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

EXPOSIÇÃO MATERNA AO DITELURETO DE DIFENILA CAUSA ALTERAÇÕES COMPORTAMENTAIS E BIOQUÍMICAS EM FILHOTES DE RATO

TESE DE DOUTORADO

Eluza Curte Stangherlin

Santa Maria, RS, Brasil
2007

EXPOSIÇÃO MATERNA AO DITELURETO DE DIFENILA CAUSA ALTERAÇÕES COMPORTAMENTAIS E BIOQUÍMICAS EM FILHOTES DE RATO

por

Eluza Curte Stangherlin

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

Orientadora: Dra. Cristina Wayne Nogueira

Santa Maria, RS, Brasil

2007

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

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

EXPOSIÇÃO MATERNA AO DITELURETO DE DIFENILA CAUSA ALTERAÇÕES COMPORTAMENTAIS E BIOQUÍMICAS EM FILHOTES DE RATO

elaborada por
Eluza Curte Stangherlin

como requisito parcial para a obtenção do grau de
Doutora em Bioquímica Toxicológica

COMISSÃO EXAMINADORA:

_____
Cristina Wayne Nogueira, Dra. (Orientadora)

_____
Diogo Onofre Gomes de Souza, Dr. (UFRGS)

_____
Luiz Valmor Cruz Portela, Dr. (UFRGS)

_____
Aldo Bolten Lucion, Dr. (UFRGS)

_____
Susana Tchernin Wofchuk, Dra. (UFRGS)

Santa Maria, 28 de agosto de 2007.

À Yasmin,
minha filha amada

AGRADECIMENTOS

Agradeço à Deus, por estar sempre ao meu lado, por ter criado a ciência e tudo mais que nos cerca.

Aos meus pais, pelo exemplo de responsabilidade, amor e dedicação que norteiam a minha vida. A vocês, que sacrificaram os seus sonhos em favor dos meus, não sei se palavras conseguiriam traduzir o que eu sinto! Muito obrigado, eu amo muito vocês!

Ao Alberto, minha porção melhor, meu chão, por existir, pelo amor incondicional, pelo carinho, pelo apoio e por ter me dado o melhor presente do mundo, nossa filha Yasmin. À Yasmin, minha razão de viver. Ao Bruno, pelo carinho e pela paciência comigo.

À Cadinha e ao Marcos, ao Fi e a Ana, por fazerem parte da minha família, pelo companheirismo, pelo "ombro amigo", em todas as horas, amo muito vocês também!

Aos meus orientadores, Cris e GZ por me darem a oportunidade de tentar ser alguém, por acreditarem nos meus sonhos, e por serem pessoas tão maravilhosas, compreensivas e dedicadas. Agradeço por terem me ensinado muito mais do que eu poderia imaginar. Muito obrigado a vocês, meus amigos...

À Cristiane, à Fran, à Ana Ardais, à Simone Pinton, à Aninha e à Bibi, pelo companherismo, humor, trabalho e principalmente pela amizade.

À todo o pessoal do laboratório, em especial à Marina, à Ethel, ao Ricardo, ao Cristiano e à Lucielli.

À Nilda e ao Prof. João Batista, pelo apoio, pelo exemplo de trabalho, de dedicação e pela sabedoria.

Ao pessoal dos laboratórios dos Professores João, Gilson e Braga, por terem compartilhado os momentos especiais.

À CAPES, pela bolsa concedida. À UFSM, pela infra-estrutura e pela qualidade do ensino público e gratuito, fundamental para a minha formação profissional e pessoal.

Aprendi que para crescer como pessoa eu preciso me cercar de gente mais inteligente do que eu.

Willian Shakespeare

RESUMO

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

EXPOSIÇÃO MATERNA AO DITELURETO DE DIFENILA CAUSA ALTERAÇÕES COMPORTAMENTAIS E BIOQUÍMICAS EM FILHOTES DE RATO

AUTORA: ELUZA CURTE STANGHERLIN
ORIENTADORA: CRISTINA WAYNE NOGUEIRA
Data e Local da Defesa: Santa Maria, 28 de agosto de 2007.

O cérebro de roedores apresenta um rápido desenvolvimento após o nascimento. Sendo assim, o funcionamento do sistema nervoso pode ser alterado pela ação de xenobióticos durante esse período. As alterações podem ser avaliadas pelo desempenho dos animais em vários testes comportamentais, os quais são as manifestações finais das funções neurais ou ainda pela análise de parâmetros bioquímicos. O xenobiótico alvo desse estudo é um composto orgânico que contém o elemento telúrio na sua estrutura, o ditelureto de difenila. Esse estudo teve como objetivo avaliar o efeito da exposição materna ao ditelureto de difenila, durante o período de amamentação, sob aspectos comportamentais e bioquímicos, nos filhotes de ratos. Os resultados obtidos revelaram tendências comportamentais desinibitórias, determinadas pelo desempenho dos animais no labirinto em cruz-elevado. Os resultados obtidos revelaram, ainda, que os animais expostos ao ditelureto de difenila apresentaram um prejuízo cognitivo, observado no teste do reconhecimento do objeto. Sendo assim, sugere-se que esse composto consegue passar para os filhotes através do leite materno, provavelmente por ter uma natureza lipídica. Uma vez nos tecidos do filhote, ele tem a capacidade de injuriar o tecido cerebral, a ponto de causar alterações que se revelam nas mudanças comportamentais observadas. A investigação dos possíveis mecanismos pelos quais o ditelureto de difenila atua revelou que ele causou uma inibição da captação de glutamato em sinaptossomas de cérebro total e não interferiu no processo de liberação de glutamato, no mesmo ensaio. Esses eventos poderiam promover um aumento de glutamato na fenda sináptica. Porém, o favorecimento da neurotransmissão glutamatérgica parece estar mais relacionado com eventos inibitórios ou ainda, de facilitação dos processos relacionados com a cognição/memória, ou seja, comportamentos contrários aos observados nesse estudo. Dessa forma, a alteração da homeostase do sistema glutamatérgico ocasionada pelo ditelureto de difenila parece não estar diretamente relacionada com as alterações comportamentais observadas. Ainda, foi observada uma inibição na atividade da enzima Na^+,K^+-ATPase cerebral. Vários estudos relacionam a inibição da atividade da Na^+,K^+-ATPase com o prejuízo da memória. Sendo assim, esse é um dos prováveis mecanismos relacionados com o prejuízo cognitivo dos animais. Além disso, a avaliação bioquímica revelou que a exposição ao ditelureto de difenila causou uma série de alterações no status oxidativo cerebral dos filhotes. As estruturas cerebrais mais afetadas foram o hipocampo e o estriado. Nessas regiões, foi observado um aumento da peroxidação lipídica e uma inibição da atividade das enzimas superóxido dismutase, catalase e δ-aminolevulinato desidratase. Provavelmente a inibição da atividade das enzimas foi uma conseqüência do estresse oxidativo. Ainda, no estriado houve um aumento dos níveis de ácido ascórbico e de grupos tióis não-protéicos. No córtex, por sua vez, houve um aumento somente

dos níveis de grupos tióis não-protéicos. O aumento dos níveis desses dois antioxidantes não-enzimáticos pode ter sido uma resposta adaptativa dos tecidos cerebrais ao estresse. Sendo assim, as alterações oxidativas localizadas estão entre os prováveis mecanismos envolvidos nas alterações comportamentais observadas. Isso porque o hipocampo e o estriado, as duas regiões mais afetadas pelo estresse oxidativo, são as regiões mais relacionadas com desinibição e cognição.

Palavras-chave: Ditelureto de difenila, estresse oxidativo, comportamento, rato.

ABSTRACT

Thesis of Doctor's Degree
Federal University of Santa Maria, RS, Brazil

MATERNAL EXPOSURE TO DIPHENYL DITELLURIDE CAUSES BEHAVIORAL AND BIOCHEMISTRY ALTERATIONS IN PUP RATS

AUTHOR: ELUZA CURTE STANGHERLIN
ADVISOR: CRISTINA WAYNE NOGUEIRA
Date and Place of the defense: Santa Maria, 2007

The brain of rodents presents a rapid development after birth. Thus, the functioning of the nervous system can be modified by action of xenobiotics during this period. The alterations can be evaluated by the performance of animals in several behavioral tests, which are the end point of neural functions or still by analysis of biochemical parameters. Diphenyl ditelluride, an organottelurium compound, was the xenobiotic target of this study. The objective of the present was to evaluate the effects of maternal exposure to diphenyl ditelluride, during the suckling period, in behavioral and biochemical parameters in rat pups. The results obtained revealed disinhibitory tendencies, evidenced by performance of animals in the elevated plus-maze. Data of this study also showed that animals exposed to diphenyl ditelluride presented cognitive impairment, observed in the object recognition memory task. Therefore, we assume that diphenyl ditelluride can pass for pups through maternal milk, probably in view of its liposolubility. The investigation of the possible mechanisms of action by which diphenyl ditelluride induced behavioral changes revealed that this compound inhibited glutamate uptake and did not alter glutamate release in sinaptossomas of total brain. These events could promote an increase of glutamate in the synaptic cleft. However, the aiding of the glutamatergic neurotransmission seems to be more related to inhibitory events or still, of facilitation of the processes related with the cognition/memory, that are, contrary behaviors to the observed ones in this study. Thus, the alteration of the homeostasis of the glutamatergic system caused by diphenyl ditelluride seems not to be directly related to the observed behavioral alterations. An inhibition in the activity of cerebral Na^+,K^+-ATPase was observed. Some studies have reported the inhibition of Na^+,K^+-ATPase activity with the impairment of memory. Thus, this is one of the probable mechanisms related to the cognitive impairment of animals. Moreover, the biochemistry evaluation revealed that the exposure to diphenyl ditelluride caused a series of alterations in the cerebral oxidative status of the pups. The most affected cerebral structures by oxidative stress were hippocampus and the striatum. In these regions, it was observed an increase in lipid peroxidation and an inhibition of enzymes superoxide dismutase, catalase and d-aminolevulinate dehydratase activities. Probably the inhibition of the activity of these enzymes was a consequence of oxidative stress. Striatum had an increase of the levels of ascorbic acid and non-protein thiols. An increase of the levels of non-protein thiols was found only in the cortex. The increase of the levels of these two non-enzymatic antioxidants can have been an adaptive response of cerebral tissues to oxidative stress. The oxidative stress found in specific cerebral regions probably is involved in the mechanisms by which diphenyl ditelluride caused behavioral alterations. In

fact, hippocampus and striatum, the most affected cerebral regions related to disinhibition and cognition.

Keywords: Diphenyl ditelluride, oxidative stress, behavior, rat.

LISTA DE ILUSTRAÇÕES

Revisão Bibliográfica

Artigo 1

Artigo 2

Artigo 3

LISTA DE TABELAS

Artigo 1

LISTA DE ABREVIATURAS

d-**ALA-D**- delta aminolevulinato desidratase ou porfobilinogênio sintase

$(PhTe)_2$ - ditelureto de difenila

ALA - ácido 5'-aminolevulínico ou ácido delta-aminolevulínico

AMPA – ácido α-amino-3-hidroxi-5-metil-4-isoxazol-propiônico

ANOVA - análise de variância

CAT – enzima catalase

DL_{50} - dose letal para 50 % dos animais

DNA – ácido desoxirribonucléico

EROs- espécies reativas de oxigênio

GPx- enzima glutationa peroxidase

GSH- glutationa reduzida

KA – ácido caínico

LTM – memória de longo prazo (long-term memory)

mGluR- receptores glutamatérgicos metabotrópicos

Mk-801- (+)-5-metil-10,11-diidro-5H-dibenzo[a,d]ciclohepten-5,10-imina ou dizolcilpina

NMDA- N-metil-D-aspartato

PBG – porfobilinogênio

rpm – rotações por minuto

SNC- sistema nervoso central

SOD – enzima superóxido dismutase

STM – memória de curto prazo (short-term memory)

NPSH – grupos tióis não-protéicos

SUMÁRIO

APRESENTAÇÃO

Os resultados que fazem parte desta tese estão apresentados sob a forma de artigos, os quais se encontram no item ARTIGO E MANUSCRITOS CIENTÍFICOS. As seções Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas encontram-se nos próprios artigos e representam a íntegra deste estudo.

Os itens DISCUSSÃO E CONCLUSÕES, encontrados no final desta tese, apresentam interpretações e comentários gerais sobre o artigo e os manuscritos científicos contidos neste trabalho.

As REFERÊNCIAS BIBLIOGRÁFICAS referem-se somente às citações que aparecem nos itens INTRODUÇÃO, REVISÃO BIBLIOGRÁFICA, e DISCUSSÃO desta tese.

No item APÊNDICE encontram-se os demais trabalhos desenvolvidos durante o Curso de Doutorado.

1. INTRODUÇÃO

O cérebro é sensível à influência de fatores ambientais durante os primeiros períodos de desenvolvimento, tais como a gestação e a lactação. O cérebro de roedores, em particular, apresenta um rápido desenvolvimento após o nascimento, caracterizado por uma intensa síntese de proteínas e de ácido desoxirribonucléico (DNA). Dentro deste contexto, o funcionamento normal do sistema nervoso pode ser alterado pela ação de xenobióticos. Essas alterações podem ser avaliadas pelo desempenho dos animais em vários testes comportamentais, os quais são as manifestações finais das funções neurais. Pela análise comportamental podem ser identificadas, por exemplo, alterações na atividade motora espontânea, na coordenação motora, no estado de ansiedade e na memória dos animais. Outra forma de avaliar as conseqüências de uma intervenção química (como é o caso do uso de xenobióticos) sobre o desenvolvimento normal do cérebro jovem é analisar parâmetros bioquímicos nesse tecido. Seguindo esse raciocínio, várias são as ferramentas experimentais validadas na literatura para tal fim: (a) avaliação do status oxidativo, (b) determinação da atividade da enzima Na^+,K^+,ATPase, como marcador cerebral e (c) determinação de parâmetros glutamatérgicos. Nesse contexto, o glutamato é importante por ser o principal neurotransmissor excitatório do SNC. Ele é encontrado em altas concentrações no cérebro de mamíferos e está envolvido em vários processos fisiológicos, tais como o aprendizado, a memória e a formação de redes neuronais durante o desenvolvimento.

