EFNS guidelines on pharmacological treatment of neuropathic pain. **Eur. J. Neurol.** 13,1153-1169, 2006.

BACKONJA, M., BEYDOUN, A., EDWARDS, K.R., SCHWARTZ, S.L., FONSECA, V., HES, M., LAMOREAUX, L., GAROFALO, E. Gabapentin for the symptomatic treatment of painful neuropathy in patients with diabetes mellitus: a randomized controlled trial. **JAMA** 280, 1831-1836, 1998.

BANKS, S.M., KERNS, R.D. Explaining hight rates of depression in chronic pain: a diathesisstress framework. **Psychol. Bull.** 119, 95-110, 1996.

BATTAGLIA, G., RUSTIONI, A. Coexistence of glutamate and substance P in dorsal root ganglion cells of the rat and monkey. **J. Comp. Neurol.** 277:302-312, 1988.

BAUER**,** M**.**E**.**, TEJANI-BUTT**,** S**.**M. Effects of repeated administration of desipramine or electroconvulsive shock on norepinephrine uptake sites measured by [^{3}H]nisoxetine autoradiography. **Brain Res.** 582, 208-214, 1992.

BEHNE, D., KYRIAKOPOULOS, A. Identification of type I iodothyronine 5'- deiodinase as a selenoenzyme. **Biochem. Biophys. Res. Com.** 173, 1143-1149, 1990.

BELLINGHAM, G.A., PENG, P.W. Duloxetine: a review of its pharmacology and use in chronic pain management. **Reg. Anesth. Pain Med.** 35, 294-303, 2010.

BERTON, O.; NESTLER, E. J. New approaches to antidepressant drug discovery: beyond monoamines. **Nat. Rev. Neurosci.** 7,137-151, 2006.

BJÖRKMAN, R. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. **Acta Anaesth. Scand.** 39,1-44, 1995.

BLACKBURN-MUNRO, G., BLACKBURN-MUNRO, R.E. Chronic pain, chronic stress and depression: coincidence or consequence? **J. Neuroendocrinol.** 13, 1009-1023, 2001.

BLAZER, D.G., KESSLER, R.C., MCGONAGLE, K.A., SWARTZ, M.S. The prevalence and distribution of major depression in a national community sample: the National Comorbitiy Survey. **Am. J. Psychiatry** 151, 979-986, 1994.

BONKALE, W.L., MURDOCK, S., JANOSKY, J.E., AUSTIN, M.C. Normal levels of tryptophan hydroxylase immunoreactivity in the dorsal raphe of depressed suicide victims. **J. Neurochem**. 88, 958-964, 2004.

BORSINI, F., MELI, A. Is the forced swimming test a suitable model for revealing antidepressant activity? **Psychopharmacology** 94, 147-160, 1988.

BORTOLATTO, C.F., JESSE, C.R., WILHELM, E.A., NOGUEIRA, C.W. Involvement of potassium channels in the antidepressant-like effect of venlafaxine in mice. **Life Sci.** 86, 372-376, 2010.

BRAS, M., DORDEVIĆ, V., GREGUREK, R., BULAJIĆ, M. Neurobiological and clinical relationship between psychiatric disorders and chronic pain. **Psychiatr. Danub.** 22, 221-226, 2010.

BRENMAN, J.E., CHAO, D.S., GEE, S.H., MCGEE, A.W., CRAVEN, S.E., SANTILLANO, D.R., WU, Z., HUANG, F., XIA, H., PETERS, M.F., FROEHNER, S.C., BREDT, D.S. Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains. **Cell** 84, 757-767, 1996.

BROCARDO, P.S., BUDNI, J., LOBATO, K.R., KASTER, M.P., RODRIGUES, A.L. Antidepressant-like effect of folic acid: Involvement of NMDA receptors and l-arginine-nitric oxide-cyclic guanosine monophosphate pathway. **Eur. J. Pharmacol.** 598, 37-42, 2008.

BUDNI, J., GADOTTI, V.M., KASTER, M.P., SANTOS, A.R., RODRIGUES, A.L. Role of different types of potassium channels in the antidepressant-like effect of agmatine in the mouse forced swimming test. **Eur. J. Pharmacol.** 575, 87-93, 2007.

BURGER, M.E., FACHINETTO, R., CALEGARI, L., PAIXÃO, M.W., BRAGA, A.L., ROCHA, J.B.T. Effects of age on reserpine-induced orofacial dyskinesia and possible protection of diphenyl diselenide. **Brain Res. Bull.** 64, 339-345, 2004.

BURGER, M.E., FACHINETTO, R., WAGNER, C., PEROTTONI, J., PEREIRA, R.P., ZENI, G., ROCHA, J.B.T. Effects of diphenyl-diselenide on orofacial dyskinesia model in rats. **Brain Res. Bull**. 70, 165-170, 2006.

BURK RF. Selenium, an antioxidant nutrient. **Nutr. Clin. Care** 5, 75-79, 2002.

CALIXTO, J.B., CABRINI, D.A., FERREIRA, J. Inflammatory pain: kinins and antagonists. **Curr. Opin. Anesth.** 14:519-526, 2001.

CALLIZOT, N., ANDRIAMBELOSON, E., GLASS, J., REVEL, M., FERRO, P., CIRILLO, R., VITTE, P.A., DREANO, M. Interleukin-6 protects against paclitaxel, cisplatin and vincristine-induced neuropathies without impairing chemotherapeutic activity. **Cancer Chemother. Pharmacol.** 62, 995-1007, 2008.

CASHMA, J.N. The mechanisms of action of NSAIDs in analgesia. **Drugs** 52,13-23, 1996.

CHAPMAN, C.R., GAVRIN, J. Suffering: the contributions of persistent pain. **Lancet** 26, 2233-2237, 1999.

CHARNEY, D.S., HENINGER, G.R., STERNBERG, D.E., REDMOND, D.E., LECKMAN, J.F., MAAS, J.W., ROTH, R.H. Presynaptic adrenergic receptor sensitivity in depression. The effect of long-term desipramine treatment. **Arch. Gen. Psychiatry** 38, 1334-1340, 1981.

CLATWORTHY, A.L., ILLICH, P.A., CASTRO, G.A., WALTERS, E.T. Role of peri-axonal inflammation in the development of thermal hyperalgesia and guarding behavior in a rat model of neuropathic pain. **Neurosci. Lett.** 184, 5-8, 1995.

COLLINO, M., ARAGNO, M., MASTROCOLA, R., GALLICCHIO, M., ROSA, A.C., DIANZANI, C. Modulation of the oxidative stress and inflammatory response by PPAR-g agonists in the hippocampus of rats exposed to cerebral ischemia/reperfusion. **Eur. J. Pharmacol.** 530, 70-80, 2006.

