UNIVERSIDADE FEDERAL DE SANTA MARIA

CENTRO DE CIÊNCIAS NATURAIS E EXATAS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA TOXICOLÓGICA

ESTUDO DA TOXICOLOGIA E DA FARMACOLOGIA DO 2-FENILETINILBUTIL-TELÚRIO EM ROEDORES

DISSERTAÇÃO DE MESTRADO

Caroline Brandão Quines

Santa Maria, RS, Brasil

2013

ESTUDO DA TOXICOLOGIA E DA FARMACOLOGIA DO 2-FENILETINILBUTIL-TELÚRIO EM ROEDORES

por

Caroline Brandão Quines

Dissertação 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

Mestre em Bioquímica Toxicológica

Orientadora: Profª Drª Cristina Wayne Nogueira

Santa Maria, RS, Brasil

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A Comissão examinadora, abaixo assinada, aprova a dissertação de Mestrado

ESTUDO DA TOXICOLOGIA E FARMACOLOGIA DO 2-FENILETINILBUTIL-TELÚRIO EM ROEDORES

elaborada por

Caroline Brandão Quines

Como requisito parcial para obtenção do grau de

Mestre em Bioquímica Toxicológica

Comissão examinadora

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Santa Maria, 05 de agosto de 2013.

A meus queridos pais, Cerize e Alexandre, e a minha irmã Victória que sempre foram meus melhores amigos, dedico este trabalho e todo o meu amor!

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Agradeço a Deus pela vida, pelo amor, por iluminar meu caminho e por todas as graças concedidas.

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"A dúvida é o principio da sabedoria"

(Aristóteles)

RESUMO

Dissertação de Mestrado

Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica

Universidade Federal de Santa Maria

ESTUDO DA TOXICOLOGIA E DA FARMACOLOGIA DO 2-FENILETINILBUTIL-TELÚRIO EM ROEDORES

AUTOR: CAROLINE BRANDÃO QUINES

ORIENTADORA: CRISTINA WAYNE NOGUEIRA

Local e Data da Defesa: Santa Maria, 05 de agosto de 2013

Os compostos orgânicos de telúrio têm despertado o interesse dos pesquisadores, devido as suas propriedades farmacológicas e toxicológicas. Acredita-se que o principal mecanismo envolvido na toxicidade desses compostos, seja a capacidade de interagir com os grupamentos sulfidrílicos de moléculas biologicamente ativas. Além dos efeitos toxicológicos, propriedades farmacológicas vêm sendo atribuídas aos compostos orgânicos de telúrio. Esse estudo teve como objetivo investigar o potencial toxicológico e farmacológico do 2- feniletinilbutil-telúrio (PEBT), através de experimentos *in vitro* e *in vivo* em roedores. Para a avaliação toxicológica, o PEBT foi utilizado em diferentes concentrações na oxidação de mono e ditiois e na determinação da atividade das enzimas Na^+K^+ -ATPase e lactato desidrogenase, *in vitro.* Ainda nesse sentido estudos de letalidade foram realizados para calcular a CL_{50} e DL_{50} desse composto para melhor compreender a sua toxicidade. Para a avaliação farmacológica, o PEBT foi administrado em camundongos, na dose de 1 mg/kg 30 minutos antes dos experimentos comportamentais, avaliação da atividade locomotora, teste do nado forçado (TNF) e teste de suspensão da cauda (TSC). Após os testes comportamentais, os animais foram mortos e o córtex cerebral foi retirado para determinação da atividade da enzima monoamina oxidase (MAO). Os resultados mostraram que o PEBT oxida tióis de baixo peso molecular e inibe a atividade da enzima Na^+K^+ -ATPase, pela oxidação de seus grupamentos sulfidrilicos, sendo essa oxidação depende da presença do átomo de telúrio na estrutura do composto. Além disso, a administração aguda do PEBT produz um efeito do tipo antidepressivo no TNF em camundongos, bem como inibe a atividade da enzima MAO-A em córtex cerebral, demonstrando o envolvimento dessa enzima no seu efeito do tipo antidepressivo.

Palavras-chave: Telúrio; Enzimas sulfidrílicas; Oxidação de mono e ditiois, Na^+K^+ -ATPase; monoamino oxidase; ratos.

ABSTRACT

Dissertation of Master's Degree

Federal University of Santa Maria, RS, Brazil

STUDY OF TOXICOLOGY AND PHARMACOLOGY OF 2-PHENYLETHYNYL BUTYLTELLURIUM IN RODENT

AUTHOR: CAROLINE BRANDÃO QUINES

ADVISOR: CRISTINA WAYNE NOGUEIRA

Place and Date of the defense: Santa Maria, August 05, 2013

The organotellurium compounds have been the subject of research due to their pharmacological and toxicological properties. It is believed that the main mechanism involved in the toxicity of these compounds is the ability to interact with sulfhydryl groups from molecules biologically active. Beyond the toxicological effects, some pharmacological properties have been attributed to organotellurium compounds. This study aimed to investigate the potential toxicological and pharmacologic of 2-phenylethynyl-butyltellurium (PEBT) through experiments *in vitro* and *in vivo* in rodents. To evaluate the toxicological effect the PEBT was used at different concentrations in the oxidation of mono and dithiols and analysis of enzyme Na^+K^+ -ATPase and lactate dehydrogenase *in vitro*. Furthermore, lethality studies were performed to calculate the LC_{50} LD_{50} of this compound and for better understanding their toxicity. To evaluate the pharmacological effect the PEBT was used at a dose of 1mg/kg 30 minutes before the behavioral experiments, evaluation of locomotor activity, forced swim test (FST) and the tail suspension test (TST), immediately after testing the cerebral cortex was removed for analysis of monoamine oxidase (MAO) enzyme. The results showed that the PEBT oxidized thiols of low molecular weight and inhibits the activity of the enzyme Na^+K^+ - ATPase by oxidation of sulfhydryl groups, and such oxidation is dependent on the tellurium atom in the structure of this compound. Moreover, the acute administration of PEBT showed an antidepressant-like effect on TNF in mice, as well inhibits the activity of MAO-A enzyme in the cerebral cortex, demonstrating the involvement of this enzyme in its antidepressant-like effect.

Keywords: Tellurium, Sulfhydryl enzymes, Oxidation of mono-and dithiols; Na^+K^+ - ATPase; monoamine oxidase; rats.

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INTRODUÇÃO

RESULTADOS COMPLEMENTARES

LISTA DE ABREVIATURAS

ATP - adenosina-5'-trifosfato

ADP – adenosina difosfato

-SH – grupamento tiólico

δ-ALA-D - δ-aminolevulinato desidratase

Na^+K^+- ATPase - sódio potássio ATPase

NPS - nitroprussiato de sódio

PEBT - 2- feniletinilbutil-telúrio

CL_{50} – concentração letal

DL_{50} – dose letal

DTT – ditiotreitol

MAO – monoamina oxidase

SUMÁRIO

1. INTRODUÇÃO

O elemento telúrio (Te) foi descoberto em 1782 e pertence ao grupo 16 da tabela periódica, denominada família dos calcogênios. Este elemento apresenta-se em diferentes estados de oxidação: Te^{+6} (telurato), Te^{+4} (telurito), Te^{0} (telúrio elementar) e Te^{+2} (telureto) (Scansetti, 1992). O Te^{0} é muito utilizado na indústria como um componente de ligas metálicas (Yarema e Curry, 2005; Abdel Aziz, 2006), na produção de microchips e de outros componentes eletrônicos. Além disso, ele também é usado na produção industrial de vidro e aço, na produção de explosivos, na vulcanização da borracha, em lubrificantes sólidos, em soluções oxidantes para polir metais e na indústria petroquímica (Taylor, 1996; Larner, 1995).

O crescente uso industrial do telúrio em produtos químicos provoca riscos ocupacionais e ambientais para a saúde humana, uma vez que o telúrio pode ser prontamente absorvido pelo organismo, através dos compostos orgânicos ou na forma de telúrio inorgânico como teluritos e teluratos. Em casos de intoxicação aguda pelo elemento telúrio, bem como por formas inorgânicas deste elemento, os sintomas são: dores de cabeça, sonolência, enjôo, alteração da frequência cardíaca, bem como odor característico de alho na respiração e na urina (Müller et al., 1989; Taylor, 1996). Um estudo recente demonstrou a citotoxicidade de compostos orgânicos e inorgânicos de telúrio em cultura de células gastrointestinais, no qual ambos os compostos de telúrio diminuíram a viabilidade celular (Vij e Hardej, 2012).

O primeiro composto orgânico de telúrio foi sintetizado por Friedrich Wöhler em 1840 (Wöhler, 1840) mas somente a partir da década de 70 as propriedades farmacológicas e toxicológicas dos compostos orgânicos de telúrio chamaram a atenção dos pesquisadores (Nogueira e Rocha, 2012). No entanto, não existem relatos na

literatura de intoxicação ocupacional, acidental ou tentativa de suicídio ou homicídio com compostos orgânicos de telúrio em humanos. Por esse motivo a necessidade de mais pesquisas envolvendo a toxicidade destes compostos tem sido realizada em modelos experimentais com animais (Nogueira et al., 2004, Nogueira e Rocha, 2012).

1.1. Propriedades toxicológicas dos compostos de telúrio

No que tange as propriedades toxicológicas, os mecanismos envolvidos na toxicidade dos organocalcogênios ainda não estão completamente entendidos. Lacerda e colaboradores (2012) demonstraram recentemente que a exposição aguda ao 3-metil-1-fenil-2-(fenilseleno) oct-2-en-1-ona, um organocalcogênio, induziu toxicidade hepática, além de causar distúrbios hematológicos em ratos.

Além disso, o seu análogo estrutural, o 3-butil-1-fenil-2-(feniltelluro) oct-en-1-ona (Figura 1) foi efetivo em inibir a atividade da enzima creatina quinase, responsável pela conversão de creatina a fosfocreatina, à custa de ATP. Corroborando com esse resultado, essa inibição pode ocorrer por dois mecanismos: através de uma competição com o ADP, ou pela oxidação dos grupamentos sulfidrílicos essenciais para o funcionamento da atividade dessa enzima (Andrade et al., 2012).

