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CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA TOXICOLÓGICA

EFEITO DO DISSELENETO DE DIFENILA SOBRE ALTERAÇÕES COMPORTAMENTAIS E BIOQUÍMICAS INDUZIDAS POR ANFETAMINA EM CAMUNDONGOS

DISSERTAÇÃO DE MESTRADO

Fernanda Hernandes Figueira

Santa Maria, RS, Brasil
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Fernanda Hernandes Figueira

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Ciências Biológicas: Bioquímica Toxicológica**.

Orientadora: Profª. Roselei Fachinetto
Co-orientador: Prof. João Batista Teixeira da Rocha

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A Comissão examinadora, abaixo assinada,
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elaborada por
Fernanda Hernandes Figueira

como requisito parcial para a obtenção de grau de
Mestre em Ciências Biológicas: Bioquímica Toxicológica.

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Santa Maria, 19 de setembro de 2012.

Dedico este trabalho aos meus pais Vera Regina Hernandes Figueira e José Otavio Figueira.

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RESUMO

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

EFEITO DO DISSELENETO DE DIFENILA SOBRE ALTERAÇÕES COMPORTAMENTAIS E BIOQUÍMICAS INDUZIDAS POR ANFETAMINA EM CAMUNDONGOS

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Data e Local da Defesa: Santa Maria, 19 de setembro de 2012.

O selênio é um elemento químico capaz importante para o funcionamento celular, envolvido também na modulação do sistema dopaminérgico. Estudos mostram que o disseleneto de difenila, um composto orgânico de selênio, possui atividade antioxidante, melhora o comportamento tipo-depressivo relacionado à inibição da atividade da enzima monoamino oxidase (MAO). No entanto, existem poucos estudos acerca dos efeitos do disseleneto de difenila sobre o sistema dopaminérgico. Desta forma, o objetivo do presente estudo foi avaliar os efeitos do tratamento agudo e sub-crônico do disseleneto de difenila sobre as alterações bioquímicas e comportamentais induzidas por anfetamina em camundongos. No tratamento agudo, os camundongos foram tratados com disseleneto de difenila (5 e 10 mg/kg, s.c.) ou veículo (10% de tween 80, s.c.) 30 minutos antes da administração de anfetamina (1,25 mg/kg, i.p.). Após 25 minutos, foram avaliados a atividade locomotora através do teste de campo aberto, além do tempo de estereotipia e imobilidade, avaliado em caixa espelhada. O tratamento subcrônico foi realizado com sete administrações de disseleneto de difenila ou veículo (5 e 10 mg/kg, s.c.) sendo uma administração por dia. No oitavo dia foi administrada a anfetamina (1,25 mg/kg, i.p.) e realizados os testes comportamentais 25 minutos após. Em ambos os tratamentos os testes *ex-vivo* realizados foram: atividade das isoformas MAO-A e MAO-B, níveis de tióis totais e não-protéico, oxidação da

diclorofluoresceína. O tratamento com anfetamina aumentou o número de cruzamentos e de levantadas no teste do campo aberto e o disseleneto de difenila preveniu somente o número de cruzamentos quando administrado agudamente aos camundongos. Além disso, o tratamento com anfetamina aumentou o tempo de imobilidade e estereotipia em camundongos. O disseleneto de difenila não preveniu estes efeitos. Pelo contrário, na dose de 10 mg/kg, a administração subcrônica de disseleneto de difenila aumentou per se o tempo de imobilidade e de estereotipia. Uma correlação positiva entre o tempo de estereotipia e de imobilidade foi também encontrada tanto para o tratamento agudo como subcrônico com disseleneto de difenila. Também foi detectada uma diminuição na atividade cerebral da MAO-B causada pelo tratamento subcrônico com disseleneto de difenila tanto per se quanto em combinação com a anfetamina. Não foram encontradas alterações em parâmetros de estresse oxidativo. Em conclusão, o tratamento subcrônico com disseleneto de difenila pode promover uma sensibilização comportamental que parece ser, pelo menos em parte, dependente da inibição da MAO-B.

Palavras-chave: disseleneto de difenila, monoamino oxidase, estereotipia, anfetamina.

ABSTRACT

Dissertation of Master's Degree
Graduating Program in Biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria

EFFECT OF DIPHENYL DISELENIDE ON BEHAVIOURAL AND BIOCHEMICAL ALTERATIONS INDUCED BY AMPHETAMINE IN MICE

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Place and date: Santa Maria, September, 19th, 2012.

Selenium is an element that can modulate the dopaminergic neurotransmission. Studies show that diphenyl diselenide, an organic compound of selenium, has antioxidant activity improves depressive-like behavior and reduce the activity of the enzyme monoamine oxidase (MAO). However, there are few studies concerning about possible alterations of diphenyl diselenide in dopaminergic system. Thus, the purpose of the present study was to evaluate the effects of acute and sub-chronic treatment of diphenyl diselenide on amphetamine-induced behavioral and biochemical alterations in mice. In the acute treatment, the mice were treated with diphenyl diselenide (5 and 10 mg/kg, s.c.) or vehicle (10% Tween 80, s.c.) 30 min before administration of amphetamine (1.25 mg/kg, i.p.). After 25 min, locomotor activity was assessed with an open field and, also, the time of stereotypy and immobility was assessed in a glass cage. Sub-chronic treatment was conducted with seven administrations of diphenyl diselenide (5 and 10 mg/kg, s.c.), or its vehicle being one administration per day. On the eighth day, amphetamine (1.25 mg/kg, i.p.) was administered and the behavioral tests were conducted after 25 min. In both treatments *ex vivo* tests were performed: isoform activity MAO-A and MAO-B, and measurement of total protein and non-protein thiol levels, oxidation of diclorofluorescein. Amphetamine increased the number of crossing and rearing in the open field test and diphenyl diselenide prevented only the increase in the number of crossings when acutely administered to mice. Furthermore, amphetamine increased

the time of immobility and stereotypy in mice. Diphenyl diselenide did not prevent these effects. By contrary, at 10 mg/kg, sub-chronic administration of diphenyl diselenide increased per se the time of immobility and stereotypy. It was also found a positive correlation between immobility and stereotypy in acute and sub-chronic treatment with diphenyl diselenide. It was also detected a decrease in brain MAO-B activity caused by sub-chronic treatment with diphenyl diselenide either alone or in combination of amphetamine. Any change was detected in oxidative stress parameters. In conclusion, sub-chronic administration of diphenyl diselenide can promote a behavioral sensitization that seems to be, at least in part, dependent of MAO-B inhibition.

Keywords: diphenyl diselenide, monoamine oxidase, stereotypy, amphetamine.

LISTA DE ABREVIATURAS

AMPc	Monofosfato cíclico de adenosine
COMT	Catecol-o-metiltransferase ácido homovanílico
DA	Dopamina
DOPA	Diidroxifenilalanina
DOPAC	Ácido 3,4-diidroxifenilacético
FAD	Dinucleotídeo flavina-adenina
MAO	Monoamino oxidase
HVA	Ácido homovanílico
MPTP	1-metil-4-fenil-1,2,3,6-tetraidropiridina
PKA	Proteína quinase A
SNC	Sistema nervoso central
TH	Tirosina hidroxilase
TDA	Transportador de dopamina
$(PhSe)_2$	Disseleneto de difenila

SUMÁRIO

1. INTRODUÇÃO

O selênio é um elemento traço que pertence ao grupo 16 (calcogênios) da tabela periódica juntamente com o enxofre, telúrio, oxigênio e polônio. O selênio foi descoberto no ano de 1817 pelo químico sueco Jöns Jacob Berzelius e cerca de um século depois, foi incorporado na tabela periódica. Inicialmente foi reconhecido como veneno para mamíferos (NOGUEIRA & ROCHA, 2011). Entretanto, em 1957, o selênio foi descrito como componente essencial na dieta de mamíferos (SCHWARZ & FOLTZ, 1957). Mais tarde, foi demonstrado que o selênio consistia num importante componente de enzimas antioxidantes, como as isoformas da glutationa peroxidase e tiorredoxina redutase (FLOHE *et al*., 1973; LU & HOLMGREN, 2009).

Tanto compostos orgânicos como inorgânicos de selênio podem apresentar propriedades farmacológicas e toxicológicas (NOGUEIRA & ROCHA, 2011; VINCETI *et al*., 2001). A toxicidade dos compostos de selênio parece ter ligação direta com a interação destes compostos com grupos tiol presentes em biomoléculas. A oxidação dos tióis endógenos por compostos orgânicos e inorgânicos de selênio desempenha um papel crucial na toxicidade destes compostos. Além disso, a oxidação dos grupos tiol pode produzir espécies reativas de oxigênio sendo este um fator a mais na toxicidade dos compostos de selênio (NOGUEIRA & ROCHA, 2011). Por outro lado, a oxidação de grupos tiol por disselenetos dá origem a grupos selenol que podem catalisar a decomposição do peróxido de hidrogênio apresentando, desta forma, propriedades farmacológicas. Este comportamento contrastante é dose-dependente e também depende da via de administração (NOGUEIRA & ROCHA, 2010).