COTRAN, C., KUMAR, T., ROBBINS, A. (1994). Patología estrutural e funcional. Rio de Janeiro: Guanabara-Koogan. **4**:35-71.

COUTAUX, A., ADAM, F., WILLER, J.C., LE BARS, D. Hyperalgesia and allodynia: peripheral mechanisms. **Joint Bone Spine** 72, 359-371, 2005.

CRYAN, J.F., MARKOU, A., LUCKI, I. Assessing antidepressant activity in rodents: recent developments and future needs. **Trends Pharmacol. Sci.** 23, 238-245, 2002.

CRYAN, J.F., MOMBEREAU, C., VASSOUT, A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. **Neurosci. Biobehav. Rev.** 29, 571-625, 2005.

CRYAN**,** J.F**.**, O'LEARY**,** O.F**.**, JIN**,** S.H**.**, FRIEDLAND**,** J.C**.**, OUYANG**,** M**.**, HIRSCH**,** B.R**.**, PAGE**,** M.E**.**, DALVI**,** A**.**, THOMAS**,** S.A**.**, LUCKI**,** I. Norepinephrine-deficient mice lack responses to antidepressant drugs, including selective serotonin reuptake inhibitors. **Proc. Natl. Acad. Sci. USA**101, 8186-8191, 2004.

CUNHA, F.Q., FERREIRA, S.H. Peripheral hyperalgesic cytokines. **Adv. Exp. Med. Biol.** 531, 22-39, 2003.

CUNHA, T.M., VERRI, W.A, JR., FUKADA, S.Y., GUERRERO, A.T., SANTODOMINGO-GARZÓN, T., POOLE, S., PARADA, C.A., FERREIRA, S.H., CUNHA, F.Q. TNF-alpha and IL-1beta mediate inflammatory hypernociception in mice triggered by B1 but not B2 kinin receptor. **Eur. J. Pharmacol.** 573, 221-229, 2007.

DA SILVA, G.D., MATTEUSSI, A.S., SANTOS, A.R.S., CALIXTO, J.B., RODRIGUES, A.L.S. Evidence for dual effects of nitric oxide in the forced swimming test and in the tail suspension test in mice. **Neuroreport** 11, 3699-3702, 2000.

DAILLY, E., CHENU, F., RENARD, C.E., BOURIN, M. Dopamine, depression and antidepressants. **Fundam. Clin. Pharmacol.** 18, 601-607, 2004.

DAVIS, M.T., BARTAY, W.J. Ebselen decrease oxygen free radical production and iron concentrations in the hearts of chronically iron-overloaded mice. **Biol. Res. Nursing** 6, 37-45, 2004.

DEAKIN**,** J.F. $5HT_2$ receptors, depression and anxiety. **Pharmacol. Biochem. Behav.** 29, 819-820, 1988.

DELGADO, P.L., MILLER, H.L., SALOMON, R.M., LICINIO, J., KRYSTAL, J.H., MORENO, F.A., HENINGER, G.R., CHARNEY, D.S. Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. **Biol. Psychiatry** 46, 212-220, 1999.

DENIKER, P. Psychopharmacology and biological psychiatry, historical review**. Soins Psychiatr**. 37, 5-6, 1983.

DENIKER, P. Psychopharmacology and biologic psychiatry. Historical review. **Soins Psychiatr.** 37, 5-6, 1983.

DENNINGER, J.W., MARLETTA, M.A. Guanylate cyclase and the NO/cGMP signaling pathway. **Biochim. Biophys. Acta** 1411, 334-350, 1999.

DESVERGNE, B., WAHLI, W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. **Endocrinol. Rev.** 20,649-688, 1999.

DHIR, A., KULKARNI, S.K. Involvement of nitric oxide (NO) signaling pathway in the antidepressant action of bupropion, a dopamine reuptake inhibitor. **Eur. J. Pharmacol.** 568, 177-185, 2007.

DICKENSON, A.H. Central acute pain mechanisms. **Ann. Med.** 27, 223-227, 1995.

DONOHUE, J.M., PINCUS, H.A. Reducing the societal burden of depression: a review of economic costs, quality of care and effects of treatment. **Pharmacoeconomics** 25, 7-24, 2007.

DREYER**,** J**.**, SCHLEICHER**,** M**.**, TAPPE**,** A**.**, SCHILLING**,** K**.**, KUNER**,** T**.**, KUSUMAWIDIJAJA**,** G**.**, MÜLLER-ESTERL**,** W**.**, OESS**,** S**.**, KUNER**,** R. Nitric oxide synthase (NOS)-interacting protein interacts with neuronal NOS and regulates its distribution and activity. **J. Neurosci.** 24, 10454-10465, 2004.

DUMONT, E., VANHAECKE, F., CORNELIS, R. Selenium speciation from food source to metabolites: a critical review. **Anal. Bioanal. Chem.** 385, 1304-1323, 2006.

DWORKIN, R.H., RICHLIN, D.M., HANDLIN, D.S., BRAND, L. Predicting treatment response in depressed and non-depressed chronic pain patients. **Pain** 24, 343-353, 1986.

DWORKIN, R.H., CORBIN, A.E., YOUNG, J.P.JR., SHARMA, U., LAMOREAUX, L., BOCKBRADER, H., GAROFALO, E.A., POOLE, R.M. Pregabalin for the treatment of postherpetic neuralgia: a randomized, placebo-controlled trial. **Neurology** 60, 1274-1283, 2003.

DWORKIN, R.H., O'CONNOR, A.B., BACKONJA, M., FARRAR, J.T., FINNERUP, N.B.,JENSEN, T.S., KALSO, E.A., LOESER, J.D.,MIASKOWSKI, C., NURMIKKO, T.J., PORTENOY, R.K., RICE, A.S., STACEY, B.R., TREEDE, R.D., TURK, D.C., WALLACE, M.S. Pharmacologic management of neuropathic pain: evidence-based recommendations. **Pain** 132**,** 237-251, 2007.

DWORKIN, S.F. Illness behavior and dysfunction: review of concepts and application to chronic pain. **Can. J. Physiol. Pharmacol.** 69, 662-671, 1991.

EHNINGER, D., KEMPERMANN, G. Neurogenesis in the adult hippocampus. **Cell Tissue Res.** 331, 243-250, 2008.

EI-MALLAKH RS, WYATT RJ. The Na^+ K^+ ATPase hypothesis for bipolar illness. **Biol. Psychiatry** 37, 235-244, 1995.