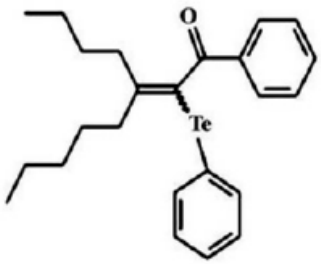

Figura 1: Estrutura química do composto 3-butil-1-fenil-2-(feniltelluro) oct-en-1-ona.

Nesse contexto, um estudo de Chaudiere e colaboradores (1992) foi de grande relevância para a elucidação da interação dos organocalcogênios com os tióis. Neste estudo foi demonstrado que a atividade tiol oxidase, a oxidação de tiois de baixo peso molecular, está intimamente relacionada com os efeitos tóxicos dos organocalcogênios.

Já foi demonstrado que a toxicidade dos compostos orgânicos de telúrio deve-se principalmente pela interação com –SH de moléculas biologicamente ativas (Blais et al., 1972; Deuticke et al., 1992). Estes compostos tem a capacidade de oxidar os grupamentos –SH, inativando enzimas e/ou diminuindo a concentração de moléculas sulfidrílicas não-protéicas (Barbosa et al., 1998; Nogueira et al., 2003a). Alguns estudos com compostos orgânicos de telúrio mostraram que estes são potentes agentes neurotóxicos, uma vez que inibem a atividade da esqualeno monooxigenase, uma enzima sulfidrílica importante na biossíntese do colesterol, o qual é precursor da mielina (Wagner-Recio et al.,1994; Laden e Porter, 2001).

De acordo com isso, nosso grupo de pesquisa demonstrou que o disseleneto de difenila $(PhSe)_2$ (Figura 2A) e o seu análogo estrutural, ditelureto de difenila $(PhTe)_2$ (Figura 2B), oxidam o ditiotreitol, um ditiol de baixo peso molecular, bem como a enzima sulfidrílica δ- aminolevulinato desidratase (δ-ALA-D). A enzima δ-ALA-D é considerada um dos alvos dos compostos orgânicos de telúrio. Esta enzima possui no seu sítio ativo dois resíduos de cisteinil, que são facilmente oxidados *in vitro* e *in vivo* por compostos de telúrio orgânicos, levando a sua inibição (Maciel et al., 2000; Meotti et al., 2003; Nogueira et al., 2003b). Maciel e colaboradores (2000) demonstraram a inibição da δ-ALA-D em fígado e cérebro de camundongos após administração aguda e subcrônica do composto $(PhTe)_2$.

A B

Figura 2: Estrutura dos compostos (A) disseleneto de difenila $(PhSe)_2$ (B) ditelureto difenila $(PhTe)_2$.

Um estudo feito por Lugokenski e colaboradores (2010) demonstrou o que o $(PhTe)_2$ e seu análogo, $(PhSe)_2$, inibem a enzima sulfidrílica lactato desidrogenase, em diferentes tecidos. Ao mesmo tempo, o $(PhTe)_2$ inibe em baixas concentrações a atividade da Na^+K^+ -ATPase, outra enzima sulfidrílica bastante importante para a manutenção da atividade neuronal normal (Borges et al., 2005). Ainda, o ditelureto de difenila altera o sistema glutamatérgico em cérebro de ratos adultos e em desenvolvimento, ao diminuir a captação de glutamato em sinaptossomas (Nogueira et al., 2002; Souza et al., 2010).

Comparsi e colaboradores (2012) demonstraram recentemente que o $(PhTe)_2$ induz peroxidação lipídica, bem como diminui a atividade de enzimas antioxidantes no cérebro. Ao mesmo tempo, nosso grupo de pesquisa demonstrou que exposição aguda ao $(PhTe)_2$ produz um dano oxidativo no pulmão de ratos jovens (Pinton et al., 2011). Além disso, Stangherlin e colaboradores (2009) demonstraram que a exposição subcrônica ao $(PhTe)_2$, através do leite materno causa estresse oxidativo em estruturas cerebrais de ratos jovens. Além de causar prejuízo cognitivo em ratos jovens após a exposição maternal ao $(PhTe)_2$ (Stangherlin et al., 2008).

Do mesmo modo, Nogueira e colaboradores (2004) mostraram que o $(PhTe)_2$ é tóxico para ratos e camundongos, e que esta toxicidade está intimamente relacionada à dose administrada e espécie animal estudada. Nesse contexto, o dietil-2-fenil-2-telurofenil vinilfosfonato (Figura 3) apresentou baixa toxicidade quando administrado em camundongos (Ávila et al., 2006). É importante ressaltar que a estrutura química do dietil-2-fenil-2-telurofenil vinilfosfonato é muito diferente do $(PhTe)_2$, e que isso pode estar envolvido com a sua baixa toxicidade.

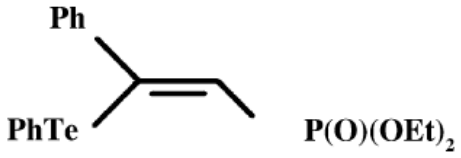

Figura 3: Estrutura química do composto dietil-2-fenil-2-telurofenil vinilfosfonato.

1.2. Propriedades farmacológicas dos compostos orgânicos de telúrio

Estudos recentes demonstram que uma série de propriedades farmacológicas tem sido atribuídas aos compostos orgânicos de telúrio (Klaman, 1990; Nogueira e Rocha, 2012). Em 1987, Sredni e colaboradores descreveram pela primeira vez uma atividade farmacológica para um composto orgânico de telúrio, ao demonstrarem as propriedades imunomoduladoras do composto codificado como AS-101 (telurato de tricloro amônio-dioxoetileno-O,O') (Figura 4) em camundongos, mediando efeitos antitumorais (Hayun et al., 2006). Sendo assim, com base na sua ação antitumoral, o composto AS-101 tem sido utilizado em ensaios clínicos de fase II contra leucemia mielóide aguda e síndrome mielodisplásica em humanos (ClinicalTrials.gov. A service of the U.S. National Institutes of Health).

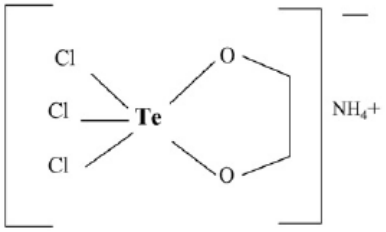

Figura 4: Estrutura química do composto AS-101 (telurato de tricloro amônio dioxoetileno-O,O').

Além disso, estudos mostraram que os compostos orgânicos de telúrio além de uma atividade imunomoduladora, apresentam propriedades anti-inflamatória e antioxidante (Frei et al., 2008; Frieadman et al., Souza et al., 2009, Nogueira e Rocha, 2012). Ao mesmo tempo, os teluretos, devido as suas propriedades citotóxicas, apresentam promissora ação antitumoral (Engman et al., 2000; Cunha et al., 2005).

De acordo com isso, o potencial antioxidante de compostos orgânicos de telúrio tem sido demonstrado em ensaios *ex vivo* e *in vitro*. Esses compostos mimetizam a atividade da enzima glutationa peroxidase (Engman et al., 1992; Andersson et al., 1993; Kanda et al., 1999; Ren et al., 2001; You et al., 2003; Braga et al., 2009), uma enzima ativada na defesa contra o dano oxidativo. Além disso, catalisam a redução de peróxidos a hidrogênio na presença de tióis (Wirth, 1998; Mishra et al., 2006, Nogueira e Rocha, 2012).

Ávila e colaboradores (2006) mostraram em um estudo *in vitro* que o dietil-2-fenil-2-telurofenil vinilfosfonato, um telureto vinílico, possui efeito antioxidante contra a peroxidação lipídica induzida por ferro. Do mesmo modo, esse composto telureto apresentou atividade antioxidante frente ao agente pró-oxidante nitroprussiato de sódio

(NPS) além de proteger contra a disfunção mitocondrial também induzida pelo NPS em estruturas cerebrais (Ávila et al., 2008). Além disso, o dietil-2-fenil-2-telurofenil vinilfosfonato quando administrado sub-agudamente, ou pelas vias subcutânea e intraperitonea em camundongos não apresentou efeitos tóxicos significativos (Ávila et al., 2006; 2007).

Outros estudos demonstraram a capacidade dos compostos orgânicos de telúrio de neutralizarem o ONOO- e inibirem a nitrosilação de proteínas em um modelo de dano oxidativo por espécies reativas de nitrogênio (Briviba et al., 1998; Jacob et al., 2000).

De acordo com isso, Pinton e colaboradores (2011) evidenciaram *in vitro* o potencial antioxidante e a atividade *scanverger* dos compostos $(PhTe)_2$ e p,p'-diclorodifenil ditelureto. Corroborando com esse estudo, Stangherlin e colaboradores (2006) mostraram que a exposição aguda e subcrônica ao $(PhTe)_2$ e seu análogo, $(PhSe)_2$, não causa toxicidade reprodutiva em ratos macho adultos. Nesse contexto, Favero e colaboradores (2007) demonstraram que a exposição subcrônica ao $(PhTe)_2$ em ratos machos não induziu toxicidade no desenvolvimento de sua progênie. Da mesma maneira, não existem dados sobre a toxicidade reprodutiva desse composto em humanos.

Além dessas importantes propriedades, a classe de compostos, alquinil vinil teluretos, administrados por via oral, mostraram um efeito do tipo antidepressivo no teste de suspensão da cauda realizado em camundongos, sem alterar a locomoção destes animais (Okoronkwo et al., 2009).

Ainda nesse contexto, nosso grupo de pesquisa demonstrou recentemente o potencial farmacológico de uma série de telúrioacetilenos, os quais apresentaram efeito antioxidante, em baixas concentrações, *in vitro* (Souza et al., 2009). Nesse sentido o

composto, 2- feniletinilbutil-telúrio (PEBT) (Figura 5), um teluroacetileno, protegeu contra o dano oxidativo cerebral causado pelo NPS em camundongos (Souza et al., 2009) e melhorou a memória de camundongos na tarefa de esquiva inibitória (Souza et al., 2012).