O xenobiótico alvo desse estudo é um composto orgânico que contém o elemento químico telúrio na sua estrutura, o ditelureto de difenila (Figura 1). Como o telúrio pertence ao grupo dos calcogênios (grupo 16) da tabela periódica, compostos orgânicos contendo esse elemento podem ser chamados genericamente de organocalcogênios. Compostos dessa natureza são reagentes muito utilizados em laboratórios de química como intermediários em reações de síntese orgânica. Recentemente, em virtude do uso de compostos contendo telúrio em diversos níveis industriais, inclusive na nanotecnologia, o risco de contaminação ocupacional motiva estudos toxicológicos. Os organocalcogênios podem afetar um grande número de processos neurais, e, além disso, se sabe que o ditelureto de difenila pode ser potencialmente teratogênico. Sendo assim, torna-se interessante o estudo dos efeitos desse organocalcogênio sobre o cérebro em desenvolvimento.

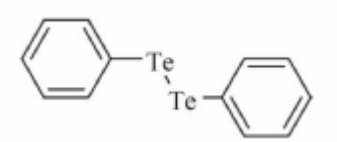


Figura 1 – Estrutura química do ditelureto de difenila.

2. REVISÃO BIBLIOGRÁFICA

2.1 Desenvolvimento Cerebral

O termo “desenvolvimento cerebral” é freqüentemente confundido na literatura com crescimento total ou ganho no peso total do cérebro. O desenvolvimento cerebral inclui a síntese de componentes celulares em paralelo à neurogênese e à gliogênese, migração de neurônios e células gliais, e diferenciação celular com aumento no tamanho da célula (Morgane, 2002). Todos estes processos são o resultado de várias reações químicas, incluindo passos críticos e limitantes.

A maturação do sistema nervoso e desenvolvimento da capacidade cognitiva dependem de três fatores essenciais: potencial genético do indivíduo, estimulação ambiental e nutrição adequada. Alterações no desenvolvimento cerebral pré-natal em humanos, a partir de um ou da combinação desses fatores, pode resultar em vários graus de disfunção cerebral (Morgane et al, 2002). Os dois maiores tipos de desordens envolvendo o desenvolvimento do SNC são: patologias causadas por lesões ou agentes tóxicos (resultando em processos destrutivos, com rápido crescimento e diferenciação) e desordens causadas por nutrição inadequada, considerando que nutrientes em quantidades apropriadas são essenciais para a formação celular e organização tecidual (Morgane et al., 2002).

Além disso, o crescimento de um órgão, incluindo o cérebro, ocorre por aumento no número de células (hiperplasia), aumento no tamanho das células (hipertrofia) ou por ambos os fenômenos. Entretanto, o cérebro não é um órgão homogêneo e o tempo de duração destas três fases difere nas várias regiões. Estas apresentam diferentes tipos celulares com características específicas de divisão e de possível migração para outros locais do sistema nervoso central (Winick, 1970). Portanto, a maturação do cérebro envolve uma série de fases que se sobrepõem temporariamente, em seqüência precisa, as quais são diferentes nas várias regiões cerebrais e dentro de uma região particular, além de variar de uma espécie animal para outra (Morgane, 2002).

No rato, a hiperplasia neuronal prevalece na vida pré-natal, ocorrendo principalmente durante a última semana de gestação (Dobbing e Sands, 1971). A neurogênese pós-natal é pequena quantitativamente, com produção de microneurônios com axônios curtos, especialmente no córtex cerebelar e hipocampo (Altman e Das, 1966; Croskerry et al., 1973). As células gliais apresentam proliferação pós-natal principalmente, ocorrendo durante o período de lactação (Figura 2). A mielinização, no rato, se dá principalmente nas duas semanas

iniciais de vida, quando declina abruptamente aos níveis adultos (Davidson e Dobbing, 1966). A sinaptogênese no cérebro do rato ocorre principalmente entre o 7º e 21º dia de vida pós-natal, podendo diferir de região a região. O aumento nos contatos sinápticos e a diferenciação destas conexões representam o começo do desenvolvimento químico e funcional do sistema nervoso central.

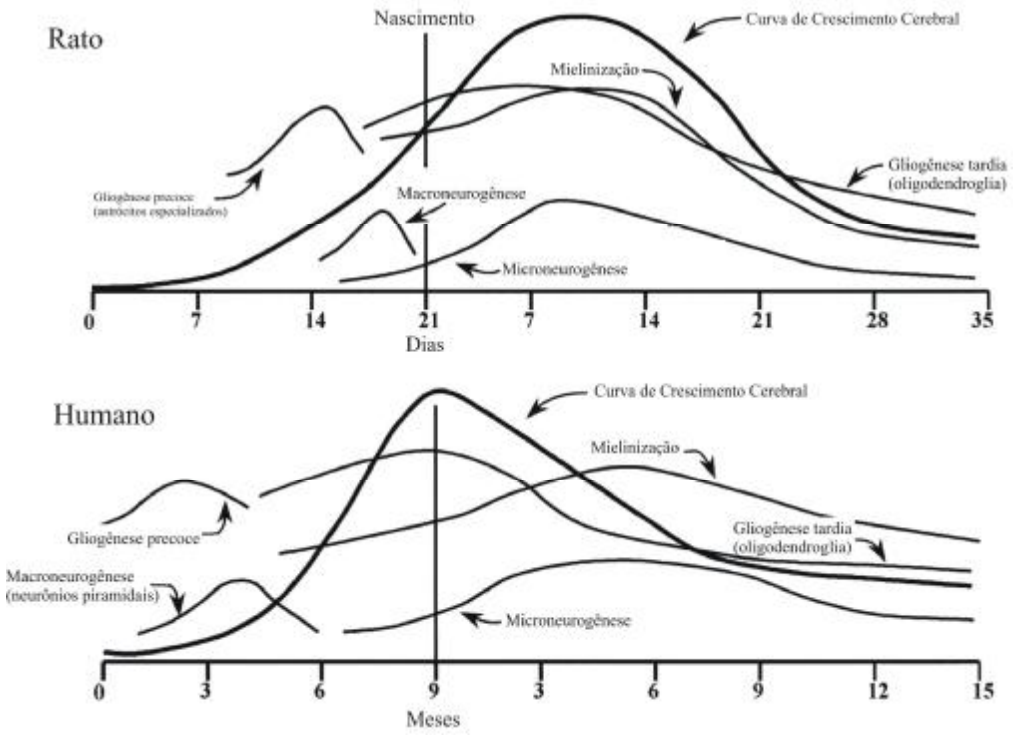


Figura 2 – Curva de Velocidade, comparando os índices relativos, duração e tempo do processo desenvolvimental específico em cérebro de ratos e humanos. As curvas de rápido crescimento cerebral (índices de mudança no peso cerebral) são sobrepostas em relação aos eventos desenvolvimentais no cérebro. Note que a gênese precoce de astroglia e células piramidais em humanos, resultando na aquisição de aproximadamente 27% do peso cerebral adulto no tempo do nascimento, comparado à aproximadamente 12% do peso cerebral adulto visto em ratos ao nascimento. A curva de rápido crescimento cerebral em ratos é alterada para a direita, comparado aos humanos. Fonte: adaptado de Morgane et al., 2002.

Sendo assim, o cérebro é extremamente sensível à influência de fatores ambientais durante os primeiros períodos de desenvolvimento, tais como a gestação e a lactação (Almeida et al., 1996; Annau e Cuomo, 1988; Rice, 1999; Rocha e Vendite, 1990). O cérebro de roedores apresenta rápido desenvolvimento após o nascimento caracterizado por uma intensa síntese de proteínas e de DNA (Gottlieb et al., 1977). Desse modo, o cérebro é sensível a alterações no seu desenvolvimento, provocadas por fatores externos durante os

períodos de rápido crescimento cerebral, após o nascimento (Annau e Cuomo, 1988; Rice, 1999; Rocinholi et al., 1997).

2.2 Análise Comportamental

O funcionamento normal do sistema nervoso pode ser alterado pela ação de xenobióticos. Essas alterações podem ser avaliadas pelo desempenho dos animais em vários testes comportamentais (Genn et al., 2003; Graeff et al., 1998; Lalonde et al., 2003), que são as manifestações finais das funções neurais.

2.2.1 Campo aberto (“open-field”)

O teste do campo aberto é uma medida da atividade motora espontânea e atividade exploratória dos animais. O aparato consiste em uma arena quadradra (45 x 45 cm) dividida em nove quadrantes. Cada animal é colocado individualmente no centro da arena e o número de segmentos atravessados (com todas as patas) é registrado durante um período de quatro minutos. Esse procedimento é repetido no dia seguinte. A exploração é um comportamento muito importante, onde o animal capta informações a respeito do ambiente que o cerca. As informações adquiridas são essenciais para a preservação da vida desse animal (Sutherland e Rudy, 1989).

2.2.2 Cilindro giratório (“rotarod”)

Esse é um teste comportamental que mede a coordenação motora dos animais. Consiste de um cilindro (7,5 cm de diâmetro) que gira a uma velocidade de 10 rpm. Os animais são colocados em cima do cilindro e o aparelho é ligado. O movimento locomotor é forçado no sentido de que se os animais não se movimentarem, eles caem do aparelho. Durante a realização do teste (que tem uma duração de quatro minutos por animal), são registrados o tempo que o animal leva para cair pela primeira vez (após, ele é imediatamente recolocado em cima do cilindro) e o número de quedas durante o tempo de observação do teste.

2.2.3 “Malabarismo” (“coat-hanger”)

Esse é um teste comportamental que também mede a coordenação motora dos animais. O aparelho consiste em um fio de aço horizontal (2 mm diâmetro x 40 cm comprimento), dividido em oito segmentos e suspenso a cerca de 50 cm de altura. Em cada uma das extremidades do fio se encontra uma plataforma. Os animais são colocados no centro do fio e o tempo para que atinjam uma das plataformas ou o tempo que levam para cair do aparelho, bem como o número de segmentos atravessados é registrado. Alterações no desempenho dos animais nesse teste são compatíveis com disfunções cerebelares (Lalonde e Strazielle, 1999).

2.2.4 Labirinto em “T” (“T-maze”)

É também chamado de teste da alternação espontânea. Consiste de um labirinto em formato de “T”, com dois braços (com 30 cm de comprimento) perpendiculares a um eixo central (com 45 cm de comprimento). Os animais são colocados na extremidade do eixo central, contrária aos braços. A latência decorrida até o animal atingir uma das extremidades de um dos braços é registrada e o animal é então retirado do aparelho. Passados dez segundos, o mesmo animal é testado novamente nas mesmas condições experimentais. Nesse teste se avalia a tendência natural que animal tem em alternar para o lado desconhecido conforme a repetição do teste. Esse é, portanto, um teste que mede a atividade exploratória dos animais. A alternação espontânea é uma conseqüência de processos cerebrais que se desenvolvem entre a segunda e a quarta semana de vida pós-natal (Egger et al., 1973). Alterações na performance dos animais nesse teste são compatíveis com retardo psicomotor (Lalonde e Strazielle, 1999).

2.2.5 Labirinto em cruz – elevado (“elevated plus-maze”)

Esse é um teste que avalia a ansiedade dos animais. O aparato consiste de quatro braços (comprimento: 50 cm, largura: 10 cm) que se cruzam em uma área comum, elevados a 50 cm do chão. Dois dos braços são fechados (com três paredes de 50 cm de altura) e os outros dois são abertos (sem paredes). Os animais são colocados para explorarem o aparato por cinco minutos. O número de entradas e o tempo gasto nos braços abertos e fechados são registrados. Esse é um teste amplamente usado para avaliação da ansiedade em modelos animais. E o aparato é farmacologicamente e etologicamente validado na literatura (Pellow et al., 1985). Nesse contexto, é avaliada a aversão natural que as animais têm ao espaço elevado.

Além disso, eles ficam explorando os braços ansiogênicos (abertos, ou “área aversiva”) ou os braços seguros (fechados). Uma interpretação alternativa para o desempenho dos animais que exploram mais os braços abertos é que eles podem estar mais desinibidos (Almeida et al., 1996; Lalonde et al., 2003; 2004). Do ponto de vista etológico, tendências desinibitórias excessivas podem não ser adaptativas e potencialmente expor o animal a situações de perigo. Além disso, esses paradigmas de alterações comportamentais podem ser interpretados mais em termos de impulsividade do que em termos de alterações no estado de ansiedade.

2.2.6 Reconhecimento do objeto

Esse teste avalia o desenvolvimento de memória de curto prazo (STM – short-term memory) e de longo prazo (LTM – long-term memory). Está baseado na capacidade que os animais possuem de reconhecer objetos familiares e novos objetos. É uma ferramenta experimental que avalia as funções neurais induzidas por drogas ou modificadas geneticamente, e é um teste de memória não aversivo, não espacial (Puma et al., 1999; Rampon et al., 2000).

2.3 Estresse Oxidativo

Durante o metabolismo basal das células aeróbicas normais existe uma produção constante de espécies reativas de oxigênio (ERO), acompanhada pela sua contínua inativação através da ação de antioxidantes, de forma a manter a integridade estrutural e funcional das biomoléculas. A extensão e o tipo de dano causado pelos ERO dependem da quantidade e da natureza dos mesmos, bem como das defesas antioxidantes celulares (Davies, 1991).

O desequilíbrio entre os fenômenos pró-oxidativos e as defesas antioxidantes celulares pode desencadear mudanças fisiológicas, denominadas genericamente de estresse oxidativo (Croft, 1998). Este pode estar relacionado com vários processos deletérios, tais como: mutagênese, carcinogênese, peroxidação lipídica, oxidação e fragmentação de proteínas e carbohidratos (Sies, 1986).

2.3.1 Defesas Antioxidantes

Halliwell e Gutteridge (1990) definem como antioxidante qualquer substância que, quando presente em baixas concentrações, comparadas a de um substrato oxidável, retarda ou inibe significativamente a oxidação deste substrato. Esta definição compreende compostos de natureza enzimática e não enzimática. Assim, por diferentes mecanismos, as ERO são inativadas de forma a impedir reações oxidativas posteriores de propagação (Sies, 1993).