EISENSAMER, B., RAMMES, G., GIMPL, G., SHAPA, M., FERRARI, U., HAPFELMEIER, G., BONDY, B., PARSONS, C., GILLING, K., ZIEGLGÄNSBERGER, W., HOLSBOER, F., RUPPRECHT. R. Antidepressants are functional antagonists at the serotonin type 3 ($5\text{-}HT_3$) receptor. **Mol. Psychiatry** 8, 994-1007, 2003.

ELHWUEGI, A.S. Central monoamines and their role in major depression. **Prog. Neuropsychopharmacol. Biol. Psychiatry** 28, 435-451, 2004.

ENGEL, G.L. Psychogenic pain and pain-prone patient. **Am. J. Med.** 26, 899-918, 1959.

ERECINSKA, M.; SILVER, I.A. Ions and energy in mammalian brain. **Prog. Neurobiol.** 43, 37-71, 1994.

ERIKSSON, P.S., PERFILIEVA, E., BJÖRK-ERIKSSON, T., ALBORN, A.M., NORDBORG, C., PETERSON, D.A., GAGE, F.H. Neurogenesis in the adult human hippocampus. **Nat. Med.** 4, 1313-1317, 1998.

FERRENDELLI, J.A., CHANG, M.M., KINSCHERF, D.A. Elevation of cyclic GMP levels in central nervous system by excitatory and inhibitory amino acids. **J. Neurochem.** 22, 535-540, 1974.

FISHBAIN, D.A., CUTLER, R., ROSOMOFF, H.L., ROSOMOFF, R.S. Chronic pain-associated depression: antecedent or consequence of chronic pain? A review. **Clin. J. Pain** 13, 116-137, 1997.

FISHBAIN, D.A. The association of chronic pain and suicide. **Semin. Clin. Neuropsychiatry** 4, 221-227, 1999.

FLOHÉ, L., GUNZLER, W.A., SCHOCK, H.H. Glutathione peroxidase: a selenium enzyme. **FEBS Lett.** 32, 132-134. 1973.

FLECK, M.P.A. Depressão. In: Duncan, B.B., Schmidt, M.I., Guigliani, E.R.J. Medicina ambulatorial: condutas de atenção primária baseadas em evidência. 3º ed. Artmed. Porto Alegre. Brasil. pp: 874-884, 2004.

FÜRST, S. Transmitters involved in antinociception in the spinal cord. **Brain Res. Bull.** 48, 129-141, 1999.

GALEOTTI, N., GHELARDINI, C., CALDARI, B., BARTOLINI, A. Effect of potassium channel modulators in mouse forced swimming test. **Br. J. Pharmacol.** 126, 1653-1659 , 1999.

GAMARO, G.D., STRECK, E.L., MATTÉ, C., PREDIGER, M.E., WYSE, A.T., DALMAZ, C. Reduction of hippocampal Na^+ K^+ ATPase activity in rats subjected to an experimental model of depression. **Neurochem. Res.** 28, 1339- 1344, 2003.

GARTHWAITE, J., BOULTON, C.L. Nitric oxide signaling in the central nervous system. **Annu. Rev. Physiol.** 57, 683-706, 1995.

GAY, B.M., PRIGOL, M., STEIN, A.L., NOGUEIRA, C.W. Antidepressant – like pharmacological profile of 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene: involvement of serotonergic system. **Neuropharmacology** 59, 172-179, 2010.

GEORGOTAS, A., SCHWEITZER, J., MCCUE, R.E., ARMOUR, M., FRIEDHOFF, A.J. Clinical and treatment effects on ^{3}H-clonidine and ^{3}H-imipramine binding in elderly depressed patients. **Life Sci.** 40, 2137-2143, 1987.

GHASEMI, M., MONTASER-KOUHSARI, L., SHAFAROODI, H., NEZAMI, B.G., EBRAHIMI, F., DEHPOUR, A.R. NMDA receptor/nitrergic system blockage augments antidepressant-like effect of paroxetine in the mouse forced swimming test. **Psychopharmacology** 206, 325-333, 2009.

GINGRICH**,** J.A**.,** HEN**,** R. Dissecting the role of the serotonin system in neuropsychiatric disorders using knockout mice. **Psychopharmacology** 155, 1-10, 2001.

GOLAN, M., SCHREIBER, G., AVISSAR, S. Antidepressants, beta-arrestins and GRKs: from regulation of signal desensitization to intracellular multifunctional adaptor functions. **Curr. Pharm. Des.** 15, 1699-1708, 2009.

GOLDENBERG, D.L. Pain/Depression dyad: a key to a better understanding and treatment of functional somatic syndromes. **Am. J. Med.** 123, 675-682, 2010.

GREENBERG, P.E., KESSLER, R.C., BIRNBAUM, H.G., LEONG, S.A., LOWE, S.W., BERGLUND, P.A., COREY-LISLE, P.K. The economic burden of depression in the United States: how did it change between 1990 and 2000? **J. Clin. Psychiatry** 64, 1465-1475, 2003.

GUIMARÃES, A.L., DE SÁ, A.R., VICTORIA, J.M., DE FÁTIMA CORREIA-SILVA, J., GOMEZ, M.V., GOMEZ, R.S. Interleukin-1beta and serotonin transporter gene polymorphisms in burning mouth syndrome patients. **J. Pain** 7, 654-658, 2006.

GUO, W.Y., TODD, K.G., BOURIN, M., HASCOET, M. The additive effects of quinine on antidepressant drugs in the forced swimming test in mice. **Psychopharmacology** 121, 173-179, 1995.

HALL, C.S. Emotional behaviour in the rat: The relationship between emotionality and ambulatory activity. **J. Comp. Psychol.** 22:345-352, 1936.

HARKIN, A.J., CONNOR, T.J., BURNS, M.P., KELLY, J.P. Nitric oxide synthase inhibitors augment the effects of serotonin reuptake inhibitors in the forced swimming test. **Eur. Neuropsychopharmacol.** 14, 274-281, 2004.

HAWKES**,** W**.**C**.**, HORNBOSTEL**,** L. Effects of dietary selenium on mood in healthy men living in a metabolic research unit. **Biol. Psychiatry** 39, 121-128, 1996.

HAWLEY, D.J., WOLFE, F. Depression in not more common in rheumatoid arthritis: a 10-year longitudinal study of 6.513 patients with rheumatic disease. **J. Rheumatol.** 20, 2025-2031, 1993.

HAYAKAWA, K., ITOH, T., NIWA, H., MUTOH, T., SOBUE, G. NGF prevention of neurotoxicity induced by cisplatin, vincristine and taxol depends on toxicity of each drug and NGF treatment schedule: in vitro study of adult rat sympathetic ganglion explants. **Brain Res.** 794, 313-319, 1998.