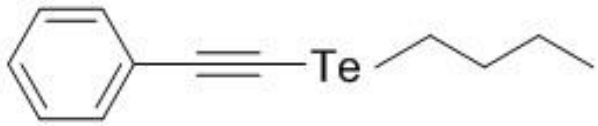

Figura 5: Estrutura química do composto 2- feniletinilbutil-telúrio (PEBT).

Um estudo realizado por Souza e colaboradores (2013a) demonstrou que uma única dose do PEBT protegeu conta o prejuízo na memória espacial induzido por escopolamina em camundongos no teste do labirinto aquático de Morris. Nesse mesmo estudo, o PEBT foi efetivo em proteger contra danos cognitivos causados pela escopolamina nas fases de consolidação e recuperação da memória na tarefa de esquiva inibitória em camundongos.

Do mesmo modo, outro estudo corroborou com o potencial farmacológico do PEBT, no qual foi demonstrado que a administração subaguda do PEBT protegeu contra o dano cognitivo induzido pelo peptídeo beta amiloide 25-35, no teste do labirinto aquático de Morris e na tarefa de esquiva inibitória em camundongos, sem alterar a atividade locomotora destes animais (Souza et al., 2013b).

Tendo em vista a constante pesquisa por novas moléculas com potencial farmacológico e o fato de que pouco se sabe sobre as propriedades toxicológicas do PEBT, este estudo pretende contribuir para o melhor entendimento dos mecanismos toxicológicos e farmacológicos deste composto.

2. OBJETIVOS

2.1. Objetivo geral

Investigar o potencial toxicológico e farmacológico do 2- feniletinilbutil-telúrio em roedores.

2.2. Objetivos específicos

Considerando os aspectos mencionados, os objetivos específicos deste estudo compreendem:

- Avaliar o efeito toxicológico do PEBT, bem como a relevância do átomo de telúrio na toxicidade deste composto na oxidação de mono e ditiois e sobre a atividade das enzimas sulfidrílicas $Na^{+}K^{+}$ -ATPase e lactato desidrogenase (LDH) *in vitro*;

- Avaliar a letalidade do PEBT, calculando a CL_{50} através do teste de mortalidade das larvas de *Artemia salina*, bem como determinar a DL_{50} em camundongos.

- Avaliar o efeito farmacológico do PEBT em modelos experimentais de depressão, tais como: o teste do nado forçado e o teste de suspensão da cauda, bem como o envolvimento das enzimas monoamina oxidase A e B.

3. RESULTADOS

Os resultados que fazem parte dessa dissertação estão apresentados na forma de um artigo científico, um manuscrito em fase de redação, e resultados complementares. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas estão dispostos de acordo com a recomendação dos periódicos científicos aos quais estes foram e serão enviados.

3.1 Artigo

Feniletinil-butiltelúrio inibe a enzima sulfidrílica Na^+, K^+-ATPase: Um efeito dependente do átomo de telúrio

PHENYLETHYNYL-BUTYLTELLURIUM INHIBITS THE SULFHYDRYL ENZYME NA^+, K^+-ATPASE: AN EFFECT DEPENDENT ON THE TELLURIUM ATOM

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Phenylethynyl-Butyltellurium Inhibits the Sulfhydryl Enzyme Na^+, K^+-ATPase: An Effect Dependent on the Tellurium Atom

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Abstract Organotellurium compounds are known for their toxicological effects. These effects may be associated with the chemical structure of these compounds and the oxidation state of the tellurium atom. In this context, 2-phenylethynyl-butyltellurium (PEBT) inhibits the activity of the sulfhydryl enzyme, δ-aminolevulinate dehydratase. The present study investigated on the importance of the tellurium atom in the PEBT ability to oxidize mono- and dithiols of low molecular weight and sulfhydryl enzymes in vitro. PEBT, at high micromolar concentrations, oxidized dithiothreitol (DTT) and inhibited cerebral Na^+, K^+-ATPase activity, but did not alter the lactate dehydrogenase activity. The inhibition of cerebral Na^+, K^+-ATPase activity was completely restored by DTT. By contrast, 2-phenylethynyl-butyl, a molecule without the tellurium atom, neither oxidized DTT nor altered the Na^+, K^+-ATPase activity. In conclusion, the tellurium atom of PEBT is crucial for the catalytic oxidation of sulfhydryl groups from thiols of low molecular weight and from Na^+, K^+-ATPase.

Keywords Tellurium · Sulfhydryl enzymes · Thiol · Toxicity · Na^+, K^+-ATPase

Introduction

The toxicity associated with specific tellurium compounds depends on the chemical structure of the compounds and the oxidation state of the tellurium atom [1]. Many tellurium-based substances are redox-active, with formal oxidation states of tellurium in these compounds ranging from −2 to + 6 [2], resulting in exciting redox and catalytic properties of organotellurium compounds.

Organotellurium compounds are known for their toxicological effects [1, 3–6]. The toxicity of organotellurium compounds has been related to their ability to catalytically oxidize sulfhydryl groups from glutathione or from different proteins or enzymes [1, 7]. In the case of enzymes, the oxidation of thiols by organochalcogen compounds can inhibit enzyme activity, which can contribute to cellular toxicity [8, 9].

In this context, our research group demonstrated that 2-phenylethynyl-butyltellurium (PEBT), a tellurium acetylene compound, inhibits the activity of the sulfhydryl enzyme, δ-aminolevulinate dehydratase (δ-ALA-D), by oxidizing important cysteinyl residues located in the active site of this enzyme [10].

However, PEBT has been reported as an antioxidant agent in a model of cerebral oxidative damage caused by sodium nitroprusside in mice [10]. Furthermore, a single low dose of PEBT improved memory on the mouse step-down inhibitory avoidance task, which was mediated by the decrease in glutamate uptake [11]. PEBT also ameliorated long-term memory deficits induced by amyloid-β peptide (25–35), in a model of Alzheimer's disease in mice [12]. The extrapolation from animal data to therapeutic effects in human should be done with caution, but in the future, PEBT could be a potential drug to be evaluated in treatment against neurological disease.

Although it is not clear whether the in vitro effect of PEBT would be consistent with the observed in vivo data, the present study attempted to better understand the importance of the tellurium atom in the PEBT ability to oxidize mono- and dithiols of low molecular weight and sulfhydryl enzymes in vitro.

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Materials and Methods

Chemical

PEBT (Fig. 1a) and 2-phenylethynyl-butyl (PEB) (Fig. 1b) were prepared according to the methods in the literature [2, 13]. Analysis of the ^{1}H-NMR and ^{13}C-NMR spectra showed that the compounds synthesized exhibited analytical and spectroscopic data in full agreement with their assigned structures. PEBT and PEB were diluted in dimethyl sulfoxide (DMSO) (for oxidation of mono- and dithiols and Na^+, K^+-ATPase activity) or ethyl alcohol (for lactate dehydrogenase activity). Control samples with 2 % of DMSO or ethyl alcohol were routinely performed. The other chemical reagents utilized for biochemical assays were obtained from Sigma Chemical (St Louis, MO, USA).

Animal

Male adult Wistar rats (200–300 g) from our own breeding colony were used. The animals were kept in a separate animal room, on a 12-h light/dark cycle, at a room temperature of 22± 2 °C, with free access to food and water. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animals of the Federal University of Santa Maria, Brazil (no. 041/2012).

Oxidation of Mono- and Dithiols

The effect of PEBT and PEB, a molecule without the tellurium atom, on the oxidation of mono- and dithiols was investigated to demonstrate if dithiothreitol (DTT) or reduced glutathione (GSH) to investigate their effect on mono- and dithiol oxidation (simulating the effect on biological thiols).

The rate of thiol oxidation of DTT and GSH was determined by measuring the disappearance of -SH groups according to the method of Ellman [14]. Potassium phosphate buffer (0.1 M), DTT (0.5 mM) or GSH (1 mM), PEBT, and PEB (100 and 200 μM) were incubated at 37 °C. Aliquots of the reactive mixture (200 μL) were removed at times of 0–120 min and checked for the amount of -SH groups at 412 nm in the presence of 10 mM 5,5-dithio-bis(2-nitrobenzoic acid). The results were expressed as micromoles of non-protein sulphydryl (NPSH) (no protein thiols).

Enzymatic Assays

The effect of PEBT was evaluated on sulfhydryl enzymes Na^+, K^+-ATPase and lactate dehydrogenase. The importance of the tellurium atom in the PEBT inhibitory effect was investigated by using PEB.

Tissue Preparation

Animals were sacrificed by decapitation, and their brains were quickly removed, placed on ice, and homogenized in 50 mM Tris/HCl at pH 7.5, 1:10 (*w*/*v*) for Na^+, K^+-ATPase and lactate dehydrogenase assay. The homogenate was centrifuged at 4,000×*g* for 10 min at 4 °C to yield a low-speed supernatant fraction (S_1). Freshly prepared S_1 was used for the enzyme assays. For each assay, we carried out three to four independent experiments performed in duplicate, in different days, using different animals.

Na^+, K^+-ATPase Activity

The reaction mixture for Na^+, K^+-ATPase activity assay contained 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl, and 50 mM Tris–HCl, at pH 7.4, in a final volume of 500 μL. An aliquot of 50 μL of S_1 was added to the reaction mixture and preincubated at 37 °C for 10 min in the presence of PEBT at different concentrations (10–300 μM) or PEB (100–300 μM). The reaction was initiated by the addition 50 μL of ATP (30 mM) to a final concentration of 3.0 mM and incubated at 37 °C for 30 min. Controls were carried out under the same conditions with the addition of 0.1 mM ouabain. Na^+, K^+-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured by the method of Fiske and Subbarow [15]. The results were expressed as nanomole of Pi per milligram of protein per minute.

Effect of DTT or GSH as a Restoring Agent for Na^+, K^+-ATPase Inhibition Caused by PEBT In order to study on the effect of DTT and GSH on restoring Na^+, K^+-ATPase activity, PEBT, at a half maximal inhibitory concentration (IC_{50}), was preincubated with S_1 at 37 °C for 10 min. After this time, the reaction was started by the addition 30 μL of DTT (3 mM) or GSH (3 mM) and substrate (3 mM ATP) and incubated at 37 °C for 30 min. The results were expressed as nanomole of Pi per milligram of protein per minute.