Entre as principais enzimas responsáveis pela defesa antioxidante do organismo destacam-se a superóxido dismutase (SOD), a catalase (CAT) e a glutationa peroxidase (GPx), que constituem a primeira defesa endógena de neutralização das ERO. Com isso, as células tentam manter baixas as quantidades do radical superóxido e de peróxidos de hidrogênio, evitando assim, a formação do radical hidroxil (Boveris e Cadenas, 1997).

A SOD, presente na quase totalidade dos organismos eucarióticos, catalisa a dismutação do radical/ânion superóxido ($O_2^{\bullet -}$) em peróxido de hidrogênio (H_2O_2) (McCord e Fridovich, 1969). O H_2O_2 por sua vez é degradado pela ação da CAT ou GPx, resultando em água e oxigênio molecular (O_2) (Farber, 1990).

Entre os antioxidantes não enzimáticos destaca-se o ácido ascórbico (vitamina C), que tem-se mostrado eficiente contra as ERO (Rose, 1987). O ácido ascórbico age protegendo biomembranas contra a peroxidação, e perpetuando desta forma, a atividade do α-tocoferol, um antioxidante não enzimático lipossolúvel. O ácido ascórbico é um dos antioxidantes mais importantes em tecidos de mamíferos (Banhegyi et al., 1997), sendo ele eficiente na redução da toxicidade de vários xenobióticos (Chakraborty et al., 1978; Chatterjee e Rudra Pal, 1975).

2.4 Enzima delta-aminolevulinato desidratase (d-ALA-D)

A enzima citoplasmática delta-aminolevulinato desidratase (δ-ALA-D, E.C.4.2.1.24), também conhecida como porfobilinogênio sintase ou 5-aminolevulinato hidroliase foi isolada na década de 50 (Dresel e Falk, 1953). Esta enzima catalisa a condensação assimétrica de duas moléculas de ácido delta-aminolevuliníco (ácido 5-aminolevulínico, ALA), com perda de duas moléculas de água, para formar o composto monopirrólico porfobilinogênio (PBG) (Jaffe, 1995). A reação catalisada pela δ-ALA-D faz parte da rota biossintética dos compostos tetrapirrólicos (corrinas, bilinas, clorofilas e hemes). A grande importância destes compostos reside na sua função como grupos prostéticos de proteínas. O heme (ferroprotoporfirina) faz

parte da estrutura de proteínas que participam do transporte de oxigênio (hemoglobina e mioglobina), transporte de elétrons (citocromos a, b e c), biotransformação de xenobióticos (citocromo P_{450}) e do sistema de proteção contra peróxidos (catalases e peroxidases) (Jaffe, 1995). A via para a biossíntese de porfirinas é semelhante em bactérias, vegetais e animais, favorecendo a ampla distribuição da δ-ALA-D na natureza (Rodrigues, 1987).

A δ-ALA-D é uma enzima de natureza sulfidrílica que pode ser inibida na presença de agentes oxidantes, como por exemplo os compostos orgânicos contendo telúrio e selênio (Barbosa et al., 1998; Maciel et al., 2000; Farina et al., 2001), que podem inibir a atividade desta enzima por oxidarem grupos sulfidrílicos. A inibição da δ-ALA-D pode prejudicar a rota biossintética do heme, resultando em conseqüências patológicas (Sassa et al., 1989; Goering, 1993). Além da redução na síntese do heme, a inibição desta enzima pode resultar no acúmulo do substrato ALA no sangue, com conseqüente aumento na excreção urinária do mesmo. O acúmulo de ALA pode estar relacionado com a superprodução de espécies reativas de oxigênio (Pereira et al., 1992; Bechara et al., 1993). Além disso, o ALA gerado no fígado e medula óssea pode atravessar a barreira hemato-encefálica, apresentando efeitos neurotóxicos (Becker et al., 1971; Cutler et al., 1979).

2.5. Enzima Na^+,K^+,ATPase

A Na^+,K^+-ATPase (EC 3.6.1.37) é uma enzima de natureza sulfidrílica, sensível a agentes oxidantes (Carfagna et al., 1996; Folmer et al., 2004). Ela se encontra embebida na membrana celular e é responsável pelo transporte ativo dos íons sódio e potássio no sistema nervoso. Sendo assim, sua ação regula as concentrações de Na^+/K^+, regulando, portanto, o gradiente iônico através da membrana plasmática. Esse processo é requerido para as funções vitais como co-transportes pela membrana, regulação do volume celular e excitabilidade (Doucet, 1988; Jorgensen, 1986).

Essa enzima dimérica tem várias isoformas no cérebro e consome grande parte da energia disponível (Bertorello e Kats, 1995). Ela está presente em altas concentrações no tecido cerebral, chegando a consumir cerca de 40 a 50 % de todo o ATP gerado nesse tecido. (Erecinska e Silver, 1994). A inativação da Na^+,K^+-ATPase leva a uma despolarização parcial da membrana, seguida de uma entrada excessiva de Ca^+ para dentro das células neuronais, o que resulta em eventos tóxicos, tais como a excitotoxicidade (Beal et al., 1993).

2.6. Glutamato

As principais vias excitatórias do sistema nervoso central utilizam glutamato como neurotransmissor (Ozawa et al., 1998; Meldrum et al, 1999). Na década de 50, foi demonstrado pela primeira vez que o L-glutamato e outros aminoácidos, de ocorrência natural, estavam envolvidos na excitação neuronal em cérebro de mamíferos (Collingridge e Lester, 1989; Bennett e Balcar, 1999).

O glutamato é encontrado em altas concentrações (10 mM) nesse tecido e está envolvido em vários processos fisiológicos, tais como o aprendizado, a memória e a formação de redes neurais durante o desenvolvimento (Ozawa et al., 1998). Recentemente, estudos têm relacionado alguns distúrbios psiquiátricos e doenças neurodegenerativas com alterações periféricas e centrais na expressão e sensibilidade dos receptores glutamatérgicos ao glutamato (Ferrarese et al., 2000, 2001; Berk et al., 2000).

Este aminoácido é sintetizado nos terminais pré-sinápticos, predominantemente a partir de glutamina através da ação da enzima glutaminase, mas pode provir do α-cetoglutarato, via glutamato desidrogenase e α-cetoglutarato aminotransferases (Kvamme et al., 1998). Um aumento nas quantidades de glutamato na fenda sináptica pode levar à estimulação excessiva dos receptores glutamatérgicos (excitotoxicidade) com conseqüente morte neuronal (Lipton e Rosenberg, 1994). Entretanto, a ação excitatória do glutamato é finalizada através de sua captação pelas células gliais ou pelos neurônios pré-sinápticos, onde é armazenado nas vesículas sinápticas.

A captação do glutamato da fenda sináptica envolve dois sistemas de transporte: um sistema de alta afinidade e dependente de Na^+, localizado nas membranas pré-sinápticas e gliais (Robinson e Dowd, 1997) e outro com baixa afinidade e independente de Na^+, nas membranas das vesículas sinápticas. A captação de glutamato apresenta uma função vital na manutenção de altos níveis de precursores de glutamato e baixas concentrações extracelulares deste neurotransmissor (Dichter e Wilcox, 1997).

O glutamato, uma vez armazenado, poderá ser liberado na fenda, desde que as membranas pré-sinápticas sejam despolarizadas. Após sua liberação, o glutamato exerce suas funções fisiológicas ativando os receptores localizados nas membranas pré e pós-sinápticas, bem como nas membranas das células gliais (Meldrum et al., 1999).

Os receptores glutamatérgicos podem ser classificados de acordo com estudos farmacológicos e moleculares, em dois grandes grupos: receptores ionotrópicos e metabotrópicos (Dichter e Wilcox, 1997; Ozawa et al., 1998). Os receptores ionotrópicos são

canais iônicos que permeiam cátions através da membrana neuronal, desencadeando uma resposta excitatória. Estes receptores são subdivididos em N-metil-D-aspartato (NMDA); ácido α-amino-3-hidroxi-5-metil-4-isoxazol-propiônico (AMPA) e ácido caínico (KA), com base na sua sensibilidade a agonistas específicos.

Os receptores NMDA medeiam a transmissão excitatória lenta, são canais com grande permeabilidade ao Ca^{2+} e baixa permeabilidade ao Na^{+} e K^{+} (Lipton e Rosemberg, 1994; Ozawa et al., 1998). Esse receptor apresenta diversos sítios para ligantes que regulam a abertura do canal: um sítio para o glutamato ou NMDA, um sítio para o co-agonista endógeno glicina (insensível à estricnina), um sítio para a união de bloqueadores (MK-801) e sítios modulatórios, tais como um sítio para o zinco, outro para as poliaminas, um sensível à modulação redox (modulado por agentes oxidantes ou redutores) e um sensível a prótons (Gozlan e Bem-Ari, 1995; Piggott et al., 1992; Euler e Liu, 1993; Ozawa et al., 1998).

Os receptores AMPA medeiam a neurotransmissão excitatória rápida e são canais com grande permeabilidade a cátions monovalentes (Na^{+} e K^{+}) e com baixa permeabilidade ao Ca^{2+} (Dichter e Wilcox, 1997). Os receptores KA diferem da maioria dos receptores AMPA por serem relativamente permeáveis aos íons Ca^{2+} (Ozawa et al., 1998) e estão concentrados em poucas áreas cerebrais, ao contrário dos AMPA, que apresentam ampla distribuição no SNC (Scatton, 1993).

Os receptores metabotrópicos (mGluRs) estão associados a sistemas de segundos mensageiros intracelulares (Conn e Pinn, 1997). Esses receptores são acoplados a proteínas G e modulam a atividade de efetores intracelulares, tais como adenilato ciclase e fosfolipase C, responsáveis pela produção de segundos mensageiros (Schoepp e Conn, 1993; Cotmann et al., 1995). Os mGluRs estão envolvidos na participação da indução da plasticidade neuronal (Bliss e Collinbridge, 1993). Entretanto, possuem papel importante na indução de convulsões e morte neuronal (Tizzano et al., 1995; Nicoletti et al., 1996). Sua ativação pode promover efeitos excitatórios e inibitórios (Ozawa et al., 1998).

2.7 Organocalcogênios

A partir da década de 30, os organocalcogênios têm sido alvo de interesse para os químicos orgânicos em virtude da descoberta de aplicações sintéticas (Petragnani et al., 1976; Comasseto, 1983) e de propriedades biológicas desses compostos (Parnham e Graf, 1991; Kanda et al., 1999), que são importantes intermediários e reagentes utilizados em síntese orgânica (Paulmier, 1986; Braga et al., 1996; 1997).

Conseqüentemente, o risco de contaminação ocupacional por organocalcogênios tem motivado estudos toxicológicos. Outro aspecto relevante é a tentativa crescente de desenvolvimento de compostos organocalcogênios que possuam atividades biológicas e aplicações farmacológicas (Parnham e Graf, 1991; Nogueira et al., 2003a).

2.7.1 Telúrio

O elemento telúrio foi descoberto em 1782. Entretanto, a inclusão desse átomo em moléculas orgânicas ocorreu no início do século XIX. No Brasil, a química de telúrio foi introduzida pelo Prof. Reinbolt, o qual se dedicou ao estudo sistemático de compostos orgânicos contendo telúrio e sua aplicabilidade como intermediários em síntese orgânica (Petragnani, 1995; Comasseto et al., 1997; Zeni et al., 2003).

O telúrio é um elemento que pertence ao grupo 16 da tabela periódica, podendo apresentar-se sob múltiplos estados de valência, que vão de –2 a +6 (apresenta-se sob quatro estados de oxidação: telurato (Te^{+6}), telurito (Te^{+4}), telúrio elementar (Te^{0}) e telureto (Te^{-2})) (Scansetti, 1992). Esse elemento é encontrado com maior frequência na forma de teluretos com ouro, bismuto, chumbo e prata.

Telúrio elementar (Te^{0}) é usado como componente de muitas ligas metálicas, na composição da borracha, na indústria de microchips, de componentes eletrônicos e em sistemas de energia fotovoltáica. Ele também é utilizado na produção industrial de vidro e aço, e como um aditivo anti-detonante na gasolina (Fairhill, 1969). O telúrio é encontrado em muitos minérios, juntamente com o selênio, e é fabricado como um sub-produto no refinamento do cobre, do chumbo, do bismuto e de outros metais (U.S. Bureau of Mines, 1985). Atualmente, telúrio inorgânico é encontrado em soluções oxidantes que servem para polir metais (Yarema e Curry, 2005) e na indústria de semicondutores particulados (Green et al., 2007; Zhang e Swihart, 2007).

2.7.1.1 Potencial farmacológico

Os efeitos do telúrio sobre o organismo animal começaram a ser estudados por Gmelin (1824). Os compostos orgânicos de telúrio apresentam propriedades imunomoduladoras, podem ser usados como drogas antitumorais e antivirais, e apresentam propriedades antiinflamatórias (Sredni et al., 1987, 1988; Nyska et al., 1989, Sun et al., 1996). Estudos recentes têm demonstrado que os diteluretos de diarila podem apresentar atividade

antioxidante (Engman et al., 1995; Andersson et al., 1994; Kanda et al., 1999) e propriedade de mimetizar a atividade da enzima GPx (Andersson et al., 1993; Engman et al., 1992), uma importante enzima endógena que participa de reações de neutralização de agentes pró-oxidantes.

Conseqüentemente, o emprego farmacológico desses agentes poderá crescer nos próximos anos.

Sabe-se que o telúrio metálico está presente na composição de organismos vegetais, particularmente em membros da família *Alium*, tais como o alho (Larner, 1995). Alguns estudos já demonstraram que pequenas quantidades de telúrio foram identificadas nos fluidos corporais, tais como sangue e urina (Siddik e Newman, 1988; Newman et al.,1989). Estudos demonstraram também que esse elemento está presente na forma de telurocisteína e telurometionina em muitas proteínas de bactérias (Boles et al., 1995; Budisa et al., 1995), leveduras (Yu et al., 1993) e fungos (Ramadan et al., 1989). Mas, até o presente momento, proteínas contendo telúrio não foram identificadas em células animais. Por isso, o telúrio não apresenta função fisiológica descrita até o momento, em mamíferos (Taylor, 1996).