O disseleneto de difenila $(PhSe)_2$ é um composto orgânico de selênio com potencial atividade antioxidante, antiinflamatória, antidepressiva, entre outras (YAMAGUCHI *et al.*, 1998; NOGUEIRA *et al.*, 2003; SAVEGNAGO *et al.*, 2008) que tem sido extensivamente estudado, principalmente por não apresentar alta toxidade (NOGUEIRA & ROCHA, 2011).

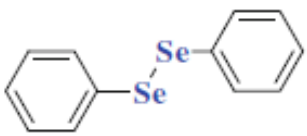

Figura 1 - Estrutura química do $PhSe)_2$ (NOGUEIRA & ROCHA, 2011).

Apesar disso, alguns estudos relatam efeitos neurotóxicos em roedores causados por este composto. Brito *et al*., em 2006 mostrou que o $(PhSe)_2$ potencializa convulsões induzidas por pentilenotetrazol em camundongos. Outros estudos demonstraram que o $(PhSe)_2$ causou inibição das enzimas Na^+, K^+-ATPase e monoamino oxidase (MAO) em cérebro de ratos (BORGES *et al.,* 2005; SAVEGNAGO *et al.,* 2007). Estas alterações na atividade da MAO e da Na^+, K^+-ATPase poderiam levar a alterações em processos fisiológicos normais. No entanto, estes resultados foram pouco explorados.

De particular importância para o nosso estudo, alguns trabalhos demonstraram a eficácia do $(PhSe)_2$ em prevenir danos motores induzidos por reserpina, haloperidol e flufenazina (BURGER *et al.*, 2004; BURGER *et al.*, 2006; FACHINETTO *et al.*, 2007) mesmo sem o envolvimento de alterações em parâmetros oxidativos. Sabe-se que estes fármacos causam danos motores através de alterações nos circuitos dopaminérgicos dos gânglios da base (KORCHOUNOV et al., 2010). No entanto, apesar destes estudos demonstrarem a eficácia do $(PhSe)_2$ nas alterações comportamentais induzidas por antipsicóticos, seu mecanismo ainda não está claro.

Dados da literatura têm demonstrado que o selênio consiste num importante elemento capaz de modular a neurotransmissão dopaminérgica (KHAN, 2010; RASEKH *et al.*, 1997). Porém os mecanismos pelos quais o selênio exerce esse efeito ainda não foram esclarecidos. Rasekh *et al.* (1997) sugere que o selênio pode potencializar a função da dopamina (DA) no sistema nervoso central de ratos. Outro trabalho, realizado por Roesler *et al*. (2006) sugere que o disseleneto de 3'3-ditrifluorometildifenila possui atividade antipsicótica por alterar a resposta comportamental estereotipada induzida por apomorfina. No entanto, existem poucos trabalhos investigando o papel do $(PhSe)_2$ sobre possíveis alterações em parâmetros relacionados ao sistema dopaminérgico.

O sistema dopaminérgico é composto por neurônios que sintetizam DA e fazem conexão com diversas regiões do cérebro. Há quatro principais vias de neurotransmissão dopaminérgica: nigroestriatal, tuberoinfundibular, mesocortical e mesolímbica. As vias dopaminérgicas partem da substância negra e área ventral tegmentar e tem como alvo estriado, córtex cerebral e áreas do sistema límbico (GOODMAN & GILMAN, 2006). Estas vias têm sido relacionadas a patologias como a esquizofrenia e doença de Parkinson, bem como aos distúrbios motores

decorrentes do tratamento com neurolépticos, por exemplo, a discinesia tardia (CRONENWETT & CSERNANSKY, 2010; SCHAPIRA & GEGG, 2011; KORCHOUNOV *et al.*, 2010).

A via nigroestriatal projeta os axônios da substância negra para o corpo do estriado e está envolvida principalmente na produção de movimento voluntário. A diminuição de neurônios dopaminérgicos na substância negra é uma das características da Doença de Parkinson (HATTORI, 1993). A via tuberoinfundibular projeta os axônios do hipotálamo para a glândula hipófise. A redução de dopamina nesta via pode aumentar os níveis de prolactina, causando lactação anormal, ciclo menstrual irregular e disfunção sexual (PORTER *et al.*, 1990). As vias mesocortical e mesolímbica partem da área ventral tegmentar. A via mesocortical tem como alvo o córtex pré-frontal que está associado à resposta emocional, motivacional e cognitiva. Já a via mesolímbica, tem como alvo as regiões do sistema límbico, núcleo accumbens, amígdala, hipocampo e está envolvida com sentimentos de recompensa, depressão e dependência (BANNON & ROTH, 1983).

A DA é sintetizada a partir do aminoácido tirosina que serve de substrato inicial para a síntese de outras catecolaminas como adrenalina (FERNSTROM, 1990). A conversão do aminoácido tirosina à DA depende da ação de duas enzimas: a tirosina hidroxilase (TH) e a L-aminoácido descarboxilase aromática (KOPIN, 1965). A enzima regulatória desta via é a TH, que converte tirosina em diidroxifenilalanina (DOPA). A DOPA, por sua vez, é convertida até DA por ação da enzima DOPA descarboxilase. Estes são passos intermediários para a biossíntese de adrenalina e noradrenalina.

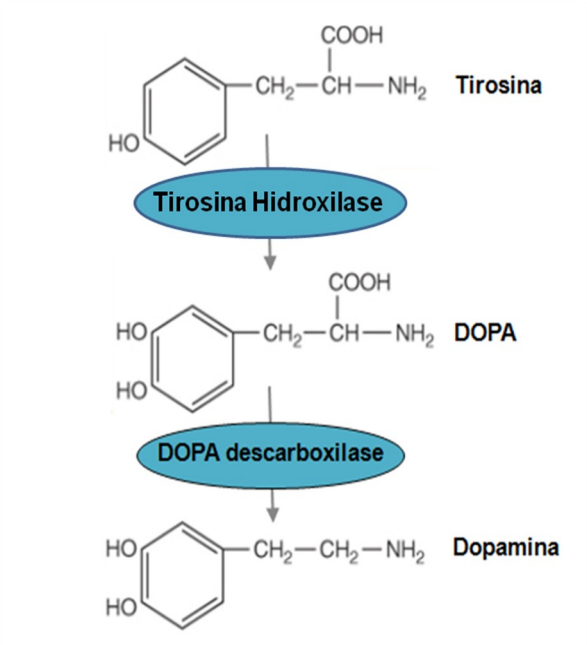

Figura 2 - Síntese de DA.

A liberação de DA na fenda ocorre em resposta ao estímulo nervoso que leva a um aumento de cálcio citosólico e à despolarização do neurônio pré-sináptico. Na fenda sináptica, a DA interage com receptores encontrados em neurônios pré e pós-sinápticos, exercendo assim suas ações celulares (GOODMAN & GILMAN, 2006).

Foram identificados cinco tipos de receptores dopaminérgicos. Esses receptores pertencem a duas famílias D_1 e D_2 ambas acopladas à proteína G. Os receptores da família D_1 estão acoplados a proteína Gs, que ativam a adenilato ciclase e os receptores da família D_2, estão acoplados a proteína Gi, que inativam a adenilato cilcase (SIBLEY *et al.*, 1982). A família D_1 é formada pelos subtipos D_1 e D_5. Os receptores D_1 encontram-se em maiores quantidades no tubérculo olfatório, estriado e núcleo accumbens. Já os receptores D_5 são menos expressos que o subtipo D_1 e encontram-se em maior densidade no hipocampo e áreas restritas do tálamo (MISSALE *et al.*, 1998). A ativação dos receptores da família D_1 acoplados a proteína Gs levam a produção de monofosfato cíclico de adenosina (AMPc) pela estimulação da adenilato ciclase. O AMPc gerado ativa a proteína quinase A (PKA) que fosforila canais de cálcio dependentes de voltagem (ROSENBAUM *et al.*, 2009).

A família de receptores D_2 é formada pelos subtipos D_2, D_3 e D_4. Os receptores D_2 são encontrados em maior densidade nas regiões do estriado,

tubérculo olfatório e núcleo accumbens. Já os receptores D_3 são encontrados de forma mais específica em áreas restritas do sistema límbico e são menos expressos que receptores D_2. Os receptores D_4 localizam-se em maior quantidade no córtex e hipocampo (MISSALE *et al.*, 1998). A ativação desses receptores que estão acoplados a proteína Gi modulam negativamente a adenilato ciclase reduzindo a concentração de AMPc e ativam canais de potássio (ROSENBAUM *et al.*, 2009).