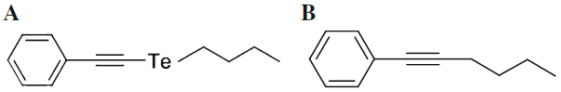

Fig. 1 Chemical structures of 2-phenylethynyl-butyltellurium (**a**), and 2-phenylethynyl-butyl (**b**)

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Lactate Dehydrogenase Activity

Lactate dehydrogenase (LDH) activity was assayed according to the method described by Pereira et al. [16] with modifications. The enzyme activity was measured by determining the amount of NADH formed at 37 °C (i.e., lactate→pyruvate reaction). To investigate the effect of PEBT, an aliquot of 50 μL of S_1, 200 μL of glycine buffer (0.001 mM, pH 7.4), and PEBT were incubated in different situations, without or with 10 min of preincubation, (1) with LDH in the presence of NAD+, with the reaction being started with the addition of lactate, and (2) with LDH in the presence of lactate, with the reaction being started with the addition of NAD+.

The reaction product (NADH) was determined spectrophotometrically at 340 nm. The results were expressed as Δ of absorbance/minute. Sodium bisulfite (BSS) (350 mM) was used as a positive control. The concentrations of substrate (lactate) and cofactor (NAD+) were 50 and 0.1 mM, respectively.

Protein Determination

The protein concentration was measured by the method of Bradford [17], using bovine serum albumin (1 mg/mL) as standard.

Statistical Analysis

Statistical analysis of data was performed using a one-way analysis of variance, followed by the Newmann–Keuls test. The half maximal inhibitory concentration (IC_{50}) was determined by linear regression from individual experiments using "GraphPad Software" (GraphPad Software, San Diego, CA, USA). The IC_{50} values were reported as means accompanied by their 95 % confidence limits. Maximal inhibition (I_{max}) values were calculated at the most effective concentration used. All data of experiments were expressed as means ± SEM. Values of $p<0.05$ were considered statistically significant.

Results

Effect of PEBT and PEB on the Oxidation of Mono- and Dithiols

PEBT significantly decreased the levels of DTT, a dithiol (Fig. 2a), at both concentrations tested. By contrast, PEB did not oxidize DTT (Fig. 2b). GSH, a monothiol, was also

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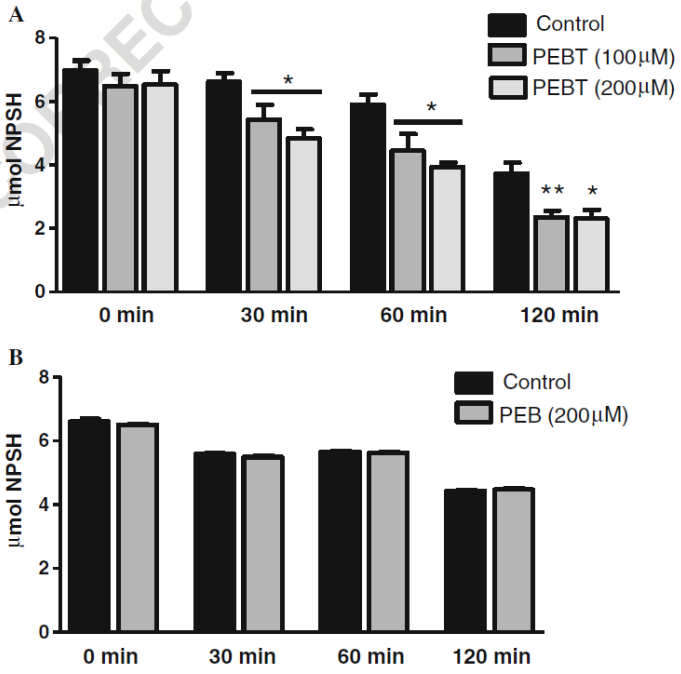

Fig. 2 Effect of PEBT (**a**) and PEB (**b**) on the oxidation of dithiothreitol. The potassium phosphate buffer (0.1 M) and DTT (0.5 mM) were incubated at 37 °C in the presence of PEBT/PEB at different concentrations. Aliquots of the reactive mixture were removed at times of 0–120 min. Results are expressed as micromole of NPSH/gram tissue. Data represent the mean ± SEM of three to four experiments. *$p<0.05$ as compared to control (basal levels)

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oxidized by PEBT at concentrations of 100 and 200 μM (data not shown).

Effect of PEBT and PEB on Na^+, K^+-ATPase Activity

PEBT inhibited cerebral Na^+, K^+-ATPase activity at a concentration of 300 μM (Fig. 3a) in rat homogenates. The calculated IC_{50} and I_{max} values were 174 μM (145.41± 207.49) and 80±7 (%), respectively. PEB, a molecule similar to PEBT but without the tellurium atom, did not inhibit cerebral Na^+, K^+-ATPase activity in the same range of concentration (Fig. 3b).

Effect of DTT or GSH as a Restoring Agent for Na^+, K^+-ATPase Inhibition Caused by PEBT

The inhibitory effect of PEBT at IC_{50} was completely restored by DTT (3 mM) (Fig. 4), but not by GSH (3 mM) (data not shown) in rat brain homogenates.

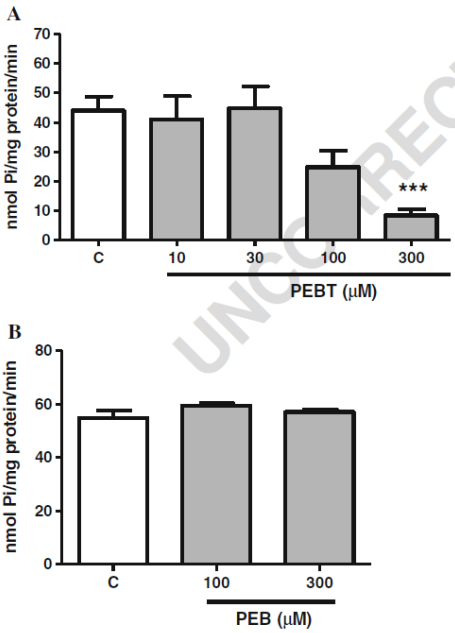

Fig. 3 Effect of PEBT (**a**) and PEB (**b**) on Na^+, K^+-ATPase activity in brain homogenates (S_1) of rats. The S_1 was preincubated in the presence of Na^+, K^+ and Mg^{2+} and PEBT/PEB at different concentrations; the reaction was started by an addition of ATP after 10 min. Results are expressed as nanomole Pi per milligram of protein per minute. Data represent the mean ± SEM of six different experiments. ***$p<0.05$ as compared to control (*C*, a tube incubated with vehicle)

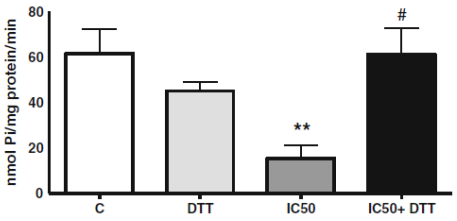

Fig. 4 Effect of DTT as a restoring agent for Na^+, K^+-ATPase inhibition caused by PEBT in brain homogenates (S_1) of rats. The S_1 was preincubated in the presence of Na^+, K^+ and Mg^{2+} and PEBT (IC_{50} 174 μM). The reaction was started by an addition of ATP and DTT (3 mM) after 10 min. Results are expressed as nanomole Pi per milligram of protein per minute. Data represent the mean ± SEM of six different experiments. **$p<0.05$ as compared to the control tube (vehicle); #$p<0.05$ as compared to the tube incubated with PEBT at IC_{50}

Effect of PEBT on LDH Activity

In both preincubation conditions, LDH activity was not altered by PEBT within the tested range concentration (Fig. 5). BSS, a positive control, inhibited LDH activity in rat brain homogenates.

Discussion

The present study clearly demonstrates that the tellurium atom of PEBT is crucial for the catalytic oxidation of sulfhydryl groups from thiols of low molecular weight and from Na^+, K^+-ATPase, an enzyme of biological significance.

PEBT even at high micromolar concentrations demonstrated to be a weak oxidant of mono- and dithiols, indicating that GSH and DTT were not good substrates for PEBT oxidation. The

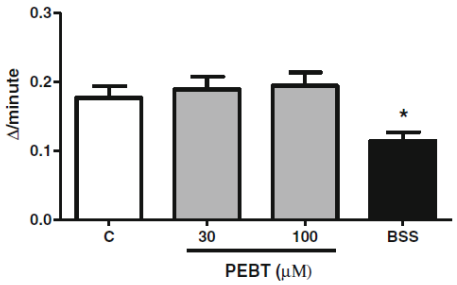

Fig. 5 Effect of PEBT on LDH activity in brain homogenates (S_1) of rats. The S1 was preincubated in the presence of NAD+ and PEBT at different concentrations or 350 mM sodium bisulfite (*BSS*), a positive control. The reaction was started by an addition of lactate after 10 min of preincubation. Results were expressed as Δ of absorbance/minute. Data represent the mean ± SEM of three different experiments. *$p<0.05$ as compared to the control tube (*C*, a tube incubated with vehicle)

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importance of the tellurium atom for the discrete oxidant effect of PEBT was demonstrated by the lack of PEB prooxidant effect on mono- and dithiols of low molecular weight.

Although mono- and dithiols of low molecular weight were not good substrates for PEBT oxidation, this organotellurium compound inhibited consistently cerebral Na^+, K^+-ATPase activity. The inhibition of Na^+, K^+-ATPase by PEBT was evident only at a high micromolar concentrations, and the IC_{50} value was 174 μM. Na^+, K^+-ATPase, or sodium pump, is a membrane protein found in animal cells, responsible for the ion transport across the plasma membrane, carrying ions of Na^+ out of the cell and ions of K^+ into the cell, and thus keeping electrolyte and fluid balance [18]. These gradients are essential for maintaining the membrane potential [19]. The inhibition of Na^+, K^+-ATPase could compromise the balance of membrane potential, characterized by elevation of intracellular Ca^{2+} and increase neuronal excitotoxicity [19]. In addition, several behavioral disorders have been associated with mutations in Na^+, K^+-ATPase α isoforms [20].