2.7.1.2 Propriedades toxicológicas

O aumento do uso industrial de produtos químicos provoca riscos ocupacionais e ambientais para a saúde humana, e cresce a preocupação em relação aos potenciais efeitos adversos desses compostos. Os primeiros relatos a respeito da toxicidade do telúrio aconteceram após o acidente de Windscale (UK) (Stewart e Crooks, 1958).

O telúrio pode ser prontamente absorvido pelo organismo, através da dieta, principalmente na forma de compostos orgânicos. Entretanto, a exposição e a absorção de telúrio inorgânico na forma de teluritos e teluratos também ocorre (Larner, 1995).

Casos de intoxicação ocupacional aguda por telúrio são raros, entretanto, quando ocorrem, os sintomas são: dores de cabeça, sonolência, náuseas, alteração da freqüência cardíaca, bem como odor característico de alho, na respiração e na urina (Müller et al., 1989; Taylor, 1996).

A toxicidade desse elemento parece estar relacionada ao seu estado de oxidação (Van Vleet et al., 1982). O mecanismo proposto para explicar essa toxicidade envolve a oxirredução de grupos –SH de moléculas biologicamente ativas (Blais et al., 1972; Young et al., 1981; Deuticke et al., 1992).

Por bloquearem a síntese do colesterol, que é um precursor da mielina, compostos que contêm telúrio são potentes agentes neurotóxicos. Os compostos de telúrio inibem a atividade da enzima esqualeno monooxigenase, responsável pela conversão do esqualeno à 2,3-epoxiesqualeno, um precursor do colesterol. Dessa forma, o esqualeno acaba se acumulando. A sensibilidade da enzima ao telúrio se deve à reação desse elemento com grupamentos sulfidrílicos e com a ligação de cisteínas vicinais (Laden e Porter, 2001). Sendo assim, o telúrio inibe a síntese de colesterol nas células de Schwann, o que resulta no bloqueio da formação de mielina e no acúmulo de esqualeno.

A conseqüência desse processo é uma desmielinização ou hipomielinização, que pode ser a causa das neuropatias ocasionadas por esses compostos (Wagner-Recio et al., 1994). Os efeitos da intoxicação com telúrio no sistema nervoso têm sido estabelecido como sugestivo de neuropatia periférica durante um período ativo de mielinogênese (Duckett et al., 1979; Harry et al., 1989; Lampert e Garrett, 1971), afetando a produção de proteínas mielínicas à nivel de gene (Morell et al., 1994). Alterações neuromusculares têm sido identificadas em animais após a administração de telurito inorgânico (Duckett et al., 1979). A suscetibilidade preferencial do sistema nervoso periférico à toxicidade do telúrio depende, provavelmente, da grande demanda de colesterol pelos nervos periféricos, e uma menor taxa de acúmulo de colesterol no cérebro (Rawlins e Smith, 1971).

Seguindo esse raciocínio, alguns autores sugerem que o ditelureto de difenila, um composto orgânico que contém telúrio, é neurotóxico para camundongos (Maciel et al., 2000; Nogueira et al., 2001; 2002; Moretto et al., 2003), além de causar toxicidade renal e hepática em roedores, quando administrado em doses muito baixas (Meotti et al., 2003). Além disso, o ditelureto de difenila é capaz de reduzir a neurotransmissão glutamatérgica em plaquetas de humanos (Borges et al., 2004) e de inibir a enzima δ-aminoluvulinato desidratase (δ-ALA-D) em eritrócitos de humanos (Nogueira et al., 2003b).

Os compostos de telúrio, que têm propriedades metálicas, atravessam a barreira cérebro-sangue, barreira placentária e a barreira ependimal-fluido cérebro espinhal-sangue fetal (Duckett e Ellem, 1971). Já foi demonstrado que telúrio metálico atravessa as membranas celulares e se localiza no citoplasma das células (Blinzinger e Hager, 1965, Mizuno, 1969), mais especificamente nas mitocôndrias, nos primeiros estágios de intoxicação (Duckett e White,1974). E que a ingestão de determinadas quantidades de telúrio por mamíferos adultos e pássaros faz com que apareçam grânulos negros ou cristais em forma de agulha no citoplasma das células do sistema urogenital, do trato alimentar, dos órgãos respiratórios, do sistema reticulo-endotelial e do sistema nervoso (Pentschew et al., 1962, Carlton e Kelly, 1967). O

metabolismo do telúrio nos tecidos não está esclarecido, mas é sugerido que os depósitos negros ou os cristais puntiformes são telúrio reduzido ou telúrio elementar (Duckett, 1972).

2.7.1.3 O telúrio e a toxicidade desenvolvimental

Estudos descreveram o aparecimento de hidrocefalia e observaram a presença de telúrio nos tecidos fetais de rato. Essa foi a primeira vez que a presença de um agente teratogênico para hidrocefalia foi demonstrado em fetos. Malformações em outros órgãos não foram verificadas nos animais hidrocefálicos no estudo em questão. Esses dados foram confirmados posteriormente (Agnew et al., 1968; Duckett, 1971).

Também foram realizados estudos que avaliaram o momento particular (período crítico) em que o telúrio metálico estaria causando dano aos tecidos fetais. Constatou-se que a administração de telúrio, em ratas, no período do 10º ao 15º dia de gestação, induziu o surgimento de malformações congênitas (Duckett e Scott, 1971). Outro estudo, porém, indicou os dias 9 e 10 da gestação, também em ratas, como sendo o período mais suscetível ao aparecimento de hidrocefalia induzida por esse composto (Agnew e Curry, 1972). Foi avaliada a arquitetura das alterações que levavam à hidrocefalia, por meio de lâminas histológicas. Nestas, foi observada uma estenose dos aquedutos cerebrais, associada com o fechamento do espaço subaracnoideo pelo aumento do volume nos ventrículos cerebrais fetais. A possível causa da hidrocefalia induzida pelo telúrio, no protocolo experimental utilizado, pode ser, entre outras, a superprodução de fluido cerebroespinhal e/ou a não absorção desse fluido. As alterações observadas foram incompatíveis com a vida (Duckett, 1972).

O dióxido de telúrio, um composto inorgânico que se demonstrou teratogênico, induziu a formação de hidrocefalia, edema, exoftalmia, hemorragia ocular, hérnia umbilical, a não descida dos testículos, rins pequenos e diminuição no tamanho corporal, de uma maneira relacionada à dose, em fetos de ratas Wistar, quando administrado diariamente em injeções s.c., na mãe, do dia 15 ao dia 19 da gestação (Perez-D'Gregorio e Miller, 1988).

Dessa forma, pode-se constatar que compostos contendo telúrio são altamente tóxicos, particularmente para mamíferos em desenvolvimento. Além disso, o ditelureto de difenila, quando administrado em ratas prenhas, causa múltiplas malformações nos fetos em desenvolvimento (Stangherlin et al., 2005). Apesar dos estudos já realizados, não existem na literatura dados referentes ao possível efeito tóxico do ditelureto de difenila sobre o desenvolvimento cerebral dos ratos após o nascimento.

3. OBJETIVOS

A partir da exposição materna ao ditelureto de difenila, durante as duas primeiras semanas do período lactacional, a presente tese teve como objetivos estudar as alterações tardias ocorridas nos filhotes, sob os seguintes aspectos:

- Avaliação comportamental: campo aberto, cilindro giratório, labirinto em T, malabarismo, labirinto em cruz elevado e reconhecimento do objeto;
- Determinação da captação e liberação de [^{3}H] glutamato em sinaptossomas de cérebro total;
- Avaliação da atividade da enzima Na^+, K^+ATPase cerebral;
- Determinação do status oxidativo em estruturas cerebrais (córtex, hipocampo e estriado): estimativa dos níveis de peroxidação lipídica, ácido ascórbico e tióis não-protéicos, e avaliação da atividade das enzimas superóxido dismutase, catalase e δ-ALA-D.

4- ARTIGO E MANUSCRITOS CIENTÍFICOS

4.1 – Artigo 1:

Exposição materna ao ditelureto de difenila durante o período de amamentação altera as tendências comportamentais da sua prole

EXPOSURE OF MOTHERS TO DIPHENYL DITELLURIDE DURING THE SUCKLING PERIOD CHANGES BEHAVIORAL TENDENCIES IN THEIR OFFSPRING

Eluza C. Stangherlin, Alexandre M. Favero, Gilson Zeni, João B.T. Rocha,
Cristina W. Nogueira*

Brain Research Bulletin 69 (2006) 311–317

ELSEVIER

Brain Research Bulletin 69 (2006) 311–317

BRAIN RESEARCH BULLETIN

www.elsevier.com/locate/brainresbull

Exposure of mothers to diphenyl ditelluride during the suckling period changes behavioral tendencies in their offspring

Eluza C. Stangherlin, Alexandre M. Favero, Gilson Zeni, João B.T. Rocha, Cristina W. Nogueira*

Departamento de Química, Universidade Federal de Santa Maria, Camobi, CCNE, 97105900 Santa Maria, Rio Grande do Sul, Brazil

Received 23 September 2005; received in revised form 22 December 2005; accepted 5 January 2006
Available online 19 January 2006

Abstract

The long-lasting possible influence of maternal exposure to 0.03 mg/kg of diphenyl ditelluride during the first 14 days of lactational period on later offspring behavior was examined in Wistar rats. Open-field locomotor activity, spontaneous alternation in the T-maze, behavior in the elevated plus-maze, motor coordination in the coat-hanger and rotorod tasks were evaluated in 30 day old pups. There were no significant specific overt signs of maternal intoxication. There were a small (less than 5%) but significant transitory differences in the body weight gain of pups between exposed and control groups, which were apparent from day 30 of suckling. Locomotor activity in the open-field task was similar between telluride and control groups. In the coat-hanger test, the latency before falling for the tellurium group was higher than that of the control group. However, the behavior of both groups was similar in the rotorod test and spontaneous alternation in the T-maze. Tellurium-treated pups presented a higher number of entries and spent more time in the open arms of the elevated plus-maze than control pups. The behavioral alterations observed here after tellurium exposure can be cautiously interpreted as an indication of behavioral disinhibition. In conclusion, this study demonstrated that dam exposure to diphenyl ditelluride can cause subtle behavioral changes in the offspring, which can be related to neurotoxic effects of diphenyl ditelluride.

Keywords: Organotellurium; Behavioral; Pups; Toxicity; Lactation

1. Introduction

Inorganic tellurium is widely used in rubber, metallurgic and electronic industries [15], which increases the risk of occupational and environmental human exposure to this element. Although still incipient, it is reasonable to presume that the occupational exposure of humans to organic tellurium compounds will increase in the near future due to tellurium importance in organic synthesis [9]. Furthermore, there are also indications that organotellurium compounds may have potential therapeutic applications [36].

Exposure of experimental animals to tellurium can cause a variety of toxic effects, including reversible hind limb paralysis due to demyelination of the sciatic nerve and spinal roots [27,26]. Tellurium exposure for more than 1 year can also be accumulated in brain lysossomes without causing overt neurological symptoms in rodents [7,10]. There is also evidence that even after cessation of exposure to tellurium the metal remains stored in tissues for long periods of time [8].

Moreover, dietary exposure to high levels (3300 ppm) of metallic tellurium causes persistent neuromotor impairment which is associated with a severe deficit in shock avoidance. Although these results indicate a clearly neurotoxic effect of tellurium, their interpretation in terms of learning were hampered because the behavioral deficits observed can be a consequence of an impaired motor ability of the subjects. Furthermore, tellurium could also cause a lowered sensitivity to noxious stimulus, which in turn would retard the learning of the active avoidance task [11]. Although the behavioral deficits observed in this aversive paradigm could be related to either motor or motivational impairments, sodium tellurite intoxication causes a consistent deficit in a non-aversive spatial learning in water maze task that could

* Corresponding author. Tel.: +55 55 32208140; fax: +55 55 32208978.
E-mail address: criswn@quimica.ufsm.br (C.W. Nogueira).

0361-9230/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2006.01.004

not be overtly linked to motor or motivational impairment in tellurium-exposed animals [57].

Dimethyltellurium, an important compound derived from inorganic tellurium metabolism in mammals, has been reported as an inducer of peripheral neuropathy in rats [18]. In fact, dimethyltellurium is a potent inhibitor of squalene monooxygenase a downstream enzyme in the cholesterol biosynthetic pathway [22]. The resulting inhibition of cholesterol synthesis blocks myelin formation and causes the accumulation of squalene in Schwann cells, leading to peripheral segmental demyelination and paralysis [56].

It is now well established that the brain is extremely sensitive to environmental factors during early periods of development such as gestation and lactation [3,6,42,43]. Rodent brain presents three stages of rapid post-natal brain development characterized by intense protein and DNA synthesis [19]. Of particular importance, brain is extremely sensitive to developmental disruption by external factor during these periods of post-natal brain growth [6,42,45].

Diphenyl ditelluride can be teratogenic to rat fetuses, causing malformations in fore- and hind-limbs, absent or short tail, subcutaneous blood clots, exophthalmia, hydrocephalus and presence of exposed brain [51]. Besides, our laboratory have obtained persuasive evidence indicating that diphenyl ditelluride is a neurotoxic compound for mice [28,31,33–36] and provided evidence for renal and hepatic toxicity of this organotellurium compound for rodents [29].

As pointed out above organotellurium compounds, such as AS-101 has been described to possess immunomodulating properties and used experimentally as an antitumor drug [37,47,49,50]. Other organotellurium compounds possess antioxidant and glutathione peroxidase-like properties [4,5,13,14]. Thus, based on their potential use as a component of a range of therapeutic agents, toxicological studies are important to determine the safety of this class of compounds.

Since there are no data about the behavioral effect of diphenyl ditelluride exposure during post-natal period, the present investigation was conducted to determine the effects of diphenyl ditelluride administration to mothers on the latter behavioral performance of their offspring in various tasks, including motor activity and habituation in the open-field, spontaneous alternation in the T-maze, anxiety in the elevated plus-maze and motor coordination in the coat-hanger and rotorod.

2. Materials and methods

2.1. Materials

Diphenyl ditelluride (Fig. 1) was synthesized according to literature method [39]. Analysis of the ^{1}H NMR and ^{13}CNMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of diphenyl ditelluride (99.9%) was determined by GC/HPLC. Diphenyl ditelluride was diluted in canola oil which was obtained from a standard commercial supplier.