A interação da DA com receptores dos neurônios pré-sinápticos leva a uma redução na atividade da TH e consequentemente à redução na síntese de DA. Outro mecanismo de controle dos níveis de DA é através da retirada da DA extracelular via transportador de dopamina (TDA) (GOODMAN & GILMAN, 2006). Aproximadamente 70% da DA é removida da fenda através da recaptação pelo TDA (BOULTON *et al.*, 1998). O TDA também é responsável pela captação de neurotoxinas como o 1-metil-4-fenil-1,2,3,6-tetraidropiridina (MPTP) e o 6-hidroxidopamina. Algumas drogas possuem a capacidade de inibir ou modular a função do transportador, como a anfetamina e a cocaína (REITH *et al.*, 1997).

A DA recaptada é enzimaticamente desaminada pela MAO. A MAO está localizada na membrana externa da mitocôndria e tem um dinucleotídeo flavina-adenina (FAD) como coenzima. Há duas isoformas da MAO, a MAO-A e a MAO-B que tem seletividade para diferentes substratos e inibidores (YOUDIM & BAKHLE, 2006).

A MAO-A em humanos catalisa, preferencialmente, a desaminação de serotonina e noradrenalina (YOUDIM & BAKHLE, 2006). Um estudo mostra que em camundongos a DA é desaminada pela MAO-A. Porém, quando em altas concentrações, a MAO-B e a MAO-A catalisam esta reação. Em ratos, a DA é sempre metabolizada pela MAO-A (FORNAI *et al.*, 1999). Alguns inibidores de MAO-A são utilizados no tratamento de depressão. Entretanto, estes inibidores não apresentam especificidade por tecidos, inibindo a enzima não só no SNC, mas também no intestino e fígado, causando efeitos colaterais indesejáveis (YOUDIM & BAKHLE, 2006).

A desaminação enzimática da DA em humanos ocorre preferencialmente pela ação da MAO-B que em roedores desamina preferencialmente serotonina e noradrenalina. Os inibidores seletivos desta isoforma são utilizados no tratamento da Doença de Parkinson por aumentar os níveis de DA já que durante o

envelhecimento ocorre um aumento na expressão de MAO-B (YOUDIM & BAKHLE, 2006).

A reação catalisada pela MAO é a desaminação oxidativa de monoaminas para formar 3,4-diidroxifenilacetaldeído. Esta reação reduz o FAD que ao ser reoxidado gera peróxido de hidrogênio (YOUDIM & BAKHLE, 2006). O peróxido de hidrogênio normalmente é neutralizado pela ação da enzima catalase. Porém, o aumento da atividade da MAO aumenta a geração de peróxido de hidrogênio que podem reagir com Fe^{2+} formando o radical hidroxila. Estes radicais são capazes de reagir com lipídeos de membrana levando a peroxidação lipídica, causando dano e morte celular (YOUDIM & BAKHLE, 2006). O 3,4-diidroxifenilacetaldeído é então oxidado pela enzima aldeído desidrogenase para produzir o ácido 3,4-diidroxifenilacético (DOPAC). Este composto é subsequentemente metilado pela enzima catecol-o-metiltransferase (COMT) para formar o ácido homovanílico (HVA).

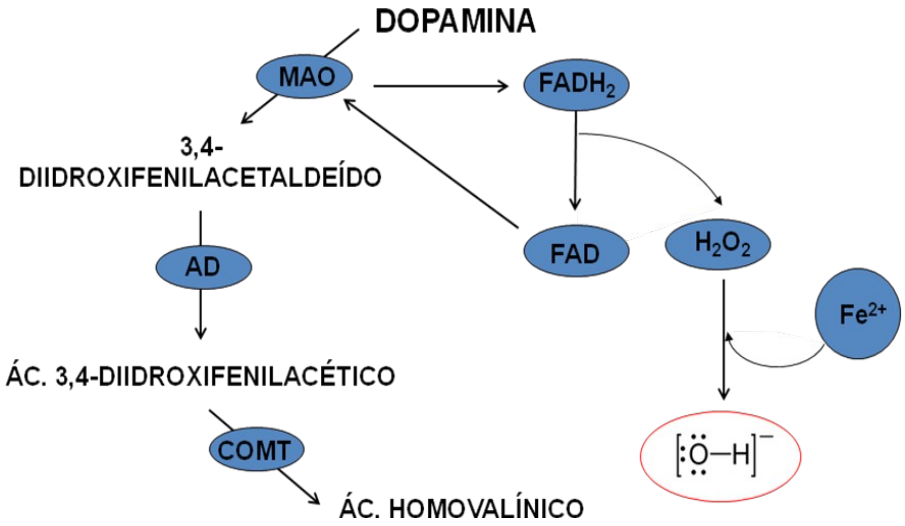

Figura 3 - Esquema da degradação da DA pela ação da MAO.

O aumento da atividade da MAO leva a um aumento na geração de espécies reativas de oxigênio. Porém, alguns neurotransmissores como a serotonina, noradrenalina e DA podem sofrer auto-oxidação espontânea quando em excesso no citosol. Este processo é uma importante fonte de espécies reativas e quinonas que

alteram o estado redox da célula e podem atacar macromoléculas importantes para a sobrevivência celular (SPENCER *et al.*, 1998).

Diversas patologias estão associadas a alterações na neurotransmissão dopaminérgica. Entre essas patologias destacam-se a Doença de Parkinson e um dos principais transtornos psicóticos, a esquizofrenia (SPENCER *et al*., 1998; DAVIS *et al.*, 1991). A esquizofrenia é um transtorno mental grave, caracterizada por diferentes sintomas classificados como positivos e negativos. O aparecimento de alucinações e delírios semelhantes aos sintomas positivos da esquizofrenia induzidos pelo uso de fármacos psicoestimulantes como a cocaína e anfetamina, que aumentam a transmissão dopaminérgica, sugere que a esquizofrenia se relaciona com alterações específicas na neurotransmissão dopaminérgica. Deste modo tem sido sugerido que os sintomas positivos estão associados ao aumento da atividade dopaminérgica na via mesolímbica e os sintomas negativos da esquizofrenia estão relacionados à redução da atividade dopaminérgica na via mesocortical (DAVIS *et al.*, 1991).

Estudos de imagem mostram um aumento da captação de DOPA no cérebro de pacientes com esquizofrenia quando comparados com pacientes controles. Em consequência, a síntese de DA e sua liberação também foi aumentada (MIYAKE *et al.*, 2011). Além disso, estudos demonstram que pacientes com esquizofrenia apresentam uma liberação de dopamina exagerada em resposta a anfetamina (LARUELLE *et al.*, 1996; BREIER *et al.*, 1997). Estes dados sugerem um desequilíbrio funcional dos neurônios dopaminérgicos em resposta à anfetamina na esquizofrenia.

A anfetamina é uma droga psicoestimulante classificada como agonista de ação indireta de DA (O'NEILL & SHAW, 1999). O efeito agudo da anfetamina é causar o transporte reverso de DA via TDA (HYMAN, 1996). A anfetamina produz liberação de DA no citoplasma do neurônio pré-sináptico e essa DA é transportada para o exterior do neurônio, aumentando a concentração de neurotransmissor disponível para interação com receptores. Outro efeito agudo da anfetamina é estimular as vesículas sinápticas contendo DA a se fundirem à membrana terminal do neurônio, liberando mais neurotransmissor. Desta forma, o uso desta droga leva a uma variedade de respostas comportamentais como euforia, ansiedade, excitação e outras, que são características de um estado hiperdopaminérgico (CRUZ *et al.*, 2011). Dados da literatura demonstram que o uso contínuo de anfetamina provoca

sensibilização comportamental, isto é, um aumento gradativo da locomoção sem aumento de dose de anfetamina. Este efeito é reflexo da hipersensibilidade dos neurônios dopaminérgicos. A administração crônica de anfetamina aumenta a expressão de TDA e transportadores vesiculares de monoaminas (LU & WOLF, 1997).

Um modelo animal experimental bastante utilizado em testes pré-clínicos de drogas com possível ação no sistema dopaminérgico é a administração de anfetamina por aumentar a liberação de DA (PONTIERI *et al.*, 1995). Esse excesso de dopamina causado pela anfetamina induz uma psicose que se assemelha aos sintomas positivos da esquizofrenia. Em função disso, a anfetamina é utilizada como modelo de estimulação dopaminérgica. No entanto apesar de alguns estudos sugerirem que o selênio possui a capacidade de alterar a função dopaminérgica, poucos trabalhos têm investigado a ação de compostos de selênio em modelos experimentais de hiperatividade dopaminérgica.

Neste contexto, apesar do $(PhSe)_2$ ser amplamente estudado, poucos trabalhos investigam os efeitos deste composto sobre suas possíveis ações no sistema dopaminérgico (BURGER *et al.*, 2006; FACHINETTO et al., 2007). Uma vez que o sistema dopaminérgico tem envolvimento com diversos estados patológicos e o $(PhSe)_2$ mostrou-se efetivo em modelos de distúrbios motores relacionados ao sistema dopaminérgico, torna-se interessante estudar melhor os efeitos deste composto em modelos experimentais de estimulação dopaminérgica. Desta forma, a anfetamina consiste numa ferramenta de estimulação dopaminérgica importante para a investigação dos mecanismos de ação do $(PhSe)_2$.