Na^+, K^+-ATPase contains thiol groups crucial to its catalytic activity [21] which could be oxidized by PEBT. The hypothesis that Na^+, K^+-ATPase inhibition is related to the ability of PEBT in oxidizing essential sulfhydryl groups of this enzyme is supported by the recovering effect of DTT, a dithiol-reducing agent, on the enzyme activity. In this study, DTT, a classical reducing agent, completely restored Na^+, K^+-ATPase activity inhibited by PEBT. This result is in accordance with previously reported data in which PEBT inhibited the cerebral δ-aminolevulinate dehydratase activity, by oxidizing important thiol groups in the active site of this enzyme [10]. The importance of the tellurium atom in the inhibitory effect of PEBT on Na^+, K^+-ATPase activity was demonstrated by the complete absence of PEB effect on the cerebral enzyme. A number of studies [7, 9, 22, 23] have revealed that the chemistry and biochemical mechanisms employed by organochalcogens to affect pharmacological responses can differ considerably under in vitro and in vivo conditions, and these mechanisms are far from being understood. However, it appears that the inhibition of Na^+, K^+-ATPase caused by high micromolar concentrations of PEBT is unlikely to be found in vivo at a pharmacologically effective dose [11].

One interesting finding of this study was the fact that PEBT did not change LDH activity; LDH is a regulatory enzyme, responsible for catalyzing the reduction of pyruvate to lactate or the oxidation of lactate to pyruvate [24, 25]. This enzyme contains essential cysteine residues, which are not localized in the catalytic site [26, 27] but in a hydrophilic area of enzyme. The cysteine residues induce a conformational change in the enzyme active site after binding of metal ions, compromising the enzymatic activity [28, 29]. By contrast, diphenyl ditelluride, another organic tellurium compound, inhibits the activity of LDH, by interaction with sulfhydryl groups [30]. PEBT, when compared to diphenyl ditelluride, is more hydrophobic, and this characteristic makes it difficult to interact with the hydrophilic area of the enzyme. Therefore, one can hypothesize that PEBT, in view of its hydrophobic character, does not interact with the hydrophilic area of enzyme, where cysteine residues are located.

The effect of organotellurium compounds on sulfhydryl enzymes has been related to the chemical structure of compounds and the oxidation state of the tellurium atom. It has been reported that diphenyl ditelluride inhibits δ-aminolevulinate dehydratase [31] and Na^+, K^+-ATPase [32]. Despite the intriguing link between the in vitro and in vivo models, the inhibition of δ-aminolevulinate dehydratase activity from different tissues of rodents by high doses of 1-butyltelurenyl-2-methylthioheptene [33] and diethyl 2-phenyl-2-tellurophenyl vinylphosphonate [34] has been associated to the toxicity of these organotellurium compounds in vivo.

Conclusion

In conclusion, the present study showed that the tellurium atom in the PEBT chemical structure is crucial for the catalytic oxidation of thiols of low molecular weight and of sulfhydryl groups of rat cerebral Na^+, K^+-ATPase activity in vitro. PEBT did not inhibit cerebral lactate dehydrogenase activity from rat.

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3.2.Manuscrito em fase de redação

Antidepressant-like action of 2-phenylethynyl butyltellurium, an inhibitor of MAO-A activity in mice

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Abstract

Depression is a serious disorder characterized by imbalance of mood and emotions, accompanied by reduction in monoaminergic signaling. Monoamine oxidases (MAO) are a family of enzymes responsible for the oxidative deamination of monoamine neurotransmitters. In this study, a possible antidepressant-like effect of 2-phenylethynyl butyltellurium (PEBT) in the forced swimming test (FST) and the tail suspension test (TST), two tests predictive of depressant activity in mice, were investigated. The involvement of cerebral MAO-A and MAO-B activities in the antidepressant-like action of PEBT was examined. Mice received PEBT (1 mg/kg, by the intragastric route, i.g.) or canola oil (10 ml/kg, i.g.) thirty minutes before behavioral tests. The results showed that PEBT was effective in increasing the latency for the first episode of immobility and in decreasing total immobility time in the FST. By contrast, PEBT at a dose of 1 mg/kg did not have antidepressant-like activity in the mouse TST. PEBT selectively inhibited MAO-A activity in cerebral cortex of mice. These results demonstrated the involvement of MAO-A activity in the antidepressant-like action of PEBT in the mouse in the FST.

Key-words: Depression, forced swimming test, monoamine oxidase, tellurium, organotellurium.

1. Introduction

Depression is a serious disorder in today's society, associated with significant levels of morbidity and mortality, characterized by imbalance of mood and emotions, accompanied by reduction in monoaminergic signaling (Meyer et al., 2006) and deregulation of the hypothalamic pituitary adrenal (HPA) axis (Kostowski, 1985; Gold and Chrousos, 1999; Ge et al., 2013).

Monoamine oxidases (MAO) are a family of enzymes present in mammalian tissues external at mitochondrial membrane, responsible for the oxidative deamination of monoamine neurotransmitters. MAO has two isoforms, MAO-A and MAO-B, which despite catalyze the same reaction, exhibit differences in selectivity for inhibitors, besides differences in the amines metabolized (Wouters, 1998; Robinson, 2002; Costa et al., 2012).

MAO-A is an isoenzyme that is selectively inhibited by clorgyline and preferentially deaminates serotonin and noradrenaline (Johnston, 1968) and MAO-B is selectively inhibited by selegiline and metabolizes preferentially phenylethylamine (Knoll and Magyar, 1972). In this context, the inhibition of MAO-A is used in the treatment of clinical depression (Robinson, 2002; Costa et al., 2012).

Organotellurium compounds have been the subject of research due to their pharmacological properties. Organotellurium compounds have been reported as antioxidants in several models of oxidative stress (Briviba et al., 1998; Jacob et al., 2000; Ávila et al., 2008). Furthermore, these compounds presented immunomodulatory and anti-inflammatory actions (Frei et al., Friedman et al., 2008; Nogueira and Rocha,

2012). In addition, the vinyl alkynyl telluride class of compounds demonstrated an antidepressant-like action in mice (Okoronkwo et al., 2009).

Recently, our research group showed the antioxidant effect of telluroacetylenes *in vitro* (Souza et al., 2009). Moreover, 2-phenylethynyl-butyltellurium (PEBT), a telluroacetylene compound, protected against oxidative damage caused by sodium nitroprusside in mouse brain, suggesting an antioxidant effect *in vivo* of this compound (Souza et al., 2012). PEBT significantly ameliorated the scopolamine-induced impairment of long-term memory and Aβ-induced learning deficits in mice, as indicated by a decrease in escape latency and an increase in the number of crossings over the platform location in the Morris Water Maze test. In addition, PEBT increased step-down latency in scopolamine-induced memory impairment in mice and Aβ-treated group (Souza et al., 2013a, 2013b).

Given the need for development of therapeutic agents for treating depression, in this study a possible antidepressant-like effect of PEBT in the forced swimming test (FST) and the tail suspension test (TST), two tests predictive of depressant activity were investigated in mice. The involvement of cerebral MAO-A and MAO-B activities in the antidepressant-like action of PEBT was examined.

2. Materials and Methods

2.1. Chemicals

PEBT (Figure 1) was prepared according to the literature methods (Petragnani, 1995; Comasseto et al., 1996). Analysis of the ^{1}HNMR and ^{13}CNMR spectra showed that the compound synthesized exhibited analytical and spectroscopic data in full

agreement with its assigned structure. PEBT was diluted in canola oil. The other chemical reagents utilized for biochemical assays were obtained from standard commercial suppliers.

2.2. Animals

The experiments were conducted using male Swiss mice (25-30 g) maintained at 22-25 °C with free access to water and food, under a 12:12 hour light/dark cycle, with lights on at 7:00 a.m. All manipulations were carried out between 08.00 a.m. and 04.00 p.m and mice were acclimated to the behavioral room at least 2 hours before the test. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil (#041/2012). All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

The animals were divided into two groups: 1) Control group (n=9): mice received canola oil (vehicle) by the intragastric (i.g.) route (10 ml/kg); 2) PEBT group (n=9): mice received PEBT at a dose of 1 mg/kg (i.g.) thirty minutes before behavioral tests

To test the hypothesis that the antidepressant-like effect of PEBT is mediated through an interaction with the monoaminergic signaling, mice were treated with PEBT or canola oil thirty minutes before being killed by cervical dislocation and the cerebral cortices were quickly removed for the determination of MAO-A and MAO-B activities.

2.3. Behavioral tests

2.3.1. Forced swimming test (FST)

The procedure is based on that described by Porsolt et al. (1977). In this test, mice were individually forced to swim in an open cylindrical container (10 × 25 cm), containing 19 cm of water at 25±1 °C. Each mouse was gently placed in the cylinder and the total duration of floating was recorded during a 6 min period. Paroxetine was used as positive control (8 mg/kg, by intraperitoneal route, i.p.). Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water.

2.3.2. Tail suspension test (TST)

Briefly, animals both acoustically and visually isolated were suspended upside down by their tails with adhesive tape to a horizontal bar 30 cm above the table. The total duration of immobility induced by tail suspension was recorded during a 6 min trial measured according to the methods described by Steru et al. (1985). Paroxetine was used as positive control, dissolved in saline and administered at a dose of 8 mg/kg (i.p.). Animals were considered immobile only when they hung passively and were completely motionless. Mice that pulled themselves up on the tail during the TST or presented tail biting and/or self-mutilation were removed from the experiment

2.3.3. Spontaneous locomotor activity

The locomotor activity monitor is a clear acrylic plastic box (50 x 48 x 50 cm) with a removable plastic lid perforated with holes for ventilation. The monitor contains photocell beams and detectors that are mounted on opposite walls (2 cm above the chamber floor). General locomotor activity and the mouse position in the chamber are detected by breaks of the photocell beams, which are recorded by Software (Insight,

Ribeirão Preto, SP, Brazil). Mice were placed in the center of the apparatus and allowed to freely explore the arena. Numbers of crossings, rearings, fecal pellets, average velocity (mm/s) and total distance traveled (mm) were recorded for a 4 min period.