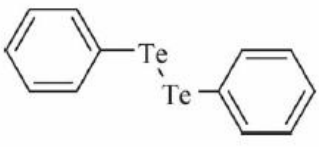


Fig. 1. Structure of diphenyl ditelluride.

2.2. Animals

Virgin female Wistar rats (180–240 g) from our own breeding colony were used. The animals were kept on a 12 h light/dark cycle, at a room temperature of 22 °C, with free access to food and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of Sao Paulo, Brazil.

2.3. Experimental procedure

Sexually naive female rats were mated with male previously tested as fertile (three females and one male in each cage). The onset of pregnancy was confirmed by the presence of sperm in vaginal smears (day 0 of pregnancy) and pregnant dams were immediately housed in individual cages. The dams received diphenyl ditelluride (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) via subcutaneous (s.c.) injection once daily during the first 14 days of lactational period. The dose of diphenyl ditelluride used in this study was 1/3 of LD_{50}, selected on the basis of LD_{50} study carried out in our laboratory [29]. Maternal body weight was recorded during this period. Pups body weight was recorded once daily until PND 30. At birth, all litters were culled to eight pups. Whenever possible, only male rats were kept within the litter and females were kept just to maintain equal litter sizes. On 21 postnatal day (PND 21), pups used for testing were weaned and placed on ad libitum standard rat chow diets.

After 1-week post-weaning period, the behavioral tests were conducted in the morning and early afternoon after PND 30. The testing schedule included spontaneous alternation in a T-maze (day 30), exploration of an open-field (days 30 and 31), motor coordination in the coat-hanger-type test (days 31 and 32) and in the rotorod (days 32 and 33) and anxiety in the elevated plus-maze (day 33). The behavioral observations were blind, and carried out under low-intensity light. Only male pups were used in the behavioral tests, litter was invariably constituted of four animals. The litters (7 control and 12 diphenyl ditelluride treated) were tested in all behavioral tests.

2.3.1. Open-field

Spontaneous motor activity was measured in the open-field test. The open-field was made of plywood and surrounded by walls 30 cm in height. The floor of the open-field, 45 cm in length and 45 cm in width, was divided by masking tape markers into 09 squares (3 rows of 3). Each animal was placed individually in the center of the arena and the number of segments crossed (4 paw criterion) were recorded during a 4-min session per 2 days. In order to evaluate intra session habituation, crossing was scored at each 2 min interval.

2.3.2. T-maze

Spontaneous alternation was tested in a T-maze made of plywood, consisting of two arms (length: 30 cm) perpendicular to a central stem (length: 45 cm). The maze was subdivided in parts of 15 cm separated for walls (30 cm in height) with holes. The external walls had 30 cm in height and the common area to arms and the central stem had 15 cm × 15 cm. On the first trial, the rats were placed in the extremity (contrary to arms) of the central stem of the T-maze for a free-choice trial. The latency to reach the extremity of the arm was recorded, as well as the side of alternation. After 10 s, the rats were placed back in the T-maze for a second trial. Spontaneous alternation was calculated as % of correct response of the litter, consequently, if one rat from a litter did not alternate, a score of 75% was attributed to this litter.

2.3.3. Elevated plus-maze

The apparatus consisted of four arms (length: 50 cm, width: 10 cm, height from floor: 50 cm) in a cross-shaped form and a central region (10 cm^2). Two of the arms were enclosed on three sides by walls (height: 50 cm), whereas the other two were not. The enclosed or open arms of the maze faced each other. The rats

were placed in the central region face an open arm and their behavior evaluated for 10 min. The number of entries and the time spent in either the enclosed or the open arms (4-paw criterion) were recorded for each 5 min interval. The rearing (count of number that the animal stood on its hind legs) was registered. As in all tests of exploration, the apparatus was wiped with a damp cloth and dried before the introduction of the next rat.

2.3.4. Coat-hanger

Motor coordination was measured in coat-hanger test with modifications. The coat-hanger consisted of a horizontal steel wire (diameter: 2 mm, length: 40 cm), divided into eight segments, terminating at each end by a platform (diameter: 5 cm), and suspended at a height of 38 cm from the cushioned floor. The rats were placed upside-down in the middle of the horizontal wire and released when all four paws gripped it, in order to ensure a stable position at the start of testing. This test was performed in 2 consecutive days of 2 trials/day with a cut-off period of 60 s and an intertrial interval of 10 min. Three types of movement time (MT) were compiled: latencies before reaching either side-platform with 2 (MT-1), 3 (MT-2) or 4 (MT-3) paws. The latencies before falling (MT-4) and the number of segments crossed were also assessed. A trial was terminated whenever a rat fell or else reached the apparatus's platform. In the latter case, it was immediately retrieved and a maximal score of 60 s given for latencies before falling.

2.3.5. Rotorod

Rotorod test was performed for measure motor coordination. The rotorod consisted of a wooden beam covered with masking tape (diameter: 7.5 cm, length: 30 cm), used for increasing the roughness of the texture and thereby providing a firm grip. The rod was flanked by two cardboard plates in order to prevent any escape and suspended at a height of 30 cm above the mat-covered table. The rats were placed on top of the already revolving beam (10 rpm) and facing away from the experimenter's view in the orientation opposite to that of the beam movement in the longitudinal axis, so that forward locomotion was necessary in order to avoid a fall. Latencies before falling, number of animals that fell and number fall were measured for two trials of 4-min per session for 2 days, with an intertrial interval of 10 min.

2.4. Statistical analysis

The litter (four animals averaged) was considered the experimental unit in all statistical analyses performed. Statistical significance was assessed by analysis of variance (ANOVA) with repeated measures, when appropriated. Post hoc Duncan's test was carried out when appropriated. A value of $p < 0.05$ was considered to be significant.

3. Results

3.1. Maternal observations and body weight of pups

There were no significant specific overt signs of maternal intoxication (tremor and reduction of body weight) following administration of diphenyl ditelluride during the suckling period (data not shown).

Regarding body weight of pups, two-way ANOVA 2 treatments (control or diphenyl ditelluride) × days revealed a significant effect of tellurium treatment ($F(1,8) = 9.84$, $p < 0.013$) and of days ($F(6,48) = 443.26$, $p < 0.000$) (Fig. 2). Interaction was not significant ($p > 0.10$). No pup lethality was noted in any group.

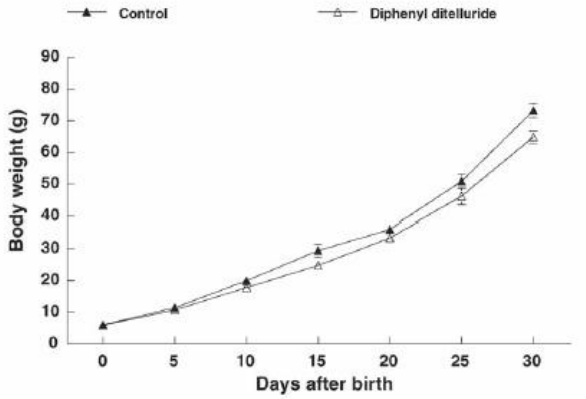


Fig. 2. Weight (g) of pups during the first 30 days postnatal. Data are reported as mean ± S.E.M.

3.2. Behavioral tests

3.2.1. T-maze

In the T-maze, the latency to reach the extremity of the arm was the same between the groups ($p > 0.10$). This was evidenced by the absence of any significant effects on ANOVA ($p > 0.10$, for trials and telluride treatment). Similarly, control and tellurium groups alternated above chance and there was no significant difference between the two groups ($p > 0.10$) (Table 1).

3.2.2. Open-field

Open-field behavior was analyzed separately for each day and two-way ANOVA 2 treatments (control or diphenyl ditelluride) × 2 intra session times (0–2 and 2–4 min) for the first day revealed a significant effect of intra session habituation ($F(1,15) = 81.32$, $p < 0.000$). Telluride treatment and interaction telluride × intra session time were not significant ($p > 0.10$). Similarly for the day 2, only the intra session main effect was significant ($F(1,15) = 49.39$, $p < 0.000004$) (Table 1).

3.2.3. Coat-hanger

Two-way ANOVA (2 treatments × 2 trials) for MT-1, MT-2 and MT-3 yielded no significant effect of treatment or trials. Interactions were also no significant ($p > 0.10$) (Fig. 3a) in the coat-hanger test. Two-way ANOVA of the latencies before falling (MT-4) revealed a significant main effect of tellurium treatment ($F(1,17) = 4.7$, $p < 0.05$). Post hoc comparisons indicated that the latencies before falling for the tellurium group were significantly higher only on the first trial ($F(1,17) = 4.59$, $p < 0.05$) (Fig. 3b). The number of segments crossed in the apparatus was similar in all groups (Fig. 3c; $p > 0.10$).

3.2.4. Rotorod

Rotorod behavior was analyzed separately for each day. Regarding the number of animals that fell, two-way ANOVA 2 treatments (control or diphenyl ditelluride) × 2 trials for the first day revealed a significant effect of trials ($F(1,17) = 50.12$, $p < 0.000002$). For day 2, ANOVA revealed a significant main effect of trials ($F(1,17) = 4.56$, $p < 0.047$) (Table 1).

In the first day, two-way ANOVA for the latency to first fall in the rotorod test revealed only a significant effect of trials ($F(1,17) = 57.05$, $p < 0.000001$) and rats from both groups increased their latencies to first fall in the second trial. A similar

Table 1
Behavioral evaluation of diphenyl ditelluride via maternal milk on T-maze, open-field and rotorod tasks

Tasks	Control	Diphenyl ditelluride
T-maze		
Latency (s)		
Trial 1	29.33 ± 6.80	35.80 ± 4.70
Trial 2	35.50 ± 8.40	31.90 ± 5.25
Correct spontaneous alternation (%)	89.28 ± 5.06	72.92 ± 6.18
Open-field		
Number of crosses		
Day 1		
0–2 min	33.65 ± 4.46	32.44 ± 1.41
2–4 min	18.04 ± 1.70	20.56 ± 1.17
Day 2		
0–2 min	27.00 ± 2.68	29.97 ± 1.71
2–4 min	17.45 ± 0.79	21.40 ± 1.05
Rotarod		
% of number of animals that fell		
Day 1		
Trial 1	67.85 ± 7.16	87.50 ± 4.86
Trial 2	32.14 ± 10.50	33.30 ± 6.42
Day 2		
Trial 1	21.43 ± 6.53	31.2 ± 6.97
Trial 2	14.28 ± 7.46	12.5 ± 5.76
Latency for first fall (s)		
Day 1		
Trial 1	104.57 ± 15.14	91.52 ± 13.99
Trial 2	203.64 ± 14.02	189.40 ± 12.35
Day 2		
Trial 1	205.57 ± 9.56	198.08 ± 11.60
Trial 2	226.39 ± 6.89	226.02 ± 4.76
Mean of falls		
Day 1		
Trial 1	2.15 ± 0.38	2.25 ± 0.16
Trial 2	1.25 ± 0.46	1.19 ± 0.19
Day 2		
Trial 1	0.86 ± 0.26	0.83 ± 0.22
Trial 2	0.71 ± 0.29	0.58 ± 0.23

Data are reported as mean ± S.E.M.

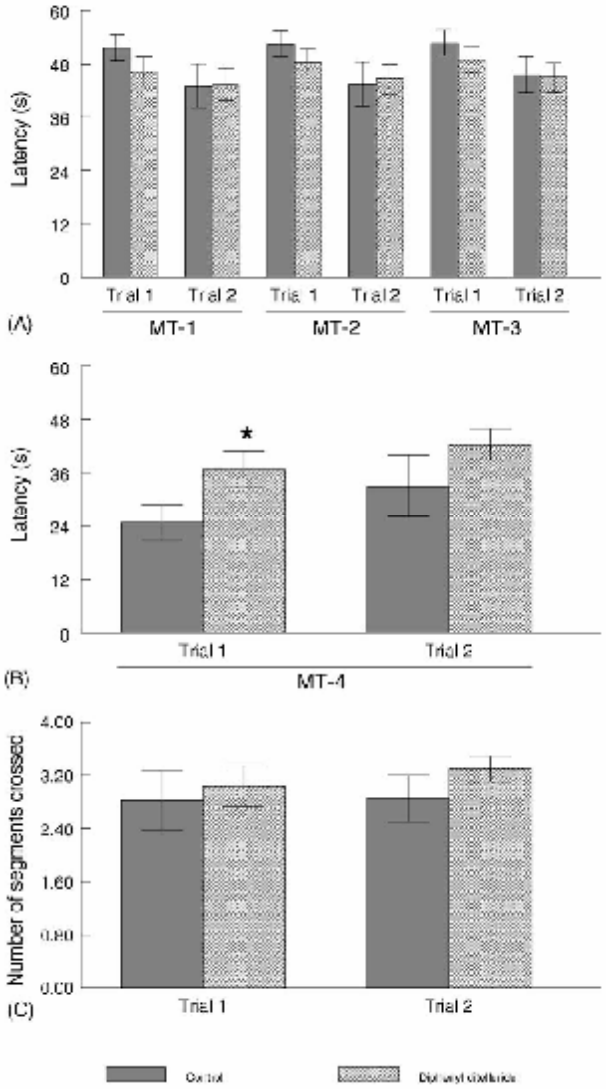


Fig. 2. Effect of diphenyl ditelluride via maternal milk on two- (MT-1), three- (MT-2) or four- (MT-3) paws movement time (A), latencies before falling (MT-4) (B) and the number of segments crossed (C) on the coat-hanger test. Data are reported as mean ± S.E.M. *$p < 0.05$ as compared to control group.

result was obtained on day 2 and ANOVA revealed a significant effect of trials ($F(1,17) = 5.88$, $p < 0.027$) (Table 1).

In the first day, two-way ANOVA for the number of falls in the rotorod test revealed only a significant effect of trials ($F(1,17) = 20.77$, $p < 0.00028$) and rats from both groups decreased the number of falls in the second trial. On day 2, ANOVA revealed no significant effects ($p > 0.10$) (Table 1).