2. OBJETIVOS

2.1. Objetivo Geral

O objetivo deste estudo foi avaliar o efeito do $(PhSe)_2$ sobre alterações comportamentais e bioquímicas induzidas por anfetamina em camundongos.

2.2. Objetivos Específicos

2.2.1. Avaliar o efeito do tratamento agudo e subcrônico com o $(PhSe)_2$ sobre alterações na atividade locomotora e exploratória induzidas por anfetamina em camundongos;

2.2.2. Investigar o efeito do tratamento agudo e subcrônico com o $(PhSe)_2$ sobre alterações no tempo de estereotipia e de imobilidade induzidas por anfetamina em camundongos;

2.2.3. Verificar o efeito do tratamento agudo e subcrônico com o $(PhSe)_2$, bem como o efeito da administração aguda de anfetamina, sobre a atividade da MAO-A e MAO-B em camundongos;

2.3.4. Quantificar possíveis alterações sobre marcadores de estresse oxidativo induzidas pelos tratamentos com anfetamina e/ou $(PhSe)_2$ administrado aguda e subcronicamente.

3. MANUSCRITO

Manuscrito em preparação

EFFECTS OF DIPHENYL DISELENIDE ON BEHAVIOURAL AND BIOCHEMICAL CHANGES INDUCED BY AMPHETAMINE IN MICE

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Abstract

Selenium organic compounds have been studied by its antioxidant and pharmacological properties. In this context, diphenyl diselenide has been studied as a potential pharmacological agent in different *in vitro* and *in vivo* models. However, there are few studies concerning about possible alterations of diphenyl diselenide in dopaminergic system. Thus, the purpose of the present study was to evaluate the effects of acute and sub-chronic treatment of diphenyl diselenide on amphetamine-induced behavioral and biochemical alterations. In acute protocol, mice were pre-treated with 5 or 10 mg/kg of diphenyl diselenide and 30 min after, amphetamine was administered. In sub-chronic protocol, mice were pre-treated with 5 or 10 mg/kg of diphenyl diselenide during 7 days and 24 h after, amphetamine was administered. Twenty five minutes after amphetamine administration, it was analyzed behavioral (locomotor activity, time of stereotypy and immobility) and biochemical (monoaminoxidase activity, delta of diclorofluorescein oxidation, protein and non-protein thiol groups) parameters. Amphetamine increased the number of crossing and rearing in the open field test and diphenyl diselenide prevented only the increase in the number of crossings when acutely administered to mice. Furthermore, amphetamine increased the time of immobility and stereotypy in mice. Diphenyl diselenide did not prevent these effects. By contrary, at 10 mg/kg, sub-chronic administration of diphenyl diselenide increased per se the time of immobility and stereotypy. It was also found a positive correlation between immobility and stereotypy in acute and sub-chronic treatment with diphenyl diselenide. It was also detected a decrease in brain MAO-B activity caused by sub-chronic treatment with diphenyl diselenide either alone or in combination with amphetamine. Any change was detected in oxidative stress parameters. In conclusion, sub-chronic administration of diphenyl diselenide can promote a behavioral sensitization that seems to be, at least in part, dependent of MAO-B inhibition.

Key-words: diphenyl diselenide, stereotypy, MAO-activity, amphetamine

3.1. Introduction

Selenium is a trace element described as an essential component in the diet of mammals (Schwarz and Foltz, 1957) and component of antioxidant enzymes, such as isoforms of thioredoxin reductase and glutathione peroxidase (Flohe et al, 1973; Engman et al, 1997). The selenoproteins are the maintenance of disulfide oxidizing thiol groups interact with metal ions and catalyze redox reactions (Arner, 2010). Furthermore, studies show that selenium is an important element that can modulate dopaminergic neurotransmission, related to its ability to potentiate dopamine (DA) function (Rasekh et al, 1997).

Dopamine (DA) is a neurotransmitter widely distributed throughout the central nervous system (CNS). More than half the content of catecholamines in the CNS is composed of DA. Among the basal ganglia, DA is found in large quantities particularly in the striatum (Palkovits and Brownstein, 1989). In the cytoplasm, monoamines are enzymatically deaminated by monoamine oxidase (MAO) to form 3,4-diidroxifenilacetaldeído (Cooper et al, 2003). MAO has two isoforms, MAO-A and MAO-B which have selectivity for different substrates and inhibitors. In human MAO B preferentially deamin DA, whereas in rodents, it is preferentially deaminated by MAO A (Ya-Li Tang et al, 2008). Alterations in the function of this enzyme are involved in psychiatric disorders such as depression (Youdim & Bakhle, 2006).

Alterations in dopaminergic neurotransmission are related to various diseases, such as Parkinson's disease and schizophrenia. Given the physiological importance of selenium, a series of compounds containing selenium such as diphenyl diselenide $(PhSe)_2$, has been studied. Nevertheless, few studies report the effects of these compounds on the dopaminergic system.

The $(PhSe)_2$ is an organic compound of selenium with potential antioxidant activity, anti-inflammatory, antidepressant (Yamaguchi et al, 1998; Nogueira et al, 2003; Savegnago et al, 2008) that has been extensively studied, mainly because they have low toxicity (Nogueira and Rocha, 2011). However, the diselenide compounds are capable of oxidizing the thiol groups which play an important role for the activity of enzymes and other proteins with biological functions such as transporters, receptors and ion channels (Nogueira and Rocha, 2011).

Furthermore, some studies have demonstrated that $(PhSe)_2$ caused a reduction in activity of MAO in rats (Savegnago et al., 2007). However, another studies show that $(PhSe)_2$ did not alter the activity of MAO in rats and mice (Rocha et al., 2012, Acker et al., 2009). $(PhSe)_2$ has demonstrated effectiveness to prevent motor damage caused by drugs that induce dopaminergic toxicity (Burger et al, 2004, Burger et al, 2006; Fachinetto et al, 2007). Of particular importance, Machado et al (2006) demonstrated that an organoselenium compound has antipsychotic activity by altering stereotyped behavior induced by apomorphine. However, $PhSe)_2$ has not been extensively investigated concerning dopaminergic mechanisms.

The administration of amphetamine is widely used as a model of dopaminergic stimulation by increasing dopamine release. Dopamine excess caused by amphetamine induces psychotic symptoms similar to schizophrenia. So, amphetamine is used in pre-clinical testing of drugs that can act on the dopaminergic system.

Considering the lack of investigation about the effects of $(PhSe)_2$ on the dopaminergic system, this study aimed to investigate the effects of acute and sub-chronic treatment of $(PhSe)_2$ on amphetamine-induced behavioral and biochemical alterations in mice.

3.2. Materials and methods

3.2.1. Animals

Albino Swiss mice weighing 25–30 g from our own breeding colony were kept in cages of 4–5 animals each, with continuous access to food and water in a room with controlled temperature (22±2ºC) and on a 12-h light/dark cycle with lights on at 7:00 a.m. Animals were maintained and used in accordance to the guidelines of the Brazilian Society of Association for Laboratory Animal Science. The experimental procedure was approved by Ethical Commission of Animal Use of Federal University of Santa Maria, Brazil under protocol number 128/2011.

3.2.2. Drugs and reagents

DL-amphetamine and diphenyl diselenide were obtained from Sigma-Aldrich. All chemical were of analytical grade.

3.2.3. Experimental procedure

This experiment was divided into two protocols: acute or sub-chronic treatment. The doses and times of administrations were performed in accordance with pilot test.

3.2.3.1. *Experiment 1 – acute pre-treatment with $(PhSe)_2$*

The mice were randomly divided into following groups (four to five animals per group): (i) control group; (ii) amphetamine group; (iii) $(PhSe)_2$-5 group; (iv) $(PhSe)_2$-5 + amphetamine group; (v) $(PhSe)_2$-10 group; and (vi) $(PhSe)_2$-10 + amphetamine group. $(PhSe)_2$ was dissolved in 10% tween 80 and administered at doses of 5 mg/kg or 10 mg/kg subcutaneously. DL-Amphetamine was dissolved in saline (0.9%) and administered at a dose of 1.25 mg/kg intraperitoneally thirty minutes after vehicle or $(PhSe)_2$. Control rats were similarly treated with the respective vehicles. All the drugs and vehicles were injected in a maximum volume of 5 mL/kg body weight. Twenty five minutes after administration of amphetamine or its vehicle, locomotor and stereotyped behavior were evaluated.

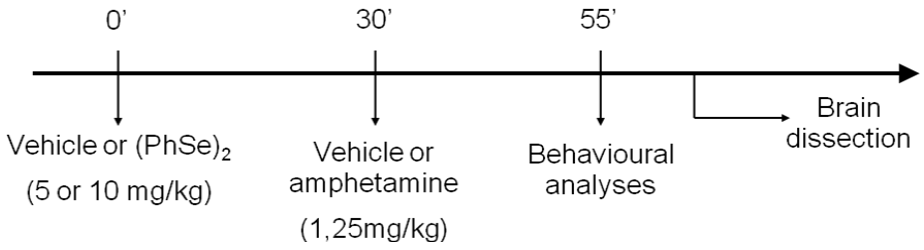

Figure 1 - Experimental procedures acute treatment.