HENRY, D., LIM, L.L., GARCIA RODRIGUEZ, L.A., PEREZ GUTTHANN, S., CARSON, J.L., GRIFFIN, M., SAVAGE, R., LOGAN, R., MORIDE, Y., HAWKEY, C., HILL, S., FRIES, J.T. Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: results of a collaborative meta-analysis. **Britis. Med. J.** 312, 1563-1566, 1996.

HENSLER, J.G. Differential regulation of 5-HT_{1A} receptor-G protein interactions in brain following chronic antidepressant administration. **Neuropsychopharmacology** 26, 565-573, 2002.

HERIN, G.A., DU, S., AIZENMAN, E. The neuroprotective agent ebselen modifies NMDA receptor function via the redox modulatory site. **J. Neurochem.** 78, 1307-1314, 2001.

HEURTEAUX, C., LUCAS, G., GUY, N., EL YACOUBI, M., THÜMMLER, S., PENG, X.D., NOBLE, F., BLONDEAU, N., WIDMANN, C., BORSOTTO, M., GOBBI, G., VAUGEOIS, J.M., DEBONNEL, G., LAZDUNSKI, M. Deletion of the background

potassium channel TREK-1 results in a depression-resistant phenotype. **Nat. Neurosci.** 9, 1134-1141, 2006.

HOLDEN, J.E., PIZZI, J.A.The Challenge of chronic pain. **Adv. Drug Deliv. Rev.** 55, 935-948, 2003.

HOLMGREN, A. Thioredoxin. **Annu. Rev. Biochem.** 54, 237-271, 1985.

HOLTZHEIMER, P.E.; NEMEROFF, C.B. Advances in the treatment of depression. **NeuroRx** 3, 42-56, 2006.

HONDA, M., UCHIDA, K., TANABE, M., ONO, H. Fluvoxamine, a selective serotonin reuptake inhibitor, exerts its antiallodynic effects on neuropathic pain in mice via 5-$HT_{2A/2C}$ receptors. **Neuropharmacology** 51, 866-872, 2006.

HOSKIN, P.J., HANKS, G.W. Opioid agonist-antagonist drugs in acute and chronic pain states. **Drugs** 41,326-344, 1991.

HUOT, P., PARENT, A. Dopaminergic neurons intrinsic to the striatum. **J. Neurochem.** 101, 1441-1447, 2007.

HYMAN, S.E. Even chromatin gets the blues. **Nat. Neurosci.** 9, 465-466, 2006.

IMAI, H., MASYASU, H., DEWAR, D., GRAHAM, D.I., MACRAE, I.M. Ebselen protects both gray and White matter in a rodent model of focal cerebral ischemia. **Stroke** 32, 2149-2154, 2001.

INAN, S.Y., YALCIN, I., AKSU, F. Dual effects of nitric oxide in the mouse forced swimming test: possible contribution of nitric oxide-mediated serotonin release and potassium channel modulation. **Pharmacol. Biochem. Behav.** 77,457-464, 2004.

JACKSON, D.L., GRAFF, C.B., RICHARDSON, J.D. Glutamate participates in the peripheral modulation of thermal hyperalgesia in rats. **Eur. J. Pharmacol.** 284,321- 325, 1995.

JACOBSEN, J.P., WEIKOP, P., HANSEN, H.H., MIKKELSEN, J.D., REDROBE, J.P., HOLST, D., BOND, C.T., ADELMAN, J.P., CHRISTOPHERSEN, P., MIRZA, N.R. SK3 K^+ channel-deficient mice have enhanced dopamine and serotonin release and altered emotional behaviors. **Genes Brain Behav.** 7, 836-848, 2008.

JESSE, C.R., SAVEGNAGO, L., NOGUEIRA, C.W. Role of nitric oxide/cyclic GMP/K($^+$) channel pathways in the antinociceptive effect caused by 2,3-bis(mesitylseleno)propenol. **Life Sci.** 81, 1694-1702, 2007.

JESSE, C.R., SAVEGNAGO, L., NOGUEIRA, C.W. Spinal mechanisms of antinociceptive effect caused by oral administration of bis-selenide in mice. **Brain Res.** 1231, 25-33, 2008.

JESSE, C.R., SAVEGNAGO, L., NOGUEIRA, C.W. Mechanisms involved in the antinociceptive and anti-inflammatory effects of bis selenide in mice. **J. Pharm. Pharmacol.** 61, 623-630, 2009.

JI, R.R., STRICHARTZ, G. Cell signaling and the genesis of neuropathic pain. **Sci. STKE**. 21, 252, 2004.

JONES, P.F. Early analgesia for acute abdominal pain. **BMJ** 305, 1020-1021, 1992.

JULIUS, D., BASBAUM, A.I. Molecular mechanisms of nociception. **Nature** 413, 203-210, 2001.

KANBAYASHI, Y., HOSOKAWA, T., OKAMOTO, K., KONISHI, H., OTSUJI, E., YOSHIKAWA, T., TAKAGI, T., TANIWAKI, M. Statistical identification of predictors for peripheral neuropathy associated with administration of bortezomib, taxanes, oxaliplatin or vincristine using ordered logistic regression analysis. **Anticancer Drugs** 21, 877-881, 2010.

KANDEL, E.R., MACK, S. A parallel between radical reductionism in science and in art. **Ann. N.Y. Acad. Sci.** 1001, 272-294, 2003.

KANDEL, E.R., SCHWARTZ, J.H., JESSEL, T.M. Princípios da Neurociência. São Paulo: Manole. 73-491, 2003.

KASTER, M.P., FERREIRA, P.K., SANTOS, A.R., RODRIGUES, A.L.S. Effects of potassium channel inhibitors in the forced swimming test: possible involvement of L-

arginine-nitric oxide-soluble guanylate cyclase pathway. **Behav. Brain Res.** 165, 204-209, 2005.

KASTER, M.P., BUDNI, J., BINFARÉ, R.W., SANTOS, A.R., RODRIGUES, A.L. The inhibition of different types of potassium channels underlies the antidepressant-like effect of adenosine in the mouse forced swimming test. **Prog. Neuropsychopharmacol. Biol. Psychiatry** 31, 690-696, 2007.

KATON, W., SULLIVAN, M.D. Depression and chronic medical illness. **J. Clin. Psychiatry** 51, 12-14, 1990.

KHASAR, S.G., MCCARTER, G., LEVINE, J.D. Epinephrine produces a betaadrenergic receptor-mediated mechanical hyperalgesia and in vitro sensitization of rat nociceptors. **J. Neurophysiol.** 81,1104-1112, 1999.