3.2.3. Elevated plus maze

In the elevated plus-maze test, ANOVA for the time spent in the open-arms revealed a significant main effect of telluride treatment ($F(1,14) = 8.12$, $p < 0.013$). Telluride-treated animals spent more time in the open-arms than control rats. ANOVA for the numbers of entries in the open-arms and closed arms revealed a significant main effect of telluride treatment ($F(1,14) = 14.16$, $p < 0.0021$) and $F(1,14) = 12.34$, $p < 0.0034$), respectively) and of time ($F(1,14) = 10.09$, $p < 0.0067$) and $F(1,14) = 31.80$, $p < 0.00006$), respectively). Telluride-treated animals entered more in the open- and closed-arms than control rats and both groups decreased the numbers of entries in the open- and closed-arms, in the second portion of the test (Table 2).

Two-way ANOVA revealed a significant interaction of treatment and open arm/total entries ratio ($F(1,14) = 6.743$, $p < 0.027$). It was observed a main effect of treatment ($F(1,14) = 6.073$, $p < 0.001$) and open arm/total entries ratio ($F(1,14) = 16.286$, $p < 0.01$). In fact, the ratio of open arm/total is higher in the tellurium-exposed than in the control group, at 0–5 min (Table 3).

A similar result was obtained for closed arm/total entries ratio, ANOVA revealed a significant interaction of treatment and closed arm/total entries ratio ($F(1,14) = 6.743$, $p < 0.021$). It

Table 2
Effect of diphenyl ditelluride via maternal milk on exploratory activity of pups in the elevated plus-maze

	Rearing	Entries in open arms	Entries in closed arms	Time spent in open arms
0–5′				
Control	12.50 ± 0.78	1.38 ± 0.36	2.08 ± 0.29	8.86 ± 1.82
Treated	14.03 ± 1.11	5.48 ± 0.39*	7.05 ± 0.43*	25.09 ± 3.26*
5′–10′				
Control	10.25 ± 1.09	1.00 ± 0.35	1.04 ± 0.24	7.64 ± 2.74
Treated	14.13 ± 0.80*	2.60 ± 0.34*	2.78 ± 0.38*	17.85 ± 4.22

Data are reported as mean ± S.E.M.
* $p < 0.05$ as compared to the control group.

Table 3
Ratio of entries in open arms/total, entries in closed arms/total and time spent in open arms/total of diphenyl ditelluride exposure, via maternal milk, on exploratory activity of pups in the elevated plus-maze

	Entries in open arms/total	Entries in closed arms/total	Time spent in open arms/total
0–5′			
Control	0.36 ± 0.045	0.64 ± 0.044	0.03 ± 0.004
Treated	0.46 ± 0.036*	0.54 ± 0.036*	0.08 ± 0.009*
5′–10′			
Control	0.48 ± 0.016	0.52 ± 0.016	0.03 ± 0.008
Treated	0.49 ± 0.009	0.51 ± 0.009	0.06 ± 0.012*

Data are reported as mean ± S.E.M.
* $p < 0.05$ as compared to the control group.

was also observed a main effect of treatment ($F(1,14) = 6.073$, $p < 0.027$) and closed arm/total entries ratio ($F(1,14) = 16.286$, $p < 0.01$). The ratio of closed arm/total is lower in the tellurium-exposed than in the control group, in the first portion of the test.

There is no intergroup difference for the ratio of entries in open arm/total and closed arm/total, in the second portion of the test (Table 3).

Regarding ratio of time spent in the open arm/total, a significant main effect of telluride treatment ($F(1,14) = 8.12$, $p < 0.013$) was reveled, in both at 0–5 and 5–10 min (Table 3).

Two-way ANOVA revealed only a significant effect of treatment ($F(1,14) = 5.41$, $p < 0.05$) for rearing. Rearing in tellurium group was higher than in the control group, at 5–10 min ($F(1,14) = 7.69$, $p < 0.014$) (Table 2).

4. Discussion

This study evaluated the effects of low level of diphenyl ditelluride administration to mothers on the behavioral performance of their offspring in various tasks and revealed that exposure to low doses of this compound may result in disinhibitory behavioral tendencies of offspring determined in the elevated plus-maze. However, in the open-field test, no sign of disinhibition was observed. In fact, the offspring of diphenyl ditelluride treated mothers showed no changes in locomotor activity and habituated to the open-field as did the control offspring. These results may indicate that the behavioral changes caused by diphenyl ditelluride is task specific and occurred only in a more complex environment.

Further, tellurium exposed pups presented a small reduction in body weight. However, considering that reduction in body weight is low and that no other signs of toxicity or lethality were found, the magnitude of this effect on pups is probably irrelevant. There were also no significant specific overt signs of maternal intoxication or lethality following administration of diphenyl ditelluride during the suckling period.

The action of nervous system and its subtle disruption functioning by xenobiotics could be evaluated through the performance of animals in several behavioral tests [17,20,25]. Exploration is a very important behavior by which the animal gains information about its environment and it is an essential life-preserving component of an animal's higher nervous functions [52]. The behavioral data presented here demonstrated that there is no difference on exploratory activity in telluride-exposed pups. Besides, general ambulation in the open-field was similar for both studied groups suggesting that motor activity was unaltered by low levels of tellurium exposure during the suckling period.

In this study, motor coordination was evaluated in coat-hanger and rotorod tests. In the rotorod test, tellurium group demonstrated behavior similar to the control group. Conversely, tellurium group presented behavior different from control group in coat-hanger test. In fact, tellurium-exposed pups presented increase in latencies before falling in coat-hanger test, which has been reported as sensitive to cerebellar dysfunction [23]. These results indicated that tellurium-treated pups did not have gross motor coordination impairments, as assessed by the rotorod performance and by an even unexpected increase in the latency to fall in coat-hanger test.

In T-maze spontaneous alternation, the tendency of rat to switch arm choices on successive trials was evaluated. Spontaneous alternation is a consequence of those brain processes that develop between the second and fourth postnatal week, consequently, it is not present during the earliest stages of development [12]. In addition, pharmacological treatment during infancy can facilitate the onset of spontaneous alternation in rats [21]. Telluride group was similar to the control group in the response to T-maze test which allows us to imply that pups exposed to diphenyl ditelluride did not present signal of psychomotor slowing [23].

The elevated plus-maze, an elected test for anxiety, is based on the natural aversion of rodents to exposed, elevated space, where rats choose to explore either anxiogenic (open- or "aver-

sive area") or safety (enclosed) arms. One of the most widely used animal models of anxiety is this apparatus which has been pharmacologically and ethologically validated [38,44]. In this study, tellurium-treated rats had a higher number of entries and spent more time in the open arms of the elevated plus-maze than control rats. These results may indicate that tellurium-treated rats were less anxious than control rats, suggesting that this drug possesses anxiolytic effects. However, an alternative interpretation to these data, that can find persuasive support in the literature [3,25,24], is that diphenyl ditelluride produces behavioral disinhibitory tendencies. From an ethological point of view, excessive disinhibitory tendencies can be not adaptative and can potentially expose the affected animal to dangerous situations. Of particular importance for the developmental neurotoxicity induced by diphenyl ditelluride, literature data indicate that in quite different animal models that are supposed to be associated with some degree of brain injure, disinhibitory tendencies in the elevated plus-maze, very similar to those observed in the present study [3,45,16]. Furthermore, in these paradigms the behavioral changes could be interpreted more in terms of high impulsiveness than in terms of alterations in the anxiety. Specifically in terms of the elevated plus-maze the malnourished animals showed lower risk-assessment behaviors [1,2,30].

Another interpretation is that the behavioral effects in the offspring may be attributable to the effect of diphenyl ditelluride on the mother. In fact, it is well established that maternal behavior in the suckling period is a powerful determinant of the pups behavior [55]. However, since the development of the offspring of telluride-exposed animals is not so different from that of controls, we realize that the maternal behavior in the home cage was similar between groups. Furthermore, the literature data also indicate that in some situations changes in dam's behavior is beneficial for the pups [54]. Another aspect that must be emphasized here is that diphenyl ditelluride could cause a direct effect on pups behavior that could change mother behavior.

Although exposure to tellurium compounds can lead to general motor impairments in experimental animals mainly by their demielinizating properties [41,46,48], in the present investigation we found neither gross, fine motor impairment of mothers exposed to diphenyl ditelluride nor in their offspring.

This study demonstrated that tellurium administered as diphenyl ditelluride can be transferred to newborn offspring through the maternal milk. Accordingly, literature evidence suggests that inorganic radiotellurium, ($H_2{}^{132}TeO_3$) can be transferred to suckling rats in proportions that varied from 2 to 5% of the administered maternal dose [32]. In the present study a lipophylic form of tellurium was used; consequently, we would expect an even higher degree of tellurium transference from mothers to their litters. Thus, it seems plausible to assume that tellurium become bioavailable to suckling rats after exposure of their mothers to diphenyl ditelluride and may cause behavioral changes in the offspring.

In conclusion, these results indicated that exposure to low levels of diphenyl ditelluride causes neurobehavioral changes, which emphasizes the potential neurotoxicity of organic tellurium compounds. The behavioral alterations observed here after tellurium exposure can be cautiously interpreted as an indication of behavioral disinhibition. However, further studies using different disinhibition behavioral paradigms, such as the Vogel conflict test [40,53], will be necessary to support this interpretation. Thus, additional investigations are necessary to determine whether the above mentioned behavioral changes are in fact manifestation of neurobehavioral toxicity of tellurium.

Acknowledgements

The financial support by FAPERGS, CAPES and CNPq is gratefully acknowledged. J.B.T.R., C.W.N. and G.Z. are the recipient of CNPq fellowships.

References

[1] S.S. Almeida, M. Araújo, G.M.S. Moreira, R.N.L. Paiva, L.M. De Oliveira, Short-term isolation does not reduce elevated plus-maze exploration in early protein malnourished rats, Nutr. Neurosci. 1 (1998) 103–110.

[2] S.S. Almeida, L.H. Duntes, L. Dye, M.L. Nunes, C. Prasac, J.B.T. Rocha, P. Wainwright, C.T.B.V. Zaia, R.C.A. Guedes, Nutrition and brain function: a multidisciplinary virtual symposium, Nutr. Neurosci. 5 (2002) 311–320.

[3] S.S. Almeida, J. Tonkiss, J.R. Galler, Prenatal protein malnutrition affects exploratory behavior of female rats in the elevated plus-maze test, Physiol. Behav. 50 (1996) 675–680.

[4] C.-M. Andersson, R. Brattsand, A. Hallberg, Diaryl tellurides as inhibitors of lipid peroxidation in biological and chemical systems, Free Radic. Res. 20 (1994) 401–416.

[5] C.-M. Andersson, A. Hallberg, R. Brattsand, I.A. Cotgreave, I. Engman, J. Persson, Glutathione peroxidase-like activity of diaryl tellurides, Bioorg. Med. Chem. Lett. 3 (1993) 2553–2558.

[6] Z. Annau, V. Cuomo, Mechanisms of neurotoxicity and their relationship to behavioral changes, Toxicology 49 (1988) 219–225.

[7] K. Henzinger, H. Hagar, Über die zellulare Speicherung von tellur und ihre Beziehungen zu den unter dem Lysosomenbegriff zusammengefaßten intrazytoplasmatischen, Köpern Verh Dtsch Ges Path 49 (1965) 357–362.

[8] E.A. Cerwenka, W.C. Cooper, Toxicology of selenium and tellurium and their compounds, Arch. Environ. Hlth. 3 (1961) 189.

[9] J.V. Comasseto, L.W. Ling, N. Petragnani, H.A. Stefani, Vinylic selenides and tellurides–preparation, reactivity and synthetic applications, Synthesis 4 (1997) 373–403.

[10] H. Cravioto, W.T. Agnew, J.A. Carregal, R.H. Pudentz, The distribution of tellurium in the nervous system of the rat, an ultrastructural study, J. Neuropathol. Exp. Neurol. 29 (1970) 158.

[11] D. Dai, W.F. Agnew, E. Greene, Effects of tellurium ingestion on learning capacity of the rat, Psychopharmacology 24 (1972) 508–515.

[12] G.J. Egger, P.J. Livesey, R.G. Dawson, Ontogenic aspects of central cholinergic involvement in spontaneous alternation behavior, Dev. Psychobiol. 6 (1973) 289–299.

[13] L. Engman, J. Persson, K. Vessman, M. Ekstrom, M. Berglund, C.M. Andersson, Organotellurium compounds as efficient retarders of lipid peroxidation in methanol, Free Radic. Biol. Med. 19 (1995) 441–452.

[14] L. Engman, D. Stern, I. Cotgreave, C.M. Andersson, Thiol peroxidase activity of diaryl ditellurides as determined by a ^{1}H NMR method, J. Am. Chem. Soc. 114 (1992) 9737–9743.

[15] L.T. Fairhill, Tellurium, in: Industrial Toxicology, Hafner Publishing Co., New York, London, 1969, p. 120.

[16] A.L. Frangolin-Silva, S.S. Almeida, The interaction of housing condition and acute immobilization stress on the elevated plus-maze behaviors of protein-malnourished rats, Braz. J. Med. Biol. Res. 37 (2004) 1035–1042.

[17] R.F. Green, S.A. Hitzemann, A. Thomas, J.E. Edwards, S.E. File, Age-associated sex differences in response to food deprivation in two animal tests of anxiety, Neurosci. Biobehav. Rev. 27 (2003) 155–161.