3.2.3.2. *Experiment 2 – sub-chronic pre-treatment with $(PhSe)_2$*

As in acute treatment, the mice were randomly divided into following groups (four to five animals per group): (i) control group; (ii) amphetamine group; (iii) $(PhSe)_2$-5 group; (iv) $(PhSe)_2$-5 + amphetamine group; (v) $(PhSe)_2$-10 group; and (vi) $(PhSe)_2$-10 + amphetamine group. $(PhSe)_2$ was dissolved in 10% tween 80 and administered at doses of 5 mg/kg or 10 mg/kg subcutaneously once a day during 7

days. DL-Amphetamine was dissolved in saline (0.9%) and administered at a dose of 1.25 mg/kg intraperitoneally on day 8. Control rats were similarly treated with the respective vehicles. All the drugs and vehicles were injected in a maximum volume of 5 mL/kg body weight. Twenty five minutes after administration of amphetamine or its vehicle, locomotor and stereotyped behavior were evaluated.

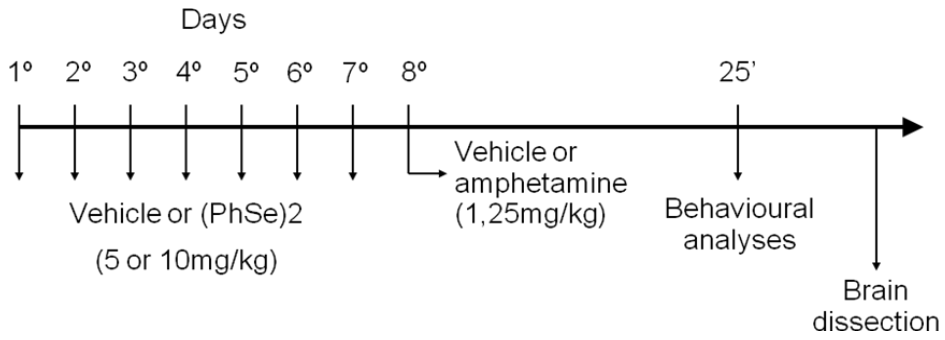

Figure 2 - Experimental procedures subchronic treatment.

3.2.4. Behavioural analyses

3.2.4.1. Open field test

To analyze possible changes in spontaneous locomotor and exploratory activity caused by amphetamine and/or $PhSe)_2$, open field test was carried out as previously described (Busanello et al., 2011; Broadhurst, 1960). Briefly, mice were placed individually in the centre of the circular arena containing 60 cm of diameter with the floor divided into 13 squares. The number of rearing and the number of line crossings were measured over 5 min by a blind experimenter.

3.2.4.2. Stereotypy

To evaluate changes in stereotyped behaviour, immediately after the open field test, mice were placed individually in glass cages (20 x 20 x 19 cm). Animals were observed during 5 min and it was counted the total time of stereotypy by a blind experimenter. The following parameters were taken to account stereotypy score: sniffing, grooming, nail biting and circling as previously described by Machado et al., 2006.

3.2.4.3. Immobility

To evaluate changes in immobility, while mice were individually in glass cages (20 x 20 x 19 cm), the total time of immobility was evaluated during 5 min.

3.2.5. Biochemical analyses

3.2.5.1. Tissue preparation

Immediately after behavioural session, mice were killed by cervical dislocation. Brain was excised and homogenized in 10 vol (w/v) of 10 mM Tris-HCl buffer, pH 7.4. Homogenates were centrifuged at 3000 rpm for 10 min and the supernatants were used for biochemical analysis.

3.2.5.2. MAO activity

Monoaminoxidase (MAO) activity was determined by measuring the kynuramine oxidation to 4-hydroxiquinoline (Sant'Anna et al., 2008) with modifications. Brain homogenate was pre-incubated during 10 min at 37ºC with MAO-A (selegiline, 250 nM) or MAO-B (chlorgiline, 250 nM) inhibitors. After this, kynuramine was added as MAO substrate in sub maximal concentrations (90 µM to MAO-A and 60 µM to MAO-B). The reaction was incubated during 30 min at 37ºC. After this time, the reaction was stopped with trichloroacetic acid (TCA) 10%. The samples were centrifuged at 3.000 rpm for 5 min and the supernatant was used to estimate the MAO activity. The product of reaction was measured spectrofluorimetrically at 315 nm for excitation and 380 nm for emission. The results are represented as percentage of control.

3.2.5.3. Thiol groups determination

Protein (P-SH) and non-protein thiol (NP-SH) groups were measured based on Ellman (1959) with minor modifications. For total thiol quantification, a supernatant aliquot was incubated with DTNB in a medium containing 1M potassium phosphate buffer, pH 7.4. For non-protein thiol quantification, supernatant was precipitated with 10% trichloroacetic acid (1:1 v/v) and centrifuged at 3000 rpm during 5 min. Then, a supernatant aliquot was incubated with DTNB in a medium containing 1M potassium phosphate buffer, pH 7.4. Data are expressed as µM of GSH/mg of tissue.

3.2.5.4. ROS levels

To evaluate the levels of reactive oxygen species (ROS), just after the centrifugation, an aliquot of supernatant was used for 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) oxidation. DCFH-DA oxidation was determined spectrofluorimetrically using the membrane-permeable fluorescent dye DCFH-DA (7 µM). Fluorescence was determined at 488 nm for excitation and 520 nm for

emission. A standard curve was carried out using increasing concentrations of 2′,7′-dichlorofluorescein (DCF) incubated in parallel (Pérez-Severiano et al. 2004). Results are shown as delta of DCFH-DA oxidation between 15 and 30 min of incubation.

3.2.6. Statistical analyses

Data were analyzed by test t or one-way ANOVA followed by Duncan's post-hoc tests when appropriate. Significance was considered when $p<0.05$.

3.3. Results

3.3.1. *Effects of amphetamine in mice treated acutely with $(PhSe)_2$ on locomotor and exploratory activity*

Amphetamine administration caused a significant increase on locomotor activity ($p<0.05$), represented by the number of crossings in the open field test (Figure 3A). Pre-treatment with $(PhSe)_2$ at a doses of 5 and 10 mg/kg prevented the amphetamine-induced hyperlocomotion in mice (Figure 3A). Moreover, crossing number was not modified in the groups treated with $(PhSe)_2$ alone. Similarly, amphetamine caused a marked increase in the exploratory activity ($p<0.05$, Figure 3B), represented by the number of rearing in the open field test. However, there were no significant protective effect of $(PhSe)_2$ on number of rearing at tested doses. As in number of crossing, $(PhSe)_2$ alone did not cause alterations in the number of rearing at tested doses (Figure 3B).

3.3.2. *Effects of amphetamine in mice treated sub-chronically with $(PhSe)_2$ on locomotor and exploratory activity*

Amphetamine administration caused a significant increase on locomotor activity ($p<0.05$), represented by the number of crossings in the open field test (Figure 3C). Pre-treatment with $(PhSe)_2$ during 7 days at a doses of 5 and 10 mg/kg did not prevent the amphetamine-induced hyperlocomotion in mice (Figure 3C). Moreover, crossing number was not modified in the groups treated with $(PhSe)_2$ alone. Similarly, amphetamine caused a marked increase in the exploratory activity ($p<0.05$, Figure 3D), represented by the number of rearing in the open field test. However, there were no significant protective effect of $(PhSe)_2$ on number of rearing at tested doses. As in number of crossing, $(PhSe)_2$ alone did not cause alterations in the number of rearing at tested doses (Figure 3D).

3.3.3. *Effects of amphetamine in mice treated acute or sub-chronically with $(PhSe)_2$ on stereotypy*

The administration of amphetamine increased the time of stereotypy in mice ($p<0.05$; Figure 4A and 4B). Pre-treatment with 10mg/kg of $(PhSe)_2$ acutely potentiated the effects of amphetamine on time of stereotypy in mice ($p<0.05$) (Figure 4A). $(PhSe)_2$ alone did not alter the time of stereotypy at doses tested.

Sub-chronic pre-treatment with both doses of $(PhSe)_2$ did not modify the time of stereotypy induced by amphetamine in mice. However, sub-chronic treatment with $(PhSe)_2$ alone at a dose of 10 mg/kg produced an increase in the time of stereotypy in mice ($p<0.05$; Figure 4B) when compared to its control group.

3.3.4. *Effects of amphetamine in mice treated acute or sub-chronically with $(PhSe)_2$ on time of immobility*

The administration of amphetamine increased the time of immobility in mice ($p<0.05$; Figure 5A and 5B). Pre-treatment with $(PhSe)_2$ acutely did not modify the effects of amphetamine on time of immobility in mice ($p<0.05$) (Figure 5A). $(PhSe)_2$ alone did not alter the time of immobility at doses tested.