KHISTI, R.T., CHOPDE, C.T. Serotonergic agents modulate antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice**. Brain Res.** 865, 291- 300, 2000.

KLIMEK, V., STOCKMEIER, C., OVERHOLSER, J., MELTZER, H.Y., KALKA, S., DILLEY, G., ORDWAY, G.A. Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. **J. Neurosci.** 17, 8451-8458, 1997.

KUMAR, A., GARG, R., GAUR, V., KUMAR, P. Nitric oxide mechanism in protective effect of imipramine and venlafaxine against acute immobilization stress-induced behavioral and biochemical alteration in mice. **Neurosci. Lett.** 467, 72-75, 2009.

KUMAR, A., GARG, R., GAUR, V., KUMAR, P. Venfalaxine involves nitric oxide modulatory mechanism in experimental model of chronic behavior despair in mice. **Brain Res.** 1311, 73-80, 2010.

LAASONEN-BALK**,** T**.**, KUIKKA**,** J**.**, VIINAMÄKI**,** H**.**, HUSSO-SAASTAMOINEN**,** M**.**, LEHTONEN**,** J**.**, TIIHONEN**,** J. Striatal dopamine transporter density in major depression. **Psychopharmacology** 144, 282-285, 1999.

LANNI, C., GOVONI, S., LUCCHELLI, A., BOSELLI, C. Depression and antidepressants: molecular and cellular aspects. **Cell Mol. Life Sci.** 66, 2985-3008, 2009.

LEE, C.H., CHOI, J.H., YOO, K.Y., PARK, O.K., MOON, J.B., SOHN,Y., CHO, J.H., HWANG, I.K., WON, M.H. Rosiglitazone, an agonist of peroxisome proliferator-activated receptor gamma, decreases immunoreactivity of markers for cell proliferation and neuronal differentiation in the mouse hippocampus. **Brain Res.** 1329, 30-35, 2010.

LEONARD, B.E. Noradrenaline in basic models of depression. **Eur. Neuropsychopharmacol.** 7, 11-16, 1997.

LESCH, K.P., AULAKH, C.S., WOLOZIN, B.L., TOLLIVER, T.J., HILL, J.L., MURPHY, D.L. Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. **Mol. Brain Res.** 17, 31-35, 1993.

LICINIO, J., WONG, M.L. The pharmacogenomics of depression. **Pharmacogenomics J.** 1, 175-177, 2001.

LIN, Z., MADRAS, B.K. Human genetics and pharmacology of neurotransmitter transporters. **Handb. Exp. Pharmacol.** 175, 327-371, 2006.

LIU, H., WANG, H., SHENG, M. Evidence for presynaptic N-methyl-Daspartate autoreceptores in the spinal cord dorsal horn. **Proc. Nat. Acad. Sci. USA** 91, 8383-8387, 1994.

LOESER, J.D., MELZACK, R. Pain: an overview. **Lancet** 353, 1607-1609, 1999.

LOPEZ, A.D., MATHERS, C.D., EZZATI, M., JAMISON, D.T., MURRAY, C.J. Global and regional burden of disease and risk factors, 2001: systematic analysis of population -health data. **Lancet** 367, 1747-1757, 2006.

MACKINNON, R. Potassium channels. **FEBS Lett.** 555, 62-65, 2003.

MACMAHON, S.B., CAFFERTY, W.B.J., MARCHAND, F. Immune and glial cell factors as pain mediators and modulators. **Exp. Neurol.** 192,444-462, 2005.

MAES, M., DE VOS, N., PIOLI, R., DEMEDTS, P., WAUTERS, A., NEELS, H., CHRISTOPHE, A. Lower serum vitamin E concentrations in major depression. Another marker of lowered antioxidant defenses in that illness. **J. Affect. Disord.** 58, 241-246, 2000.

MAGYAR, K.; KNOLL, J. Selective inhibition of the "B form" of monoamine oxidase**. Pol. J. Pharmacol. Pharm.** 29,233-246, 1977.

MANN, J.J., MCBRIDE, P.A., MALONE, K.M., DEMEO, M., KEILP, J. Blunted serotonergic responsivity in depressed inpatients. **Neuropsychopharmacology** 13, 53-64, 1995.

MANTOVANI, M., PÉRTILE, R., CALIXTO, J.B., SANTOS, A.R., RODRIGUES, A.L. Melatonin exerts na antidepressant-like effect in the tail suspension test in mice: evidence for involvement of N-methyl-D-aspartate receptors and the L-arginine –nitric oxide pathway. **Neurosc. Lett.** 343,1-4, 2003.

MARTIN, J.L., GERLACK, M.L. Selenium metabolism in animals. **Ann. NY Acad. Sci.** 192, 193-199, 1972.

McARTHUR, R., BORSINI, F. Animal models of depression in drug discovery: A historical perspective. **Pharmacol. Biochem. Behav**. 84,436-452, 2006.

MEANA, J.J., BARTUREN, F., GARCÍA-SEVILLA, J.A. Alpha 2-adrenoceptors in the brain of suicide victims: increased receptor density associated with major depression. **Biol. Psychiatry** 31, 471-490, 1992.

MENDELL, J.R., SAHENK, Z. Painful sensory neuropathy. **N. Engl. J. Med.** 348, 1243-1255, 2003.

MEOTTI, F.C., MISSAU, F.C., FERREIRA, J., PIZZOLATTI, M.G., MIZUZAKI, C., NOGUEIRA, C.W., SANTOS, A.R. Anti-allodynic property of flavonoid myricitrin in models of persistent inflammatory and neuropathic pain in mice. **Biochem. Pharmacol.** 72, 1707-1713, 2006.

MERSKEY, H., BOGDUK, N. Classification of Chronic Pain. Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms**. IASP Press**, 1994.

MEYER, J.H., GINOVART, N., BOOVARIWALA, A., SAGRATI, S., HUSSEY, D., GARCIA, A., YOUNG, T., PRASCHAK-RIEDER, N., WILSON, A.A., HOULE, S. Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. **Arch. Gen. Psychiatry** 63,1209-1216, 2006.

MEYER**,** S**.**E**.**, CHROUSOS**,** G**.**P**.**, GOLD**,** P**.**W. Major depression and the stress system: a life span perspective. **Dev. Psychopathol.** 13, 565-580, 2001.

MICO, J.A., ARDID, D., BERROCOSO, E., ESCHALIER, A. Antidepressants and pain. **Trends Pharmacol. Sci.** 7,348-354, 2006.