[18] J.P. Goeckritz, Role of organotelluronium species in tellurium neuropathy, Neurochem. Res. 23 (1998) 1313–1319.
[19] A. Gottlieb, I. Keydar, H.T. Epstein, Rodent brain growth stages: an analytical review, Neonatology 32 (1977) 166–176.
[20] F.G. Graeff, F.C. Netto, H. Zangrossi, The elevated T-maze as an experimental model of anxiety, Neurosci. Biobehav. Rev. 23 (1998) 237–246.
[21] A. Isseroff, Facilitation of delayed spontaneous alternation behavior in adult rats following early hydroxyzine treatment: differential sensitivity in late infancy, Psychopharmacology 69 (1980) 179–181.
[22] B. Laden, T. Porter, Inhibition of human squalene monooxygenase by tellurium compounds: Evidence of interaction with vicinal sulfhydryls, J. Lipid Res. 42 (2001) 235–240.
[23] R. Lalonde, C. Strazielle, Motor performance of spontaneous murine mutations with cerebellar atrophy, in: W. Crusio, E. Gerlai (Eds.), Handbook of Molecular-Genetic Techniques for Brain and Behavior Research (Techniques in the Behavioral and Neural Sciences, vol. 13), Elsevier, Amsterdam, 1999, pp. 627–637.
[24] R. Lalonde, H.D. Kim, K. Fukuchi, Exploratory activity, anxiety, and motor coordination in bigenic APPswe+PS1/Delta E9 mice, Neurosci. Lett. 369 (2004) 155–160.
[25] R. Lalonde, S. Qian, C. Strazielle, Transgenic mice expressing the PS1-A246E mutation: effects on spatial learning, exploration, anxiety, and motor coordination, Behav. Brain Res. 138 (2003) 71–79.
[26] P.W. Lamper, R.S. Garratt, Mechanism of demyelination in tellurium neuropathy. Electron microscopic observations, Lab. Invest. 25 (1971) 383–388.
[27] P. Lampert, F. Garro, A. Pentschew, Tellurium neuropathy, Acta Neuropathol. 15 (1970) 308–317.
[28] E.N. Maciel, R.C. Borzan, A.L. Braga, J.B.T. Rocha, Diphenyl diselenide and diphenyl ditelluride differentially affect anti-oxidant delta-aminolevulinate dehydratase from liver, kidney and brain of mice, J. Biochem. Mol. Toxicol. 14 (2000) 310–319.
[29] F.C. Meotti, V.C. Borges, G. Zeni, J.B.T. Rocha, C.W. Nogueira, Potential renal and hepatic toxicity of diphenyl diselenide, diphenyl ditelluride and ebselen for rats and mice, Toxicol. Lett. 143 (2003) 9–16.
[30] G.M.S. Moreira, M. De-Araújo, L.V. De-Oliveira, S.S. Almeida, The behavior of protein-calorie-malnourished rats on the elevated plus-maze test: an ethopharmacological analysis, Psychobiology 25 (1997) 180–185.
[31] M.B. Morente, J.I. Rossato, C.W. Nogueira, G. Zeni, J.B.T. Rocha, Ebselen and diorganochalcogenides inhibition of $^{45}Ca^{2+}$ efflux into brain synaptosomes is voltage-dependent, J. Biochem. Mol. Toxicol. 17 (2003) 154–160.
[32] Y. Nishimura, S.K. Sahoo, H.-S. Kim, S. Horiuma-Takeda, Y. Watanabe, J. Inaba, Biokinetics of radiotellurium in rats, Radiat. Protect. Dosim. 105 (2003) 285–290.
[33] C.W. Nogueira, E.M. Maciel, G. Zeni, D. Graça, J.B.T. Rocha, Biochemical toxicology of simple diorganoyl chalcogenides, IECSOC Electronic Conference on Synthetic Organic Chemistry, 2001 (http://www.mdpi.net/ecsoc-5/d00151).
[34] C.W. Nogueira, L.N. Rotta, M.L. Perry, D.O. Souza, J.B.T. Rocha, Diphenyl diselenide and diphenyl ditelluride affect the rat glutamatergic system in vitro and in vivo, Brain Res. 906 (2001) 157–163.
[35] C.W. Nogueira, L.N. Rotta, G. Zeni, D.O. Souza, J.B.T. Rocha, Exposure to ebselen changes glutamate uptake and release by rat brain synaptosomes, Neurochem. Res. 27 (2002) 283–288.
[36] C.W. Nogueira, G. Zeni, J.B.T. Rocha, Organoselenium and organotellurium compounds: toxicology and Pharmacology, Chem. Rev. 104 (2004) 6255–6285.
[37] A. Nyska, T. Waner, M. Pirak, M. Albeck, B. Srecni, Toxicity study in rats of a tellurium based immunomodulating drug, AS-101: a potential drug for AIDS and cancer patients, Arch. Toxicol. 63 (1989) 386–393.
[38] S. Pellow, P. Chopin, S.E. File, M. Briley, Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat, J. Neurosci. Meth. 14 (1985) 149–167.
[39] N. Petragnani, Preparation of the principal classes of organic tellurium compounds, in: A.R. Katritzky, O. Meth-Cohn, C.W. Rees (Eds.), Tellurium in Organic Synthesis, Academic Press, London, 1994, pp. 9–88.
[40] H. Piantadosi, S. Khan, Characterization of anxiety and habituation profile following global ischemia in rats, Physiol. Behav. 84 (2005) 543–552.
[41] D.A. Rawlins, M.B. Smith, Myelin synthesis in vitro: a comparative study of central and peripheral nervous tissue, J. Neurochem. 18 (1971) 1861–1870.
[42] D.C. Rice, Effect of exposure to 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) throughout gestation and lactation on developmental and spatial delayed alternation performance in rats, Neurotoxicol. Teratol. 21 (1999) 59–69.
[43] J.B.T. Rocha, D. Vendite, Effects of undernutrition and handling during suckling on shuttle avoidance and footshock escape behavior and on plasma glucose levels of young rats, Dev. Psychobiol. 23 (1990) 157–168.
[44] J.B.T. Rocha, L.K. Rocha, T. Emanuelli, M.M. Pereira, Effect of mercuric chloride and lead acetate treatment during the second stage of rapid postnatal brain growth on the behavioral response to chlorpromazine and on δ-ALA-D activity in weaning rats, Toxicol. Lett. 125 (2001) 143–150.
[45] L.C. Rocinholi, S.S. Almeida, L.M. De-Oliveira, Response threshold to aversive stimuli in stimulated early protein-malnourished rats, Braz. J. Med. Biol. Res. 30 (1997) 407–413.
[46] T.J. Rodgers, B.-J. Cao, A. Dalvi, A. Holmes, Animals models of anxiety: an ethological perspective, Braz. J. Med. Biol. Res. 30 (1997) 289–304.
[47] G. Said, S. Ducket, Tellurium-induced myelinopathy in adult rats, Muscle Nerve 4 (1981) 319–325.
[48] G. Said, S. Ducket, B. Sauron, Proliferation of Schwann cells in tellurium-induced demyelination in young rats. A radioautographic and teased nerve fiber study, Acta Neuropathol. (Berl.) 155 (1981) 173–179.
[49] B. Srecni, R.R. Caspi, A. Klein, Y. Kalechman, Y. Danziger, M. Ben Ya'akov, T. Tamari, F. Shalit, M. Albeck, A new immunomodulating compound (AS-101) with potential therapeutic application, Nature 330 (1987) 172–176.
[50] B. Srecni, R.R. Caspi, S. Lustig, A. Klein, Y. Kalechman, Y. Danziger, M. Ben Ya'akov, T. Tamari, F. Shalit, M. Albeck, The biological activity and immunotherapeutic properties of AS-101, a synthetic organotellurium compound, Nat. Immun. Cell Growth 7 (1988) 163–168.
[51] E.C. Stangherlin, A.M. Favero, G. Zeni, J.B.T. Rocha, C.W. Nogueira, Teratogenic vulnerability of rat fetuses to diphenyl ditelluride: prenatal assessment, Toxicology 207 (2005) 251–258.
[52] R.J. Sutherland, J.W. Rudy, Configural association theory: the role of the hippocampal formation in learning, memory and amnesia, Psychobiology 17 (1989) 129–144.
[53] A.L. Svensson, P. Akesson, J.A. Engel, B. Soderpalm, Testosterone treatment induces behavioral disinhibition in adult male rats, Pharmacol. Biochem. Behav. 75 (2003) 481–490.
[54] J. Tonkiss, J.L. Smart, R.F. Massey, Effects of early life undernutrition in artificially-reared rats. 3. Subsequent behaviour, Physiol. Behav. 41 (1987) 555–562.
[55] J. Voglenova, S. Biru, N.M. Fandella, C.R. Breese, An assessment of the long-term developmental and behavioral teratogenicity of prenatal nicotine exposure, Behav. Brain Res. 150 (2004) 159–170.
[56] M. Wagner Recio, A.D. Toews, P. Morell, Tellurium blocks cholesterol synthesis by inhibiting squalene metabolism: Preferential vulnerability of this metabolic block leads to peripheral nervous system demyelination, J. Neurochem. 57 (1991) 1851–1901.
[57] E. Wicy-Tyszkiewicz, A. Piechal, B. Gajkowska, M. Smialek, Tellurium-induced cognitive deficits in rats are related to neuropathological changes in the central nervous system, Toxicol. Lett. 131 (2002) 203–214.

4.2 – Manuscrito 1:

Ditelureto de difenila induz prejuízo da memória de reconhecimento

DIPHENYL DITELLURIDE INDUCES IMPAIRMENT OF RECOGNITION MEMORY

Eluza Curte Stangherlin, João Batista Teixeira Rocha, Cristina Wayne Nogueira*

Submetido à Life Sciences

Diphenyl ditelluride induces impairment of recognition memory

Eluza Curte Stangherlin[a], João Batista Teixeira Rocha[a], Cristina Wayne Nogueira[a,*]

[a]Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, SM, RS, CEP 97105-900 Santa Maria, Brazil

*Correspondence should be sent to:
Cristina Wayne Nogueira
Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brasil.
Phone: 55-55 3220-8140
FAX: 55-55-3220-8978
E-mail: criswn@quimica.ufsm.br (Nogueira CW)

Abstract

In the present study, the possible influence of maternal exposure to 0.03 mg/kg of diphenyl ditelluride $(PhTe)_2$ during the first 14 days of lactational period in Wistar rats was investigated. Object recognition memory task, evaluation of synaptosomal glutamate uptake and release as well as cerebral Na^+, K^+-ATPase activity were evaluated in 30 day old pups. There were no significant specific overt signs of maternal intoxication. The body weight gain of pups was similar among groups. $(PhTe)_2$-exposed group showed a significantly lower time exploring the novel object when compared to the performance of the control group in short-term memory (STM) test. In addition, $(PhTe)_2$ significantly inhibited the synaptosomal glutamate uptake and the cerebral Na^+, K^+-ATPase activity in animals. The synaptosomal glutamate release was similar between $(PhTe)_2$ and control groups. In conclusion, the present study establishes that litters presented cognitive impairment after maternal exposure to $(PhTe)_2$ via maternal milk, demonstrated by the performance of animals in object recognition memory task. The possible mechanism involved in $(PhTe)_2$ action in memory of recognition can involve inhibition in cerebral Na^+,K^+-ATPase activity.

Keywords: Organotellurium, Na^+,K^+-ATPase, Object Recognition Memory, Glutamate Uptake and Release.

Introduction

Although the tellurium (Te) element rarely occurs in the free state in nature, silver and bismuth tellurides do occur (Larner, 1995a; Schroeder et al., 1967). Moreover, this metallic element is known to be present in plant material, particularly in members of the Alium family, such as garlic (Larner, 1995b). Currently, inorganic Te is used in metal-oxidizing solutions to blacken or tarnish metals (Yarema and Curry, 2005) and in industry of nanoparticulate semiconductors (Green et al., 2007; Zhang and Swihart, 2007). Moreover, the use of organic Te compounds will increase due to its importance in organic synthesis (Comasseto et al., 1997).

A number of studies have shown that trace amounts of tellurium are present in body fluids, such as blood and urine (Siddik and Newman, 1988, Newman et al., 1989). Furthermore, Te has been shown to be present as tellurocysteine and telluromethionine in several proteins in bacteria (Boles et al., 1995; Budisa et al., 1995), yeast (Yu et al., 1993) and fungi (Ramadan et al., 1989). But to date, no telluroproteins have been identified in animal cells. By contrast, attention has been drawn to the toxicity of tellurium.

Nowadays, in the literature it was reported two cases of toxicity in young children from ingestion of metal-oxidizing solutions that contained substantial concentrations of tellurium (Yarema and Curry, 2005). Clinical features of acute tellurium toxicity include a metallic taste, nausea, nausea, blackened oral mucosa and skin and garlic odor of the breath (Muller et al., 1989).

Exposure of experimental animals to tellurium can cause a variety of toxic effects, including reversible hind limb paralysis due to demyelination of the sciatic nerve and spinal roots (Lampert et al., 1970; Lampert and Garret, 1971). This has been proposed to be primarily due to blockage of cholesterol biosynthesis at squalene epoxidase (Wagner-Recio et al., 1994), which sequentially affects the transcription of the myelin proteins themselves at the gene level (Morell et al., 1994). Moreover, dietary exposure to high levels (3300 ppm) of metallic tellurium causes persistent neuromotor impairment which is associated with a severe deficit in shock avoidance. Furthermore, tellurium could also cause a lowered sensitivity to noxious stimulus, which in turn would retard the learning of the active avoidance task (Dru et al., 1972). Sodium tellurite intoxication causes a consistent deficit in a non-aversive spatial learning in water maze task that could not be overtly linked to motor or motivational impairment in tellurium exposed animals (Widy-Tysziewicz et al., 2002). Dimethyltellurium, an important compound derived from inorganic tellurium metabolism in mammals, has been reported as an inducer of peripheral neuropathy in rats (Goodrum, 1998). Moreover, data of

our group of research suggest that exposure to mothers to low doses of diphenyl ditelluride $(PhTe)_2$, an organotellurium compound, may result in disinhibition behavior of their offspring on elevated plus maze task (Stangherlin et al., 2006). Besides, $(PhTe)_2$ can be teratogenic, causing various morphologic abnormalities in rat fetuses in development (Stangherlin et al., 2005).

Of particular importance, our research group has obtained persuasive evidences indicating that $(PhTe)_2$ causes marked neurotoxic effects in mice after acute or prolonged exposure either by subcutaneous or intraperitoneal routes (Nogueira et al., 2004). $(PhTe)_2$ affects a number of neuronal processes and modifies the functionality of the glutamatergic system in vitro and in vivo (Nogueira et al., 2001) as well as inhibits the cerebral Na^+, K^+-ATPase activity (Borges et al., 2005).

Despite nervous system, glutamate is known to play an important role in cognition, learning and memory (Davis et al., 1994; Maren, 1996; LeDoux, 1994) and in the neural plasticity of synaptic connections (Kaczmarek et al., 1997). Moreover, Na^+,K^+-ATPase is an enzyme embedded in the cell membrane, responsible for the generation of the membrane potential through the active transport of sodium and potassium ions in the central nervous system necessary to maintain neuronal excitability (Erecinska and Silver, 1994).