As in stereotypy, sub-chronic pre-treatment with both doses of $(PhSe)_2$ did not modify the time of stereotypy induced by amphetamine in mice. However, sub-chronic treatment with $(PhSe)_2$ alone at a dose of 10 mg/kg produced an increase in the time of stereotypy in mice ($p<0.05$; Figure 5B) when compared to its control group.

It was also found a positive correlation between time of immobility and time of stereotypy in acute ($r=0.74$ and $p<0.05$; Figure 6A) and sub-chronic ($r=0.91$ and $p<0.05$; Figure 6B) treatment with $(PhSe)_2$.

3.3.5. *MAO activity in brain of mice pre-treated acute or sub-chronically with $(PhSe)_2$ and exposed to amphetamine*

In mice acutely or sub-chronically treated with $(PhSe)_2$ and exposed to amphetamine, any change was observed in brain MAO-A activity (Figure 7A and 7C). In the same way, in mice acutely treated with $(PhSe)_2$ and exposed to amphetamine, any change was observed in brain MAO-B activity (Figure 7C). However, sub-chronic treatment with $(PhSe)_2$ alone decreased MAO-B activity of brain at both tested doses

($p<0.05$; Figure 7D). However, this effect occurred only at a dose of 10 mg/kg of $(PhSe)_2$ when the animals were pre-treated with $(PhSe)_2$ during 7 days and exposed to amphetamine ($p<0.05$; Figure 7D).

3.3.6. *Oxidative stress parameters in brain of mice pre-treated acute or sub-chronically with $(PhSe)_2$ and exposed to amphetamine*

There was not a significant difference among the groups in DCFH-DA oxidation delta, P-SH or NP-SH levels in brain of mice pre-treated acute or sub-chronically with $(PhSe)_2$ and exposed to amphetamine .

3.4. Discussion

Our current results show that sub-chronic treatment with $(PhSe)_2$ increases the time of stereotypy (Fig. 4) and immobility (Fig. 5) in mice. Furthermore, $(PhSe)_2$ sub-chronically administered reduces MAO-B in brain of mice (Fig. 7). MAO-B inhibition was not associated to thiol oxidation (Tab. 2).

It has been demonstrated that the level of Se in the brain is an important factor in the etiology of several neurodegenerative diseases (Chang 1983; Zafar et al. 2003). In addition, studies from our group have previously shown that the organoselenium compounds demonstrate protective effects on neurotoxicity models (for review, see Nogueira et al. 2004). $(PhSe)_2$ has been extensively studied concerning its pharmacological properties. Besides many studies demonstrating the actions of $(PhSe)_2$ on central nervous system little is known about its effects on models of dopaminergic alterations. Thus, we investigated in the present study the effects of acute and sub-chronic treatment of $(PhSe)_2$ on amphetamine-induced behavioral and biochemical alterations in mice.

It is well known that the amphetamine is a drug capable of inducing stereotypy and hyper-locomotion (Wolgin, 2011) by increasing the release of DA (Pontieri et al, 1995). For this purpose, amphetamine is used as a tool to test drugs with possible effect in dopaminergic system. In our study, amphetamine, as previously described in the literature, caused an increase in locomotion (Fig. 3), time of stereotyped behavior (Fig. 4) and immobility time (Fig. 5). Acute administration of $(PhSe)_2$ prevented the hyper-locomotion (Fig. 3A) and potentiated the stereotypy induced by amphetamine at highest dose tested (Fig. 4A), suggesting an interaction at same receptors than

amphetamine. Otherwise, sub-chronic treatment with $(PhSe)_2$ per se increased the time of stereotyped behavior (Fig. 4B) and immobility time (Fig. 5B) suggesting a behavioral sensitization promoted by $(PhSe)_2$.

These effects suggest that the $(PhSe)_2$ could act in different ways: interacting with the same pathway of amphetamine to induce behavioral alterations; increases the dopaminergic neurotransmission in mesolimbic pathway, increasing the stereotypy and reducing the neurotransmission in mesocortical pathway, which could increase the time of immobility. This hypothesis could not be tested. However, the positive correlation between the stereotyped behavior and immobility time shows that it is unlikely that these events occur dependent each other (Fig. 6).

Some studies have attributed to $(PhSe)_2$ an antidepressant-like effect (Savegnago et al, 2008; Acker et al, 2009) and antimanic-like (Bruning et al, 2012) due to its capacity in to involve alterations in MAO activity. Thus, we also investigated if the MAO-activity is involved in behavioral responses promoted by $(PhSe)_2$. Sub-chronic treatment with $(PhSe)_2$ reduced the activity of MAO-B in brain of mice (Fig. 7D). The reduction of MAO-B may explain, at least in part, why the behavioral alterations of $(PhSe)_2$ in subchronic treatment were more significant.

The effects of $(PhSe)_2$ on the activity of MAO are contradictory (Ya-Li Tang et al, 2008; Savegnago et al., 2007, Rocha et al., 2012, Acker et al., 2009). In MAO-A, any effect was observed in the present study (Fig. 7A and 7C). This effect can reflect a preference of $(PhSe)_2$ to inhibit an isoform of MAO. Considering that $(PhSe)_2$ alone caused an increase in stereotypy only in sub-chronic treatment, this result could suggest modifications in specific pathways by $(PhSe)_2$.

It is known that diselenide compounds are capable of oxidizing the thiol groups which play an important role for the activity of enzymes and other proteins with biological functions such as transporters, receptors and ion channels (Nogueira and Rocha, 2011). Considering that MAO is an enzyme that could be inhibited by thiol oxidation, we investigated if the tested doses of $(PhSe)_2$ are able to oxidize thiol groups or alter species oxygen reactive production. However, we have not detected alterations in these parameters (Table 1 and 2). Thus, we can speculate that $(PhSe)_2$ is inhibiting MAO by a mechanism independente of thiol oxidation.

Taken together, our results indicate that in acute administration $(PhSe)_2$ can caused behavioral alteration manly by interaction at same amphetamine receptor, but in sub-chronic administration $(PhSe)_2$ could be interacting with neurotransmitter

systems related to behavioral sensitization that seems to be, at least in part, dependent of MAO-*B* inhibition.