MILLAN, M.J. The induction of pain: an integrative review. **Prog. Neurobiol.** 57, 1-164, 1999.

MILLAN, M.J. Descending control of pain. **Prog. Neurobiol.** 66, 355-474, 2002.

MILLAN, M.J. The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. **Eur. J. Pharmacol**. 500, 371-384, 2004.

MITANI, H., SHIRAYAMA, Y., YAMADA, T., KAWAHARA, R. Plasma levels of homovanillic acid, 5-hydroxyindoleacetic acid and cortisol, and serotonin turnover in depressed patients. **Prog. Neuropsychopharmacol. Biol. Psychiatry** 30, 531-534, 2006.

MORENO, S., FARIOLI-VECCHIO, S., CERU, M.P. Immunolocalization of peroxisome proliferator activated receptor and retinoic X receptors in the adult rat CNS. **Neuroscience** 123, 131-145, 2004.

MUGESH, G., DU MONT, W.W., SIES, H. Chemistry of biologically important Organoselenium compounds. **Chem. Rev.** 101, 2125-2179, 2001.

MUNCE, S.E,, WELLER, I., ROBERTSON BLACKMORE, E.K., HEINMAA, M., KATZ, J., STEWART, D.E. The role of work stress as a moderating variable in the chronic pain and depression association. **J. Psychosom. Res.** 61, 653-660, 2006.

MUSCETTOLA, G., POTTER, W.Z., PICKAR, D., GOODWIN, F.K. Urinary 3-methoxy-4-hydroxyphenylglycol and major affective disorders. A replication and new findings. **Arch. Gen. Psychiatry** 41, 337-342, 1984.

NAIR, A., VAIDYA, V.A. Cyclic AMP response element binding protein and brain-derived neurotrophic factor: molecules that modulate our mood? **J. Biosci.** 31, 423-434, 2006.

NEMEROFF, C.B. The burden of severe depression: a review of diagnostic challenges and treatment alternatives. **J. Psychiatr. Res.** 41,189-206, 2006.

NOGUEIRA, C.W., ZENI, G., ROCHA, J.B. Organoselenium and organotellurium compounds: toxicology and pharmacology. **Chem. Rev**. 104, 6255-6285, 2004.

NOGUEIRA, C.W., ROCHA, J.B.T. Diphenyl diselenide a Janus-Faced molecule. **J. Braz. Chem. Soc.** 21, 2055-2071, 2010.

NOWAK, P.M, SALUK-JUSZCZAK, J., OLAS, B., KOLODZIEKCZYK, J., WACHOWICZ, B. The protective effects of selenoorganic compounds against peroxynitrite-induced changes in plasma proteins and lipids. **Cel. Mol. Biol. Lett.** 11, 1-11, 2006.

OQUENDO, M.A., PLACIDI, G.P., MALONE, K.M., CAMPBELL, C., KEILP, J., BRODSKY, B., KEGELES, L.S., COOPER, T.B., PARSEY, R.V., VAN HEERTUM, R.L., MANN, J.J. Positron emission tomography of regional brain metabolic responses to a serotonergic challenge and lethality of suicide attempts in major depression. **Arch. Gen. Psychiatry** 60, 14-22, 2003.

OZAKI, M., NAKAMURA, M., TERAOKA, S., OTA, K. Ebselen, a novel anti-oxidant compound, protects the rat liver from ischemia-reperfusion injury. **Transpl. Int.** 10, 96-102, 1997.

PAAKKARI, I., LINDSBERG, P. Nitric oxide in the central nervous system. **Ann. Med.**, 27:369-377, 1995.

PAPAKOSTAS, G.I. Dopaminergic-based pharmacotherapies for depression. **Eur. Neuropsychopharmacol.** 16, 391-402, 2006.

PARNHAM, M.J., GRAF, E. Seleno-organic compounds and the therapy of hydroperoxide-linked pathological conditions. **Biochem. Pharmacol.** 36, 3095-3102, 1987.

PARNHAM, M.J., GRAF, E. Pharmacology of synthetic organic selenium compounds. **Prog. Drug. Res.** 36, 10-47, 1991.

PORCIÚNCULA, L., ROCHA, J.B.T., BOECK, C.R., VENDITE, D.A., SOUZA, D.O. Ebselen prevents excitotoxicity provoked by glutamate in rat cerebellar granule neurons. **Neurosci. Lett.** 299, 217-220, 2001.

PORSOLT, R.D., LE PICHON, M., JALFRE, M. Depression: a new animal model sensitive to antidepressant treatments. **Nature** 266, 730-732, 1977.

POSSER, T., KASTER, M.P., BARAÚNA, S.C., ROCHA, J.B., RODRIGUES, A.L., LEAL, R.B. Antidepressant-like effect of the organoselenium compound ebselen in mice: evidence for the involvement of the monoaminergic system. **Eur. J. Pharmacol.** 602, 85-91, 2009.

PYTLIAK, M., VARGOVÁ, V., MECHÍROVÁ, V., FELŠÖCI, M. Serotonin receptors - from molecular biology to clinical applications. **Physiol. Res.** 60, 15-25, 2011.

RAMAMOORTHY, S., SHIPPENBERG, T.S., JAYANTHI, L.D. Regulation of monoamine transporters: Role of transporter phosphorylation. **Pharmacol. Ther.**

RAYMAN M.P. The importance of selenium to human health. **Lancet** 356, 23-41, 2000.

REDROBE, J.P, BOURIN, M. Partial role of 5-HT_2 and 5-HT_3 receptors in the activity of antidepressants in the mouse forced swimming test. **Eur. J. Pharmacol.** 325, 129-135, 1997.

RICE, A.S.C., HILL, R.G. New treatment of neuropathic pain. **Annu. Rev. Med**. 57, 535-541, 2006.

ROSA, A.O., KASTER, M.P., BINFARÉ, R.W., MORALES, S., MARTÍN-APARICIO, E., NAVARRO-RICO, M.L., MARTINEZ, A., MEDINA, M., GARCÍA, A.G., LÓPEZ, M.G., RODRIGUES, A.L. Antidepressant-like effect of the novel thiadiazolidinone NP031115 in mice. **Prog. Neuropsychopharmacol. Biol. Psychiatry** 32, 1549-1556, 2008.

ROSSATO, J., KELTZER, L. A., CENTURIÃO, F. B. Antioxidant properties of new chalcogenides against lipid peroxidation in rat brain. **Neurochem. Res.** 318, 137-140, 2002.

ROWBOTHAM, M.C. Mechanisms of neuropathic pain and their implications for the design of clinical trials. **Neurology** 65, 66-73, 2005.