2.3.4. Rotarod

The rotarod test was used to investigate motor coordination. The rotarod consisted of a wooden beam covered with masking tape (diameter, 3 cm), used to increase the roughness of the texture and thereby providing a firm grip. The rod was flanked by two cardboard plates to prevent any escape and suspended at a height of 30 cm above the mat-covered table. The mice were placed on top of the already revolving beam (10 rpm) and facing away from the investigator, in the orientation opposite to that of the beam movement in the longitudinal axis, so that forward locomotion was necessary to avoid a fall. Latencies before falling were measured for three trials, with an inter trial interval of 10 min.

2.4. *Ex vivo* assays

2.4.1. Monoamine oxidase (MAO) activity

Mitochondria preparation

A preparation of mitochondria was used for MAO assay as previously described (Soto-Otero et al., 2001). After removal, the cerebral cortices from mice were washed in ice-cold isolation medium (pH 7.4, Na_2PO_4/KH_2PO_4 isotonized with sucrose). Mitochondria were then obtained by differential centrifugation. Briefly, cerebral tissue was manually homogenized with four volumes (w/v) of the isolation medium. Then, the homogenate was centrifuged at 900 × g at 4 $^{\circ}$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.

Enzyme assay

MAO activity was determined as described by Krajl (1965) with some modifications of Matsumoto et al. (1984). Aliquots of samples were incubated at 37 $^{\circ}$C for 5 min in a medium containing buffer solution (Na_2PO_4/KH_2PO_4 isotonized with KCl, pH 7.4) and specific inhibitors, pargyline (a MAO-B inhibitor, 250 nM) or chlorgyline (a MAO-A inhibitor, 250 nM), at a final volume of 600 µl. Then kynuramine dihydrobromide (final concentration 90 mM to MAO-A assay and 60 mM to MAO-B assay) was added to the reaction mixture as substrate. Samples were then incubated at 37 $^{\circ}$C for 30 min. After incubation, the reaction was terminated by adding of 10% thichloroacetic acid. After cooling and centrifugation at 3000 × g for 15 min, an aliquot of supernatant was added to 1M NaOH. The fluorescence intensity was detected spectrofluorimetrically with excitation at 315 nm and emission at 380 nm. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve of 4-hydroxyquinoline. MAO-A and MAO-B activities were expressed as nmol of 4-hydroxyquinoline formed/mg protein/min.

2.5. Protein determination

Protein concentration was measured according to Bradford (1976), using bovine serum albumin (1mg/ml) as the standard.

2.6. Statistical analysis

All experimental results are given as the mean ± S.E.M. Comparisons between experimental and control groups were analyzed using unpaired Student's t-test. Probability values less than 0.05 ($p<0.05$) were considered as statistically significant. All analyses were performed using the GraphPad software (GraphPad software, San Diego, CA, USA).

3. Results

3.1. Effect of PEBT on the FST

PEBT was effective in increasing the latency for the first episode of immobility ($p<0.01$, Figure 2A) and in decreasing the total immobility time ($p<0.001$, Figure 2B) in the mouse FST. In addition, the positive control paroxetine (administered at a dose of 8 mg/kg i.p.) decreased the immobility time in the FST (data not shown).

3.2. Effect of PEBT on the TST

PEBT at a dose of 1 mg/kg did not alter the immobility time in the mouse TST (Figure 3). By contrast, the positive control paroxetine decreased the immobility time in the TST (data not shown).

3.3. Effect of PEBT on spontaneous locomotor activity and rotarod test

The average velocity (mm/s), the number of crossings, rearings, and fecal pellets were not altered by PEBT treatment (Table 1). By contrast, the total distance traveled ($p<0.05$, Table 1) was increased in the PEBT group during the spontaneous locomotor

activity. In addition, treatment with PEBT did not alter the latency to fall during the rotarod test (Figure 4).

3.4. Effect of PEBT on MAO-A and MAO-B activities from cerebral cortex

PEBT at a dose of 1 mg/kg selectively inhibited MAO-A activity ($p<0.05$, Figure 5A) from cerebral cortices of mice. By contrast, PEBT at the same dose did not alter MAO-B activity (Figure 5B).

4. Discussion

The results of the present study showed that treatment with PEBT produced an antidepressant-like effect in mice, which was demonstrated by an increase in the latency for the first episode of immobility and the decrease in the total immobility time in the FST. PEBT selectively inhibited MAO-A activity in cerebral cortices of mice, suggesting an involvement of monoaminergic signaling in the antidepressant-like effect of this organotellurium compound.

In the present study we demonstrated that a single PEBT administration (1 mg/kg, i.g) produced antidepressant-like effects in mice. PEBT treatment increased the latency for the first episode of immobility besides decreasing the total immobility time in the FST in mice.

The FST is the most widely used model for assessing antidepressant-like action and is very well accepted as a reliable indicator of this kind of pharmacological activity. The reduction in the immobility time in the FST is demonstrated by therapeutically antidepressant drugs used on treatment of this disorder (Crayn et al., 2005a; 2005b). Additionally, there are some differences concerning the sensitivity of FST and TST. In the case of the TST the stressful situation involves the haemodynamic stress of being

hung in an uncontrollable fashion by their tail whereas in the FST mice are placed in a cylinder filled with water (Thierry et al., 1986). Furthermore, some antidepressants commonly used for the treatment of depression demonstrated some differences to the immobility-reducing effects when evaluated in these two tests (Crayn et al., 2005a; 2005b). In accordance to that, in the present study PEBT treatment did not alter the immobility time of mice in TST.

Moreover, it is important to mention that the treatment with PEBT did not cause impairment in the locomotor activity and exploratory behavior of mice, since animals treated with PEBT showed no alterations in the locomotor activity monitor and rota rod test. In agreement, Souza et al. (2012, 2013a, b) demonstrated that PEBT did not affect locomotor and exploratory behavior of mice in the open field test.

In addition to the organotellurium chemistry versatility (Zeni et al., 2004; Stein et al., 2008; Okoronkwo et al., 2009), the most varied pharmacological properties of these compounds have been demonstrated (Cunha et al., 2005). In fact, immunomodulatory, anti-inflammatory, antioxidant, antidepressant-like, and antitumoral properties (Rossato et al., 2002; Puntel et al., 2007, Frei et al., 2008; Frieadman et al., 2008; Okoronkwo et al. 2009) have been attributed to organotellurium compounds. Regarding the pharmacological properties of PEBT, antioxidant and nootropic activities have been reported *in vivo* (Souza et al., 2009, 2012).

Depression is a serious disorder with symptoms manifested at the psychological, behavioral, and physiological level, accompanied by reduction in monoaminergic signaling (Costa et al., 2012). Monoamine neurotransmitters, such as serotonin, norepinephrine, and dopamine, are believed to be involved in depressive disorders and

play important roles in mediating behavioral effects of antidepressant drugs (Elhwuegi, 2004; Millan, 2004).

MAO is an enzyme responsible for the oxidative deamination of monoamine neurotransmitters. MAO inhibitors are used on the treatment of depression, since it has been determined that the capacity to inhibit MAO could increase the levels of serotonin in the brain. The increased level of serotonin leads to improvements in the depressive state of the patient (Robinson, 2002; Costa et al., 2012). The MAO inhibitors are divided by their specificity for MAO-A or MAO-B isoenzymes and whether their inhibition is reversible or not (Costa et al., 2012).

The treatment response to MAO inhibitors is often superior to other antidepressants, and they may be effective when other treatments have failed, therefore justifying the need for the research of new antidepressant drugs that acts by inhibiting MAO activity (Amsterdam and Shults, 2005; Costa et al., 2012).

In the present study it was demonstrated the selective inhibition of MAO-A activity from cerebral cortices of mice treated with PEBT, which could be a possible mechanism for the antidepressant-like action of PEBT.

Interestingly, mice treated with PEBT did not alter MAO-B activity. One possible explanation for this fact is that the MAO isoenzymes have differences, which differ in their substrate affinities and inhibitor sensitivities. Besides, the three-dimensional arrangements of MAO isoforms suggest that the structure of the active site for both enzymes is different (Medvedev et al., 1996; Costa et al., 2012).

5.Conclusion

In summary, the present results demonstrated an antidepressant-like action of PEBT in the mouse FST. Furthermore, we also demonstrated that the selective inhibition of MAO-A activity by PEBT could be a possible mechanism for the antidepressant-like action of PEBT. However, further studies are needed to better understand the mechanisms involved in the pharmacological effect of PEBT.

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Conflict of interest

The authors declare they have no conflicts of interest to disclose.

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Figure legends

Figure 1: Chemical structure of 2-phenylethynyl-butyltellurium (PEBT)

Figure 2: Effect of PEBT treatment on the latency for the first episode of immobility (A) and total immobility time (B) in the FST. Values are expressed as mean ± S.E.M. for 9 animals per group. (**) Denotes $p<0.01$ compared to the control group; (***) denotes $p<0.001$ compared to the control group (unpaired Student's t test).

Figure 3: Effect of PEBT treatment on the immobility time in the TST. Values are expressed as mean ± S.E.M. for 9 animals per group.

Figure 4: Effect of PEBT treatment on the rotarod test. Values are expressed as mean ± S.E.M. for 9 animals per group.

Figure 5: Effect of PEBT treatment on the MAO-A (A) and MAO-B (B) activities from cerebral cortices. Values are expressed as mean ± S.E.M. for 9 animals per group. (*) Denotes $p<0.05$ compared to the control group (unpaired Student's t test).

Table 1 Parameters of spontaneous locomotor activity

Test parameters	Control	PEBT
Number of crossing	274.7 ± 45.41	377.4 ± 31.58
Number of rearing	9.0 ± 2.23	11.89 ± 1.86
Velocity (mm/s)	33.54 ± 6.39	36.71 ± 2.89
Distance (mm)	4948 ± 921.4	8115 ± 779.8*
Number of fecal pellets	1.22 ± 0.59	2.22 ± 0.32

Data are reported as means ± S.E.M. for nine animals per group. *Compared to the Control group, $p < 0.05$, (unpaired Student's *t* test).