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3.6. Figures and tables

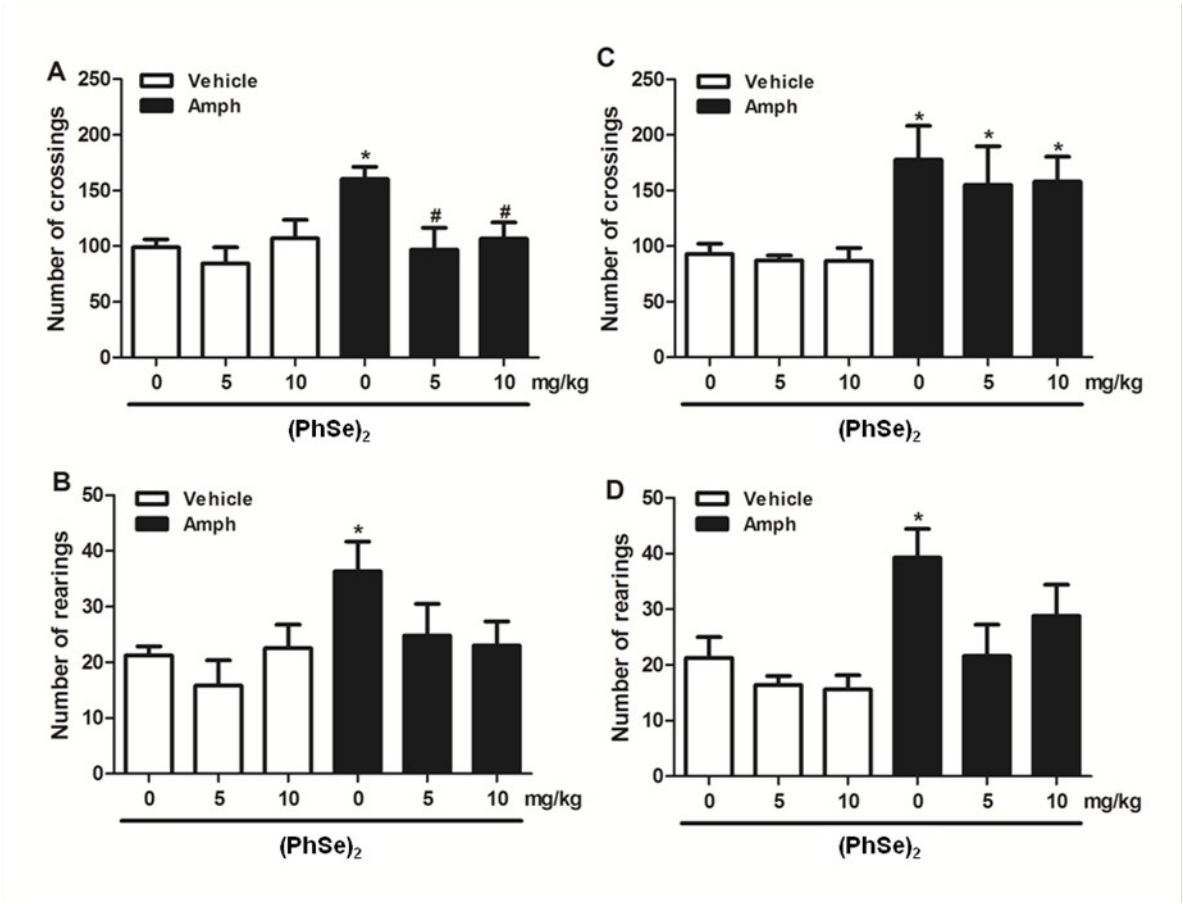

Figure 3 - A and B) Effects of acute treatment $(PhSe)_2$ (5 or 10 mg/kg, s.c., 55 min before the test) and/or amphetamine (1.25 mg/kg, i.p., 25 min before the test) treatment in the open field test in mice. C and D) Effects of subchronic treatment $(PhSe)_2$ (5 or 10 mg/kg, s.c., administered during 7 days, behavioral evaluation 24 h before the last administration) and/or amphetamine (1.25 mg/kg, i.p., 25 min before the test) treatment in the open field test in mice. A and C) Number of crossings and B and D) number of rearings in 5 min. Values are means±S.E.M. of 4-5 animals per group. One-way ANOVA followed by Duncan's post-hoc tests. (*) $p<0.05$ compared with control group; ($^{\#}$) $p<0.05$ compared with amph group.

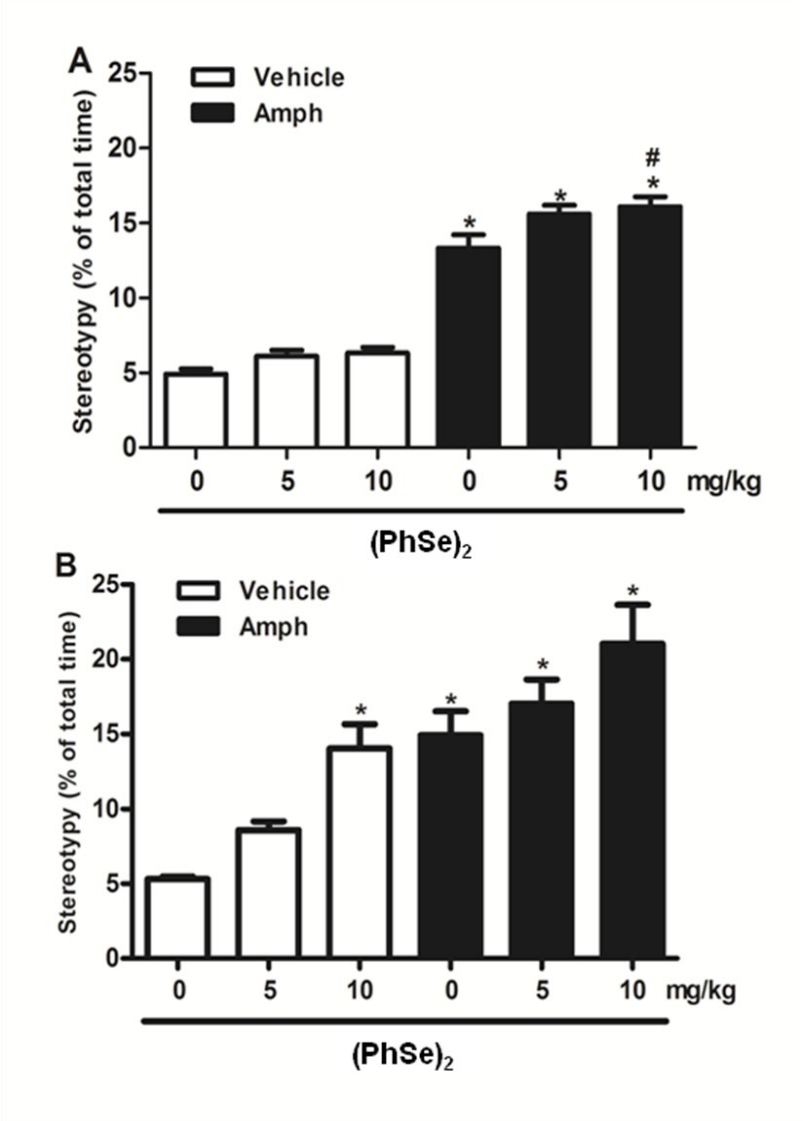

Figure 4 - A) Effects of acute treatment $(PhSe)_2$ (5 or 10 mg/kg, s.c., 55 min before the test) and/or amphetamine (1.25 mg/kg, i.p., 25 min before the test) treatment on stereotypy in mice. B) Effects of subchronic treatment $(PhSe)_2$ (5 or 10 mg/kg, s.c., administered during 7 days, behavioral evaluation 24 h before the last administration) and/or amphetamine (1.25 mg/kg, i.p., 25 min before the test) treatment on stereotypy in mice observed in a glass cages. Stereotypy is represented by the percentage of time the animal presents sniffing, grooming, nail biting and circling during 5 min. Values are means±S.E.M. of 4-5 animals per group. One-way ANOVA followed by Duncan's post-hoc tests. (*) $p<0.05$ compared with control group; ($^{\#}$) $p<0.05$ compared with amphetamine group.

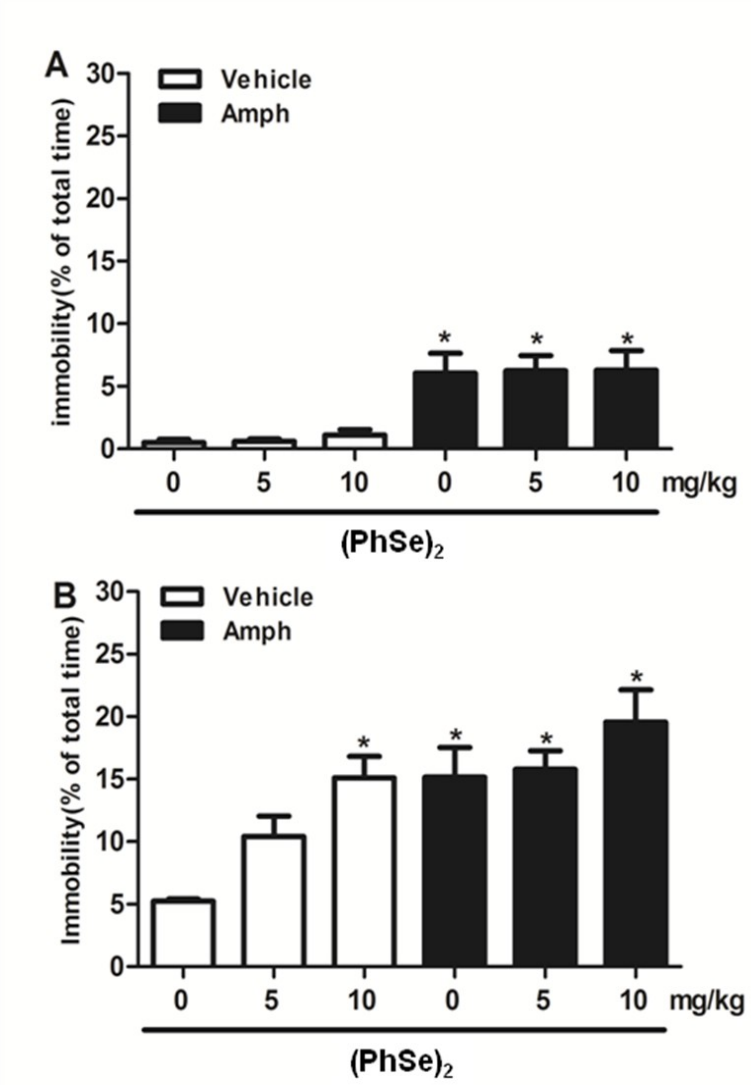

Figure 5 - A) Effects of acute treatment $(PhSe)_2$ (5 or 10 mg/kg, s.c., 55 min before the test) and/or amphetamine (1.25 mg/kg, i.p., 25 min before the test) treatment on immobility in mice. B) Effects of subchronic treatment $(PhSe)_2$ (5 or 10 mg/kg, s.c., administered during 7 days, behavioral evaluation 24 h before the last administration) and/or amphetamine (1.25 mg/kg, i.p., 25 min before the test) treatment on immobility in mice observed in a glass cages during 5 min. Values are means±S.E.M. of 4-5 animals per group. One-way ANOVA followed by Duncan's post-hoc tests. (*) $p<0.05$ compared with control group.