RUSSO**,** C.M**.**, BROSE**,** W**.**G. Chronic pain. **Annu. Rev. Med.** 49, 123-133, 1998.

SAIKA, F., KIGUCHI, N., KOBAYASHI, Y., FUKAZAWA, Y., MAEDA, T., OZAKI, M., KISHIOKA, S. Suppressive effect of imipramine on vincristine-induced mechanical allodynia in mice. **Biol. Pharm. Bull.** 32, 1231-1234, 2009.

SANCHEZ-BAHILLO, A., BAUTISTA-HERNANDEZ, V., BARCIA GONZALEZ, C., BAÑON, R., LUNA, A., HIRSCH, E., HERRERO, M.T. Increased mRNA expression of cytochrome oxidase in dorsal raphe nucleus of depressive suicide victims. **Neuropsychiatr. Dis. Treat.** 4, 413-416, 2008.

SARANDOL, A., SARANDOL, E., EKER, S.S., ERDINC, S., VATANSEVER, E., KIRLI, S. Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative antioxidative systems. **Hum. Psychopharmacol. Clin. Exp.** 22, 67-73, 2007.

SARTORIUS, N. The economic and social burden of depression. **J. Clin. Psychiatry** 15, 8-11, 2001.

SAVEGNAGO, L., JESSE, C.R., MORO, A.V., BORGES, V.C., SANTOS, F.W., ROCHA, J.B., NOGUEIRA, C.W. Bis selenide alkene derivatives: A class of potential antioxidant and antinociceptive agents. **Pharmacol. Biochem. Behav.** 83, 221-229, 2006.

SAVEGNAGO, L., PINTO, L.G., JESSE, C.R., ROCHA, J.B., NOGUEIRA, C.W. Diphenyl diselenide attenuates acute thermal hyperalgesia and persistent inflammatory and neuropathic pain behavior in mice. **Brain Res.** 1175, 54-59, 2007a.

SAVEGNAGO, L., PINTO, L.G., JESSE, C.R., ROCHA, J.B., NOGUEIRA, C.W., ZENI, G. Spinal mechanisms of antinociceptive action caused by diphenyl diselenide. **Brain Res.** 1162, 32-37, 2007b.

SAVEGNAGO, L., PINTO, L.G., JESSE, C.R., ALVES, D., ROCHA, J.B., NOGUEIRA, C.W., ZENI, G. Antinociceptive properties of diphenyl diselenide: evidences for the mechanisms of action. **Eur. J. Pharmacol.** 555, 129-138, 2007c.

SAVEGNAGO, L., PINTO, L.G., JESSE, C.R., ROCHA, J.B.T., NOGUEIRA, C.W., ZENI, G. Monoaminergic agents modulate antidepressant-like effect caused by diphenyl diselenide in rats. **Prog. Neuropsychopharmacol. Biol. Psychiatry** 31, 1261-1269, 2007d.

SAVEGNAGO, L., PINTO, L.G., JESSE, C.R., ROCHA, J.B.T., NOGUEIRA, C.W., ZENI, G. Diphenyl Diselenide exerts antidepressant–like and anxiolytic-like effects in mice: Involvement of L-arginine-nitric oxide-soluble guanylate cyclase pathway in its antidepressant-like action. **Pharmacol. Biochem. Behav.** 88, 488-496, 2008.

SCHILDKRAUT, J.J. The catecholamine hypothesis of affective disorders: a review of supporting evidence. **Am. J. Psychiatry** 122, 509-522, 1965.

SCHLOSS, P., HENN, F.A. New insights into the mechanisms of antidepressant therapy. **Pharmacol. Ther.** 102, 47-60, 2004.

SCHOLZ, J., WOOLF, C.J. The neuropathic pain triad: neurons, immune cells and glia. **Nat. Neurosci**. 10, 1361-1368, 2007.

SCHWARTZ, K., FOLTSZ, P.J. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. **J. Am. Chem. Soc.** 79, 200-214, 1957.

SHARP, T.J. Chronic pain: a reformulation of the cognitive-behavioural model. **Behav. Res. Ther.** 39, 787-800, 2001.

SHER, L. Depression and suicidal behavior in alcohol abusing adolescents: possible role of selenium deficiency. **Minerva Pediatr.** 60, 201-209, 2008.

SHERWOOD ER, TOLIVER-KINSKY T. Mechanisms of the inflammation response. **Best Pract. Res. Clin. Anaesthesiol.** 18, 385-405, 2004.

SHIH, J. C.; CHEN, K.; RIDD, M. J. Monoamine oxidase: from genes to behavior. **Annu. Rev. Neurosci.** 22,197-217, 1999.

SINDRUP, S.H., JENSEN, T.S. Pharmacotherapy of trigeminal neuralgia. **Clin. J. Pain** 18, 22-27, 2002.

SONENREICH, C.; ESTEVÃO, G.; FRIEDRICH, S.; SILVA FILHO, L.M.A. A Evolução Histórica do Conceito de Depressão. **Rev. ABP-APAL**, 17, 29-40, 1995.

SORKIN, L.S., MCADOO, D.J., WILLIS, W.D. Raphe magnus stimulation-induced antinociception in the cat is associated with release of amino acids as well as serotonin in the lumbar dorsal horn. **Brain Res.** 618, 95-108, 1993.

SPALLHOLZ, J. E. On the nature of selenium toxicity and carcinostatic activity. **Free Rad. Biol. Med.** 20, 131-143, 1993.

STADTMAN, T.C. Selenium-dependent enzymes. **Annu. Rev. Biochem.** 49, 93-110, 1980.

STERNBACH, R.A. Psychological aspects of pain and the selection of patients. **Clin. Neurosurg.** 21, 323-333, 1974.

STERU, L., CHERMAT, R., THIERRY, B., SIMON, P. The tail suspension test: a new method for screening antidepressants in mice. **Psychopharmacology** 85, 367-370, 1985.

SULLIVAN, M.J., REESOR, K., MIKAILM, S., FISHER, R. The treatment of the depression in chronic low back pain: review and recommendations. **Pain** 50, 5-13, 1992.

TIERNEY JG. Treatment-resistant depression: managed care considerations. **J. Manag. Care Pharm.** 13, 2-7, 2007.

TRACEY**,** I**.**, MANTYH**,** P**.**W. The cerebral signature for pain perception and its modulation. **Neuron** 55, 377-391, 2007.

TSUBOI, H. Possible connections among job stress, depressive symptoms, lipi modulation and antioxidants. **J. Affect. Disord.** 91, 63-70, 2006.