Figure 1

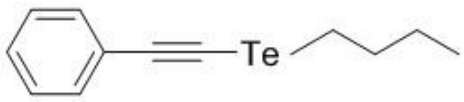
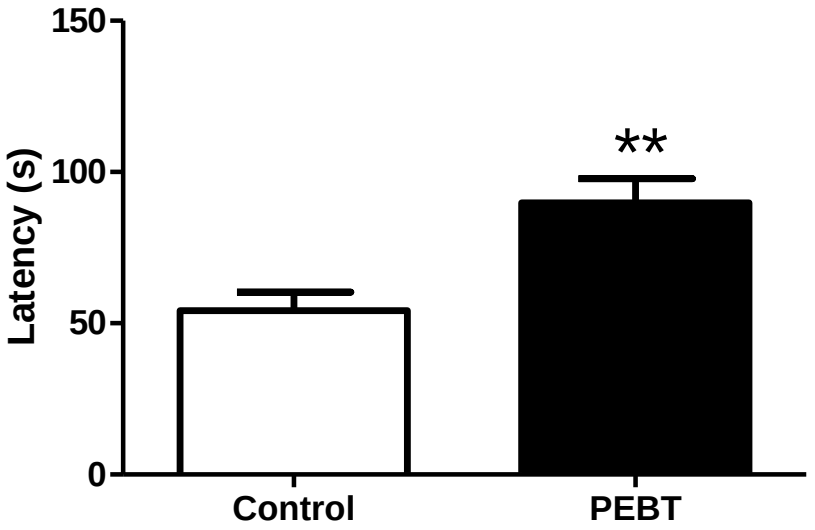


Figure 2

A


150
100
50
0
Latency (s)
**
Control
PEBT

B

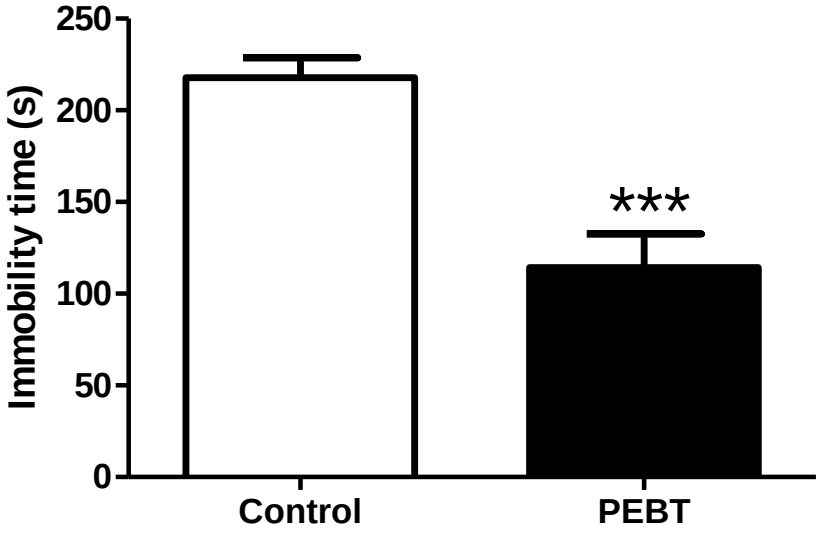

250
200
150
100
50
0
Immobility time (s)
***
Control
PEBT

Figure 3

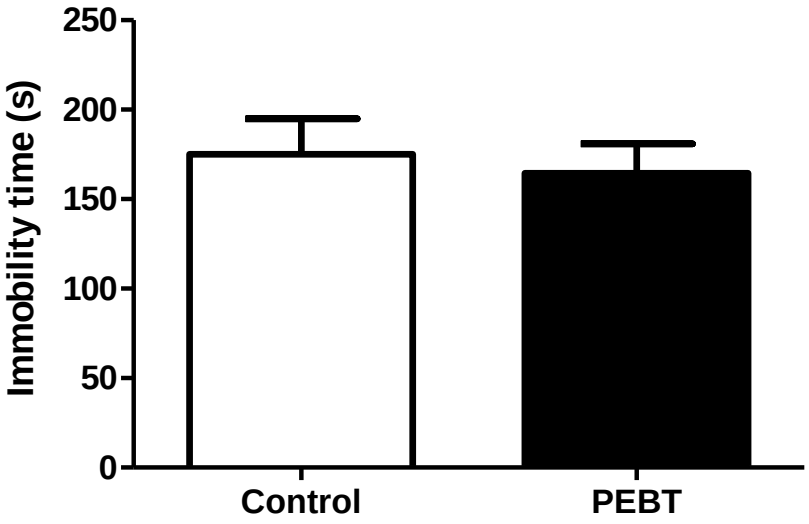


Figure 4

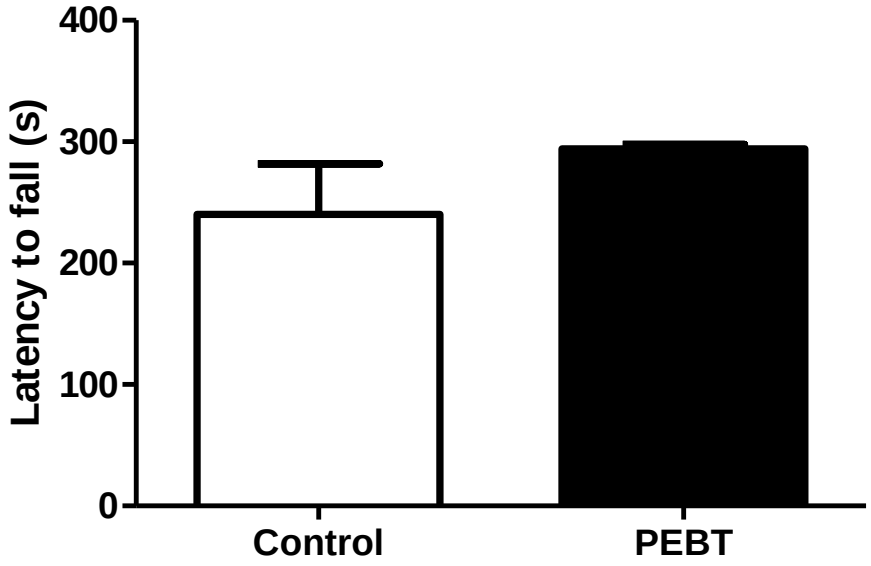


Figure 5

A


nmol 4-OH quinoline / mg ptn / min
2.0
1.5
1.0
0.5
0.0
*
Control
PEBT

B


nmol 4-OH quinoline / mg ptn / min
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Control
PEBT

4. RESULTADOS COMPLEMENTARES

4.1.Estudos de toxicidade

4.2.Materiais e métodos

4.2.1. *Artemia salina* - teste de letalidade

Diferentes concentrações do PEBT (0,01-10 μM) foram avaliadas quanto a letalidade para as larvas de *Artemia salina* de acordo com o procedimento descrito por Meyer et al., (1982). Resumidamente, os ovos de artemias foram colocados em meio salino (35 g de sal marinho, pH 8,0), sob condições controladas de arejamento e de iluminação. Após 24 horas, as larvas (10 por frasco) foram transferidas para frascos de 5 mL, contendo o PEBT e o meio salino. Devido à falta de estudos de toxicidade relatados, o PEBT foi testado em vários ensaios, realizado com cinco repetições de cada concentração. Após 24 h de incubação, o número de sobreviventes foi contado e a percentagem de morte calculada. O número de larvas mortas foi registrado e utilizado para calcular a concentração letal de 50% (CL_{50}), pelo programa Spearmam-Karber test.

4.2.2.Dose letal

Camundongos adultos albinos *Swiss* machos foram tratados com uma administração intragástrica do PEBT em 4 diferentes doses (1, 10, 18 e 30 mg/kg). Para determinar o potencial de letal do PEBT, os animais foram observados por 24 horas após a administração do composto. A DL_{50} foi calculada pelo método da tabela de Dixon (Dixon, 1982).

4.3.Resultados

A figura 6 mostra o efeito do PEBT sobre a sobrevivência das *Artemia salina* após uma exposição de 24 horas. A CL_{50} do PEBT foi 0,27 μM (0,14 - 0,50) e foi calculada através do programa Spearmam-Karber test. n: cinco ensaios diferentes.

Figura 6

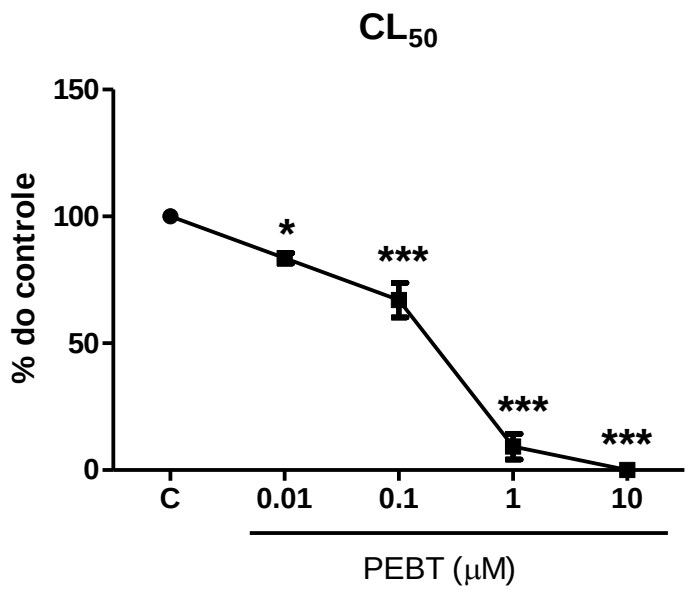


Figura 6: Efeito da exposição das larvas de *Artemia salina* a diferentes concentrações do PEBT durante 24 horas, n: 5 experimentos. Os resultados obtidos foram expressos como média ± DP. A análise estatística dos dados foi realizada através do teste de uma via (ANOVA) seguida pelo teste de Newmann-Keuls. (*) $p<0.05$ quando comparado ao controle; (***) $p<0.001$ quando comparado ao controle.