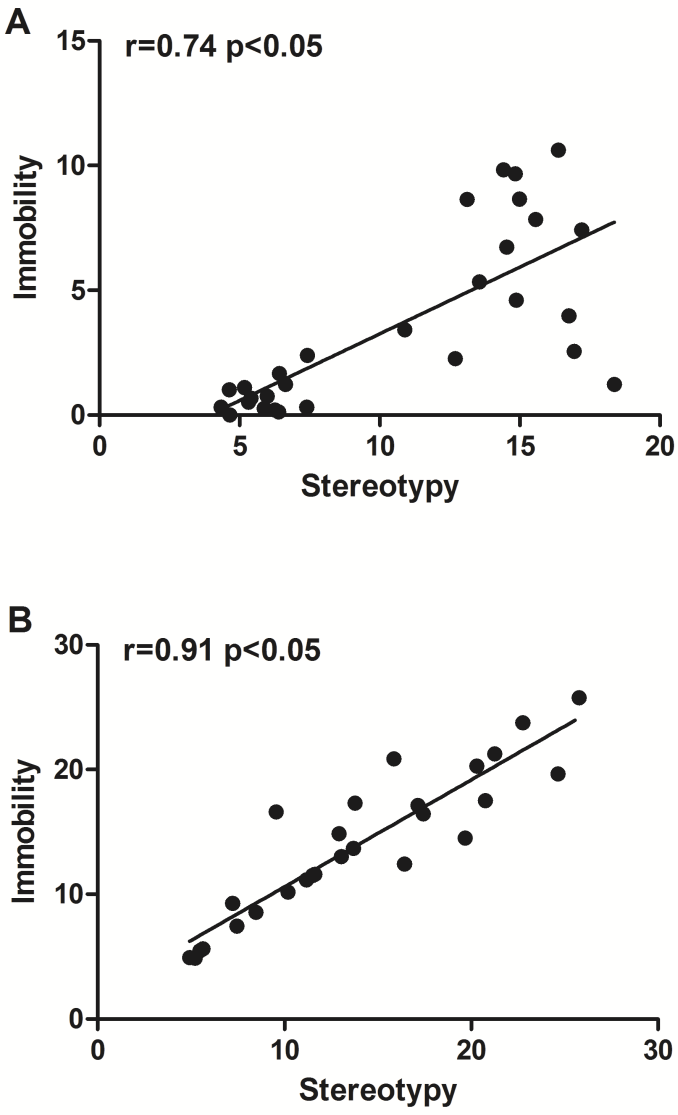

Figure 6 - A) Linear regression analysis between immobility and stereotypy after acute (A) or sub-chronic (B) treatment with $(PhSe)_2$ (5 or 10 mg/kg, s.c.) with or without co-treatment with amphetamine (1.25 mg/kg, i.p.). Significance was considered when $p<0.05$.

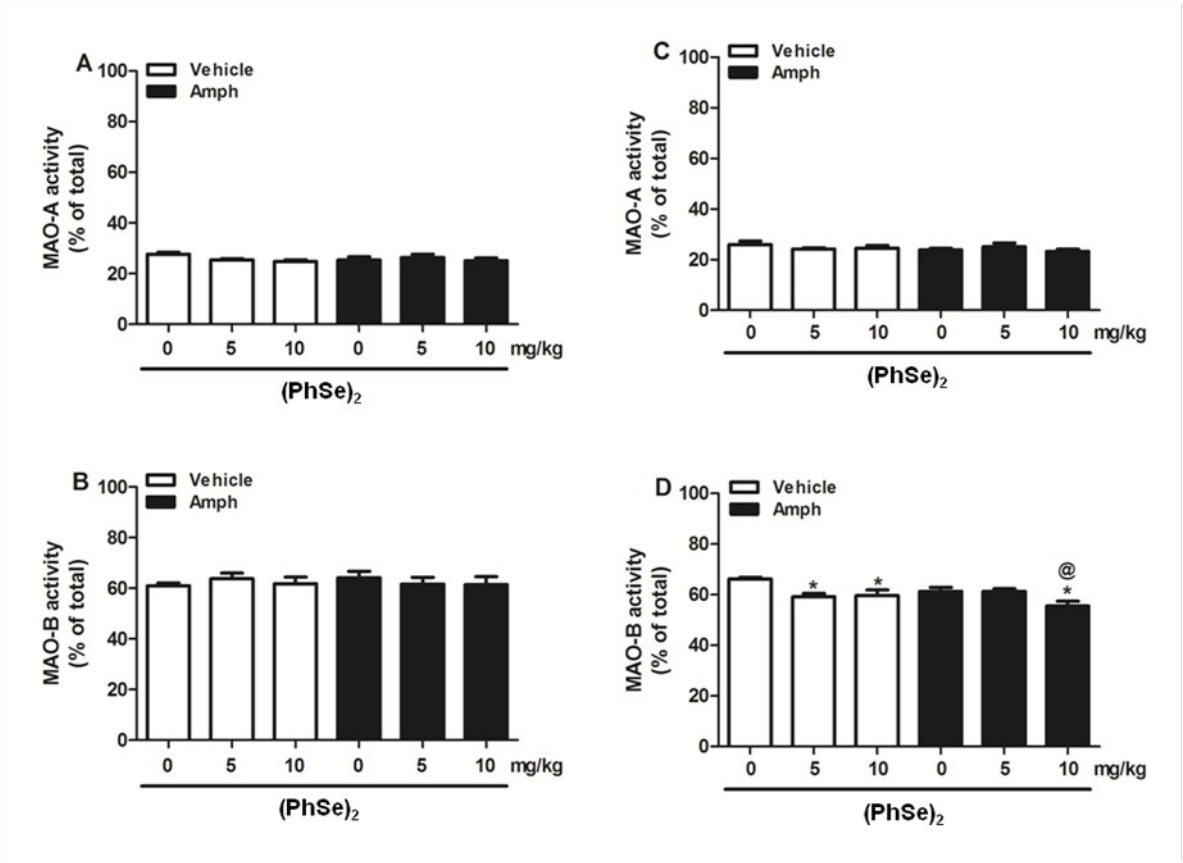

Figure 7 - Effects of acute (A and B) or sub-chronic (C and D) treatment $(PhSe)_2$ (5 or 10 mg/kg, s.c.) and/or amphetamine (1.25 mg/kg, i.p.) on MAO-A (A and C) or MAO-B (B and D) activity of brain homogenate of mice. Values are means±S.E.M. of 4-5 animals per group. One-way ANOVA followed by Duncan's post-hoc tests. (*) $p<0.05$ compared with control group; ($^{@}$) $p<0.05$ compared with amphetamine+$(PhSe)_2$ 10 mg/kg group.

TABLE 1 - Effects of acute treatment $(PhSe)_2$ (5 or 10 mg/kg, s.c.) with or without co-treatment with amphetamine (1.25 mg/kg, i.p.) on P-SH, NP-SH and DCF levels of brain homogenate of mice.

	P-SH (µmol/mg protein)	**NP-SH** (µmol/mg protein)	**DCF** (delta)
Control	14.43±0.56	1.32±0.04	166.50±13.17
$(PhSe)_2$ 5 mg/kg	15.05±0.99	1.23±0.09	163.20±18.20
$(PhSe)_2$ 10 mg/kg	16.37±0.77	1.31±0.09	177.30±19.52
Amph	14.73±1.21	1.38±0.08	157.50±10.71
Amph+$(PhSe)_2$ 5 mg/kg	15.50±0.96	1.31±0.07	173.30±20.51
Amph+$(PhSe)_2$ 10 mg/kg	17.65±1.72	1.34±0.08	162.50±13.32

Values are means±S.E.M. of 4-5 animals per group.

TABLE 2 - Effects of sub-chronic treatment $(PhSe)_2$ (5 or 10 mg/kg, s.c.) with or without co-treatment with amphetamine (1.25 mg/kg, i.p.) on P-SH, NP-SH and DCF levels of brain homogenate of mice.

	P-SH (µmol/mg protein)	**NP-SH** (µmol/mg protein)	**DCF** (delta)
Control	16.10±1.35	1.84±0.37	297.70±35.85
$(PhSe)_2$ 5 mg/kg	17.74±1.59	1.98±0.42	305.10±31.18
$(PhSe)_2$ 10 mg/kg	17.16±1.43	1.56±0.35	286.80±24.64
Amph	15.92±1.09	1.66±0.43	301.60±35.89
Amph+$(PhSe)_2$ 5 mg/kg	17.89±1.73	1.89±0.41	295.20±25.45
Amph+$(PhSe)_2$ 10 mg/kg	17.04±0.74	1.53±0.35	306.00±21.51

Values are means±S.E.M. of 4-5 animals per group.

4. CONCLUSÕES

Considerando o conjunto de resultados obtidos neste estudo, pode-se concluir que:

- O $(PhSe)_2$ interage com sistemas de neurotransmissores relacionados a sensibilização comportamental.
- A sensibilização comportamental induzida pelo tratamento subcrônico parece ser dependente da redução da atividade da isoforma MAO B.

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