URSINI, F., HEIM, S., KIESS, M., MAIORINO, M., ROVERI, A., WISSING, J., FLOHÉ, L. Dual function of the seleno-protein PHGPx during sperm maturation. **Science** 285, 1393-1396, 1990.

VERRI, W.A. JR., CUNHA, T.M., PARADA, C.A., POOLE, S., CUNHA, F.Q., FERREIRA, S.H. Hypernociceptive role of cytokines and chemokines: targets for analgesic drug development? **Pharmacol. Ther.** 112, 116-138, 2006.

VOLKE, V., WEGENER, G., BOURIN, M., VASAR, E. Antidepressant- and anxiolytic-like effects of selective neuronal NOS inhibitor 1-(2-trifluoromethylphenyl)-imidazole in mice. **Behav. Brain Res.** 140, 141-147, 2003.

VOLKOW, N.D., FOWLER, J.S., WANG, G.J., DING, Y.S., GATLEY, S.J. Role of dopamine in the therapeutic and reinforcing effects of methylphenidate in humans: results from imaging studies. **Eur. Neuropsychopharmacol.** 12, 557-566, 2002.

VON KNORRING, L., PERRIS, C., EISEMANN, M., ERIKSSON, U., PERRIS, H. Pain as a symptom in depressive disorders. II. Relationship to personality traits as assessed by means of KSP. **Pain** 17, 377-384, 1983.

WANGER, P.D., PEDERSEN, N.D., HATFIELD, J., WESWING, P.H. Absortion of selenite and selenomethionine from ligated digestive tract segments in rats. **Proc. Soc. Exp. Biol. Med.** 153, 295, 1976.

WATSON, C.P., EVANS, R.J., REED, K., MERSKEY, H., GOLDSMITH, L., WARSH, J. Amitriptyline versus placebo in postherpetic neuralgia. **Neurology** 32, 671-673, 1982.

WENDEL, A., FAUSEL, M., SAFAYI, H., OTTER, R. A novel biologically active seleno-organic compound II. Activity of PZ 51 in relation to glutathione peroxidase. **Biochem. Pharmacol.** 33, 3241-3245, 1984.

World Health Organization - WHO. Mental health: new understanding, new hope. Geneva: World Health Organization; 2001.

World Health Organization – WHO. Mental health. 2010 – Available from: http:// http://www.who.int/topics/mental_health/en/ [18/11/2010].

WICKENDEN, A. K^+ channels as therapeutic drug targets, **Pharmacol. Ther.** 94:157- 182, 2002.

WILBER, C.G. Toxicology of selenium: a review. **Clin. Toxicol.** 17, 171-230. 1980.

WILHELM, E.A., JESSE, C.R., BORTOLATTO, C.F., BARBOSA, N.B., NOGUEIRA, C.W. Evidence of the involvement of K^+ channels and PPARgama receptors in the antidepressant-like activity of diphenyl diselenide in mice. **J. Pharm. Pharmacol.** 62, 1121-1127, 2010.

WONG, D.T., PERRY, K.W., BYMASTER, F.P. Case history: the discovery of fluoxetine hydrochloride (Prozac). **Nat. Rev. Drug Discov.** 4, 764-774, 2005.

WOOLF, C.J., MANNION, R.J. Neuropathic pain: aetiology, symptoms, mechanisms, and management. **Lancet** 353, 1959-1964, 1999.

WOOLF, C.J., SALTER, M.W. Neuronal plasticity: increasing the gain in pain. **Science** 288, 1765-1769, 2000.

WÖRZ, R. Pain in depression-depression in pain. **Pain clinical updates** 11, 1-4, 2003.

YALCIN, I., TESSIER, L.H., PETIT-DEMOULIÈRE, N., DORIDOT, S., HEIN, L., FREUND-MERCIER, M.J., BARROT, M. Beta2-adrenoceptors are essential for desipramine, venlafaxine or reboxetine action in neuropathic pain. **Neurobiol. Dis**. 33, 386-394, 2009.

YOSHIZUMI, M., KOGAME, T., SUZAKI, Y., FUJITA, Y., KYAW, M., KIRIMA, K., ISHIZAWA, K., TSUCHIYA, K., KAGAMI, S., TAMAKI, T. Ebselen attenuates oxidative stressinduced apoptosis via the inhibition of the c-Jun N-terminal kinase and activator protein-1 signalling pathway in PC12 cells. **Br. J. Pharmacol.** 136, 1023-1032, 2002.

YOSHIZUMI, M., FUJITA, Y., IZAWA, I., SUZAKI, Y., KYAW, M., ALI N., TSUCHIYA, K., KAGAMI, S., YANO, S., SONE, S., TAMAKI, T. Ebselen inhibits tumor necrosis factor-alpha-induced c-Jun N-terminal kinase activation and adhesion molecule expression in endothelial cells. **Exp. Cell Res.** 292, 1-10, 2004.

YOUDIM, M. B.; EDMONDSON, D.; TRIPTON, K. F. The therapeutic potential of monoamine oxidase inhibitors. **Nat. Rev. Neurosci.** 7,295-309, 2006.

ZASSO, F.B., GONÇALES, C.E.P., JUNG, E.A.C., ARALDI, D., ZENI, G., ROCHA, J.B.T., NOGUEIRA, C.W. On the mechanisms involved in antinociception induced by diphenyl diselenide. **Environm. Toxicol. Pharmacol.** 19, 283-289, 2005.

ZEILHOFER, H.U. Synaptic modulation in pain pathways. **Rev. Physiol. Biochem. Pharmacol.** 154, 73-100, 2005.

ZHU, L., CASTRANOVA, V., HE, P. fMLP-stimulated neutrophils increase endothelial $[Ca^{2+}]_i$ and microvessel permeability in the absence of adhesion: role of reactive oxygen species. **Am. J. Physiol. Heart Circ. Physiol.** 288, 1331-1338, 2005.

ZIMMERMANN, M. Pathobiology of neuropathic pain. **Eur. J. Pharmacol.** 429, 23- 37, 2001.

ZOMKOWSKI, A.D.E., ROSA, A.O., LIN, J., SANTOS, A.R.S., CALIXTO, J.B., RODRIGUES, A.LS. Evidence for serotonin receptor subtypes involvement in agmatine antidepressant like-effect in the mouse forced swimming test. **Brain Res.** 1023, 253-263, 2004.

ZOMKOWSKI, A.D., ENGEL, D., GABILAN, N.H., RODRIGUES, A.L. Involvement of NMDA receptors and l-arginine-nitric oxide guanosine monophosphate pathway in the antidepressant-like effects of escitalopram in the forced swimming test. **Eur. Neuropsychopharmacol.** 20, 793-801, 2010.