A figura 7 mostra o efeito do PEBT após 24 horas da administração em camundongos. A DL_{50} do PEBT foi de 11,91 mg/kg (11,36-12,46) e foi calculada através da tabela de Dixon (Dixon, 1982).

Figura 7

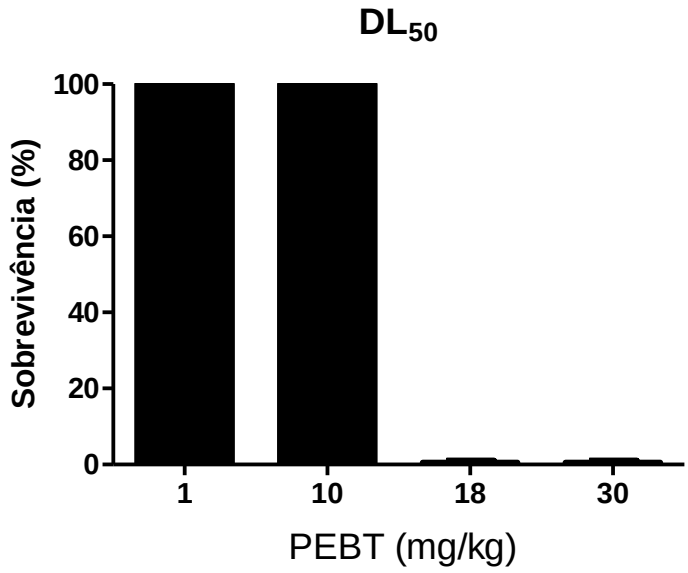


Figura 7: Efeito de diferentes doses do PEBT após 24 horas da administração em camundongos, n: 6 animais.

5. DISCUSSÃO

A busca constante por novos compostos com atividade farmacológica despertou o interesse dos pesquisadores pelos compostos orgânicos de telúrio a partir da década de 70, devido as suas propriedades toxicológicas e farmacológicas. Primeiramente os compostos orgânicos de telúrio eram relatados na literatura devido ao seu potencial toxicológico.

Acredita-se que um dos mecanismos envolvidos na toxicidade dos organocalcogênios seja a interação destes compostos com tióis. Chaudiere e colaboradores (1992) demonstraram que a oxidação de tióis de baixo peso molecular está intimamente relacionada com os efeitos tóxicos dos organocalcogênios. Além disso, esses compostos têm a capacidade de oxidar grupamentos –SH de moléculas biologicamente ativas, como enzimas sulfidrílicas (Blais et al., 1972; Deuticke et al., 1992).

Nosso grupo de pesquisa demonstrou que o $(PhTe)_2$, um composto orgânico de telúrio, oxida o ditiotreitol (DTT), um ditiol de baixo peso molecular, bem como inibe a atividade da enzima sulfidrílica δ-ALA-D. Essa enzima é considerada um dos alvos dos compostos orgânicos de telúrio, por possuir no seu sítio ativo dois resíduos de cisteinil, que são facilmente oxidados *in vitro* e *in vivo* por compostos de orgânicos telúrio (Maciel et al., 2000; Meotti et al., 2003; Nogueira et al., 2003b).

Nesse contexto Souza e colaboradores (2009) demonstraram pela primeira vez a capacidade do PEBT em interagir com grupamentos –SH, já que o PEBT inibiu a atividade da enzima sulfidrílica δ-ALA-D *in vitro*, e essa inibição foi revertida na

presença do DTT, confirmando que a inibição ocorreu pela oxidação dos grupamentos –SH.

Corroborando com esse estudo, os resultados desta dissertação demonstraram que o PEBT oxida grupamentos –SH de moléculas de baixo peso molecular, além de inibir a atividade da enzima sulfidrílica Na^+K^+ - ATPase em altas concentrações, e esta inibição é revertida na presença do DTT. Adicionalmente, este estudo também confirmou a importância do átomo de telúrio na interação com tiois, uma vez que sem a presença do átomo de telúrio, o seu análogo não oxida tióis de baixo peso molecular, bem como não inibe a atividade da enzima Na^+K^+ - ATPase.

Esses resultados ressaltam o envolvimento do átomo de telúrio nas propriedades toxicológicas do composto PEBT, uma vez que já foi demonstrado que a oxidação dos grupamentos –SH de moléculas biologicamente ativas, além da oxidação de tióis de baixo peso molecular está intimamente relacionada aos efeitos tóxicos dos organocalcogênios.

Entretanto, nenhuma alteração foi observada na atividade da enzima sulfidrílica LDH. Esse resultado demonstra que o PEBT pode ser menos tóxico quando comparado ao $(PhTe)_2$, uma vez que o $(PhTe)_2$ inibe a atividade dessa enzima em diferentes tecidos (Lugonkeski et al., 2010). Além disso, o PEBT inibe a atividade da Na^+K^+ - ATPase e δ-ALA-D somente em altas concentrações (Souza et al., 2009), enquanto o $(PhTe)_2$ inibe ambas as enzimas em baixas concentrações (Nogueira et al., 2003a; Borges et al., 2005). É importante ressaltar também que esses compostos apresentam diferenças quanto as suas estruturas químicas, uma vez que o PEBT possui apenas um átomo de telúrio na sua estrutura química e o $(PhTe)_2$ possui dois átomos, fato que pode estar envolvido com a baixa toxicidade do PEBT quando comparado com o $(PhTe)_2$.

Além das propriedades toxicológicas envolvendo esse composto, Souza e colaboradores (2009) demonstraram a atividade antioxidante do PEBT em ensaios *in vitro* e *in vivo*. Estudos recentes corroboram com os efeitos farmacológicos do PEBT, uma vez que a administração aguda e subcrônica desse composto foram efetivas em melhorar a memória de camundongos em diferentes modelos de danos cognitivos (Souza et al 2013a, 2013b).

A escolha da dose de 1mg/kg para o estudo da propriedade antidepressiva, deveu-se ao fato da DL_{50} desse composto ser de 11,91 mg/kg (11,36 - 12,46) (Figura 7), além desse composto apresentar uma CL_{50}: 0,27 μM (0,14 - 0,50) (Figura 6). A dose escolhida é portanto 10x menor que a DL_{50}. Corroborando com isso, em um estudo feito por Souza e colaboradores (2013a), o PEBT, administrado de maneira subcrônica, com essa mesma dose, foi efetivo em exercer seu efeito farmacológico, além de não induzir alterações locomotoras nos animais.

O teste de toxicidade das larvas de *Artemia salina* é um teste bem aceito para estabelecer a CL_{50} de compostos *in vitro*, entretanto a exposição das larvas de *Artemia salina* ao PEBT demonstrou que elas são muito sensíveis a presença desse composto. Contudo, Souza e colaboradores (2012; 2013b) demonstraram um efeito farmacológico desse composto com a dose de 10mg/kg, sendo possível, então, estabelecer que a janela terapêutica do PEBT encontra-se entre 1mg/kg e 10mg/kg. Sendo assim, é importante ressaltar que, a apesar da DL_{50} calculada para este composto ser baixa a dosagem utilizada nesse estudo foi 10x menor que a DL_{50}.

Adicionalmente esses resultados demonstram um novo efeito farmacológico do PEBT, no qual a administração de apenas uma dose do composto (1 mg/kg) mostrou um efeito do tipo antidepressivo no teste do nado forçado. Além disso, nesse estudo foi

evidenciado o envolvimento da enzima sulfidrílica monoamina oxidase A, como um possível mecanismo desse efeito do tipo antidepressivo. Esse resultado ressalta a importância do átomo de telúrio em interagir com enzimas sulfidrílicas.

Da mesma maneira que o PEBT, outros organocalcogênios demonstraram um efeito do tipo antidepressivo em roedores, além de uma inibição da atividade da MAO-A e MAO total (Savegnago et al. 2007; Bruning et al. 2011).

Dentre os medicamentos mais utilizados para o tratamento da depressão estão os inibidores da atividade enzima MAO, uma vez que essa enzima é responsável pela desaminação oxidativa dos neurotransmissores monoaminérgicos. A MAO apresenta duas isoformas, a MAO-A e a MAO-B, que apesar de catalisarem a mesma reação, apresentam diferenças quanto a seletividade a inibidores e substratos. Desta maneira, a MAO-A é seletivamente inibida pela clorgilina e desamina preferencialmente serotonina e noradrenalina, enquanto que a MAO-B degrada preferencialmente feniletilamina e é inibida seletivamente por selegilina. A dopamina e a tiramina são desaminadas por ambas as enzimas (Wouters, 1998; Costa et al., 2012).

Nesse contexto, os inibidores da MAO-A são mais utilizados no tratamento da depressão, uma vez que a inibição na atividade da MAO-A resultaria em um aumento nos níveis de serotonina no cérebro. Além disso, em humanos já foi relatado que um aumento nos níveis de serotonina leva a uma melhora no seu estado depressivo (Robinson, 2002; Costa et al, 2012).

Apesar dos resultados obtidos nesse estudo, mais pesquisas se fazem necessárias para esclarecer o potencial farmacológico e toxicológico do PEBT, bem como seus possíveis mecanismos de ação.

6. CONCLUSÃO

Podemos concluir com os resultados apresentados nesta dissertação que

- A presença do átomo de telúrio na estrutura química PEBT é fundamental para a oxidação catalítica de tióis de baixo peso molecular, bem como a oxidação dos grupos sulfidrílicos da enzima Na^+K^+ -ATPase cerebral *in vitro*.

- A avaliação de letalidade do PEBT estabeleceu que esse composto apresentou baixa toxicidade para camundongos na dose de 1mg/kg, contudo é tóxico para as larvas de *Artemia salina*.

- A administração aguda do PEBT em camundongos demonstrou um efeito do tipo antidepressivo no modelo experimental de depressão, teste do nado forçado. Além disso, foi demonstrado que a inibição seletiva da atividade da MAO-A pelo PEBT pode ser um possível mecanismo para a ação do tipo antidepressiva desse composto.

7. PERSPECTIVAS

Com base nos resultados obtidos neste trabalho, as perspectivas para trabalhos posteriores são:

- Estudar possíveis mecanismos envolvidos no efeito do tipo antidepressivo do PEBT, além de investigar mais as propriedades toxicológicas desse composto.

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