Thus, the present investigation was carried out to determine the effects of $(PhTe)_2$ on the behavioral performance of rat pups in object recognition memory task. The possible involvement of glutamatergic system and of cerebral Na^+,K^+-ATPase activity in $(PhTe)_2$ effect was evaluated.

Materials and Methods

Materials

Diphenyl ditelluride-$(PhTe)_2$ was synthesized according to literature method (Petragnani, 1994). Analysis of the ^1H NMR and ^{13}CNMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of $(PhTe)_2$ (99.9%) was determined by GC/HPLC. $(PhTe)_2$ is solid compound, very stable and can be stored in the lab, in the simple flasks for long time. $(PhTe)_2$ was diluted in canola oil which was obtained from a standard commercial supplier.

Animals

Virgin female Wistar rats (180–240 g) from our own breeding colony were used. The animals were kept on a 12 h light/dark cycle, at a room temperature of 22 ºC, with free access

to food and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, Federal University of Santa Maria, Brazil.

Experimental procedure

Sexually naive female rats were mated with male previously tested as fertile (three females and one male in each cage). The onset of pregnancy was confirmed by the presence of sperm in vaginal smears (day 0 of pregnancy) and pregnant dams were immediately housed in individual cages. At birth, the dams received $(PhTe)_2$ (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) via subcutaneous (s.c.) injection once daily during the first 14 days of lactational period. The dose of diphenyl ditelluride used in this study was selected on the basis of LD_{50} study carried out in our laboratory (Meotti et al., 2003). Maternal body weight was recorded during this period. The body weight of pups was recorded once daily until PND 30. At birth, all litters were culled to eight pups. Whenever possible, only male rats were kept within the litter and females were kept just to maintain equal litter sizes. On 21 postnatal day (PND 21), pups used for testing were weaned and placed on ad libitum standard rat chow diets. After 1-week post-weaning period, the object recognition task was conducted (in the morning of PND 30). The behavioral observations were blind, and carried out under low-intensity light. Only male pups were used in the behavioral tests, litter was invariably constituted of four animals. In PND 31, the animals were euthanized for evaluating neurochemical parameters.

Behavioral analysis

The object recognition task took place in a 45 x 45 cm^2 open field surrounded by 30 cm height walls, made of brown plywood. All animals were given a habituation session where they were left to freely exploring the open field for 5 min. No objects were placed in the box during the habituation trial (Fig. 1a). Twenty-four hours after habituation, training was conducted by placing individual rat for 5 min into the field, in which two identical objects (objects A_1 and A_2; Duplo Lego toys) were positioned in two adjacent corners, 10 cm from the walls (Fig. 1b). In a short-term memory (STM) test given 1.5 h after training, the rats explored the open field for 5 min in the presence of one familiar (A) and one novel (B) object (Fig. 1c). All objects presented similar textures, colors, and sizes, but distinctive shapes. The percentage of the total exploration time that the animal spent investigating the novel object was the measure of recognition memory. Between trials the objects were washed with 10% ethanol solution. In a long-term memory (LTM) test given 24 h after training, the same rat

explored the field for 5 min in the presence of familiar object A and a novel object C (Fig. 1d). Recognition memory was evaluated as for the STM test. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Data are expressed as the mean ± SE percentage time exploring any of the objects (training) or the novel objects. Exploratory preference in: *Training*= $(A_2/(A_1+A_2))*100$; *STM*= $(B/(A_1+B))*100$; *LTM* = $(C/(A_1+C))*100$.

Preparation of Synaptosomes

Twenty-four hours after the last behavioral test, three animals of each group were decapitated. After that, the whole brain was removed and used to prepare synaptosomes on a discontinuous Percoll gradient according to Dunkley et al. (1988). Protein concentration was measured according to the method of Lowry et al. (1951).

[^{3}H]Glutamate Release by Synaptosomes

Determination of [^{3}H]glutamate release was accomplished according to the method described by Migues et al. (1999). The synaptosomal preparation was loaded with 0.25 µCi [^{3}H]glutamate (Amersham, specific activity 53 mCi/mmol, final concentration 5 µM) by pre incubation in Tris/HCl buffered salt solution (composition in mM: Tris/HCl 27, NaCl 133, KCl 2.4, $MgSO_4$ 1.2, KH_2PO_4 1.2, Glucose 12, $CaCl_2$ 1.0) pH 7.4 (adjusted with HCl), for 15 min at 37°C. Aliquots of labeled synaptosomes (1.4 mg protein) were centrifuged at 16,000 g for 1 min. Supernatants were discarded, and the pellets were washed four times in Tris/HCl buffer by centrifugation at 16,000 g for 1 min (at 4°C). To assess the basal release of [^{3}H]glutamate, the final pellet was resuspended in Tris/HCl buffer and incubated for 60 s, at 37°C. Incubation was terminated by immediate centrifugation (16,000 g, 1 min, 4°C). Radioactivity present in supernatants and pellet was separately determined in a scintillation counter. The released [^{3}H]glutamate was calculated as a percentage of the total amount of radioactivity present in the synaptosomes at the start of the incubation period. K^+-stimulated [^{3}H]glutamate release was assessed as described for basal release, except for the fact that the incubation medium contained 40 mM KCl to induce synaptosomal depolarization.

[^{3}H]Glutamate Uptake by Synaptosomes

The synaptosomal preparation was washed twice by suspending in 3 volumes of 0.3 M sucrose, in 15 mM Tris/acetate buffer (pH 7.4) and centrifuging at 35,000 g for 15 min. The final pellet was suspended in 0.3 M sucrose, 15 mM Tris/acetate buffer (pH 7.4), and incubated in Tris/HCl buffer (composition in mM: Tris/HCl 27, NaCl 133, KCl 2.4, $MgSO_4$ 1.2, KH_2PO_4 1.2, Glucose 12, $CaCl_2$ 1.0) pH 7.4 (adjusted with HCl), in the presence of

[^{3}H]glutamate (final concentration 100 mM) for 1 min at 37°C. The reaction was stopped by centrifugation (16,000 g, 1 min, 4°C), and the pellets were washed three times in Tris/HCl buffer by centrifugation at 16,000 g for 1 min (at 4°C). Radioactivity present in pellet was measured in a scintillation counter. Specific [^{3}H]glutamate uptake was calculated as the difference between the uptake obtained in the incubation medium described above, and the uptake obtained with a similar incubation medium in which NaCl was replaced by choline chloride.

Na^+, K^+-ATPase activity

Immediately after the euthanized, the whole brain was removed and the homogenate was prepared in 0.05M Tris–HCl buffer (pH 7.4). The homogenate was centrifuged at 4000×g at 4ºC for 10 min and supernatant was used for assay of protein Na^+, K^+-ATPase. The reaction mixture for Na^+, K^+-ATPase activity assay contained 3 mM MgCl, 125 mM NaCl, 20 mM KCl and 50 mM Tris–HCl, pH 7.4, in a final volume of 500 μL. The reaction was initiated by addition of ATP to a final concentration of 3.0 mM. Controls were carried out under the same conditions with the addition of 0.1mM 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 (1925). All the experiments were conducted at least four times and similar results were obtained.

Statistical analysis

The litter (four animals averaged) was considered the experimental unit in all statistical analyses performed. Statistical significance was assessed by analysis of variance (ANOVA) with repeated measures, when appropriated. Post hoc Duncan's test was carried out when appropriated. A value of $p < 0.05$ was considered to be significant.

Results

General analysis

There were no significant specific overt signs of maternal intoxication. The pups demonstrated normal body weigh gain (data not shown here).

Object recognition task

Results for recognition memory task are shown in Fig. 2. There were no significant differences among groups in the time exploring any of the two identical objects during training

($p > 0.05$) or in the time exploring the novel object during the LTM test ($p > 0.05$). In the STM test, $(PhTe)_2$-exposed group showed a significantly lower time exploring the novel object ($p > 0.05$) when compared to the performance to the control group. In addition, control animals showed a significantly higher time exploring the novel object, in the STM test, when compared to the performance of control animals during training ($p < 0.05$).

Synaptosomal [^{3}H]glutamate release and uptake

Results for synaptosomal [^{3}H]glutamate uptake are shown in Fig. 3. The [^{3}H]glutamate uptake by synaptosomes was significantly decreased (around 15%) in the $(PhTe)_2$-exposed group when compared to the control group ($p<0.05$).

[^{3}H]glutamate release from synaptosomes are shown in Fig. 4. The basal and K^+-stimulated [^{3}H]glutamate release by synaptosomes from whole brain of young rats were not different between control and $(PhTe)_2$-exposed groups ($p>0.05$).

Na^+, K^+-ATPase activity

Na^+, K^+-ATPase activity was significantly decreased (around 34%) in the $(PhTe)_2$-exposed group when compared to the control group ($p<0.05$) (Fig. 5).

Discussion

The present study establishes that pups presented cognitive impairment after maternal $(PhTe)_2$ exposure, demonstrated by the performance of animals in object recognition memory task. Another finding of this study is that $(PhTe)_2$ significantly inhibited the synaptosomal glutamate uptake and the cerebral Na^+, K^+-ATPase activity in these animals.

A point that must be discussed here is that a lipophylic form of tellurium was used and we would expect an even higher degree of tellurium transference from mothers to their litters. In fact, literature evidence suggests that inorganic radiotellurium can be transferred to suckling rats in proportions that varied from 2 to 5% of the administered maternal dose (Nishimura et al., 2003). Thus, it seems plausible to assume that tellurium become bioavailable to suckling rats after exposure of their mothers to $(PhTe)_2$ and may cause behavioral changes in the offspring. In this context, during experimental protocol, $(PhTe)_2$-exposed pups via maternal milk presented normal body weight gain and no signs of toxicity or lethality. There were also no significant specific overt signs of maternal intoxication or lethality following administration of $(PhTe)_2$ during the suckling period.

The action of nervous system and its subtle disruption functioning by xenobiotics could be evaluated through the performance of animals in several behavioral tests (Annau and Cuomo, 1988; Graeff et al., 1998; Lalonde et al., 2003). The object recognition memory task in rodents has been shown to be a very useful experimental tool for assessing changes in neuronal function induced by drugs or genetic modifications. Novel object recognition is a type of non-aversive and non-spatial memory (Puma et al., 1999; Rampon et al., 2000). Evidence in the literature has shown that systemic administration of diphenyl diselenide, $(PhSe)_2$, a selenium compound analogous to $(PhTe)_2$, induces a cognitive enhancers in the object recognition task in mice (Rosa et al., 2003).

In the present study, a behavioral performance of animals suggests that the exposure to $(PhTe)_2$ induced a cognitive impairment. Our data corroborated with findings reported in the literature that showed a consistent deficit in spatial learning following inorganic tellurium intoxication using the water maze task (Widy-Tyszkiewicz et al., 2002). Moreover, dietary exposure to high levels (3300 ppm) of metallic tellurium causes persistent neuromotor impairment which is associated with a severe deficit in shock avoidance. Furthermore, tellurium could also cause a lowered sensitivity to noxious stimulus, which in turn would retard the learning of the active avoidance task (Dru et al., 1972). Additionally, data of our research group suggest that mother exposure to low doses of $(PhTe)_2$ may result in disinhibitory behavior of their offspring on elevated plus maze task (Stangherlin et al., 2006).

In addition, our research group has obtained persuasive evidence indicating that $(PhTe)_2$ affects a number of neuronal processes and modifies the functionality of the glutamatergic system in vitro and in vivo (Nogueira et al., 2001). Glutamate is known to play an important role in cognition, learning and memory (Davis et al., 1994; Maren, 1996; LeDoux, 1994) and in the neural plasticity of synaptic connections (Kaczmarek et al., 1997). Therefore, we examine if glutamatergic neurotransmission is involved in the $(PhTe)_2$ behavioral effect. In general, glutamate is the most dominant transmitter across tasks of learning and memory and has been linked to associative processes (Myhrer, 2003). In this context, one candidate for improve of memory is a persistent enhancement of glutamate release (Dolphin et al., 1982), triggered by a retrograde messenger or messengers following activation of postsynaptic glutamatergic receptor/channels (Bliss et al., 1990). Several studies have reported facilitated glutamate release with consequent increase in learning (Daisley et al., 1998; Lhullier et al., 2004; Mameli et al., 2005; McGahon et al., 1996). Different from these data, in the present study, synaptosomal glutamate release was not affected by $(PhTe)_2$. On the other hand, $(PhTe)_2$ inhibited glutamate uptake, which can increase the levels of extracellular glutamate.

According to the literature data, this event could improve memory in animals. However, the behavior observed in the present study is exactly the opposite. Taken together, these results pointed out that neurochemical mechanisms involved in the action of $(PhTe)_2$ in the impairment of memory exclude, at least in the whole brain, synaptosomal glutamate release and uptake processes. Several other studies have reported the involvement of glutamatergic system in learning/memory in specific cerebral structures, such as hippocampus (Izquierdo and Medina, 1997; Lisman et al., 2005; Rosenzweig and Barnes, 2003; Wu and Yamaguchi, 2004).

Our results provide evidence for the involvement of Na^+,K^+-ATPase in $(PhTe)_2$ effect. In fact, Na^+,K^+-ATPase activity was found inhibited in pups exposed to $(PhTe)_2$. Na^+,K^+-ATPase is an enzyme embedded in the cell membrane, responsible for the generation of the membrane potential through the active transport of sodium and potassium ions in the central nervous system necessary to maintain neuronal excitability (Erecinska and Silver, 1994). In this context, inhibition of Na^+,K^+-ATPase activity may be involved in the memory consolidation of step-down inhibitory avoidance in the hippocampus (Wyse et al., 2004). Moreover, stimulation of Na^+,K^+-ATPase activity was shown to inhibit neurotransmitter release (Vizi and Vyskocil, 1979). Since Na^+,K^+-ATPase is crucial for maintaining ionic gradients in neurons and is reported to be critically involved in potassium buffering after periods of hyperstimulation (Xiong and Stringer, 2000), it is well acceptable that a reduction in this enzyme activity may impair neuronal activity and memory storage.

Although the fact that a parallelism of effects was verified between Na^+,K^+-ATPase activity and memory, it does not necessarily mean that the reduced activity of this enzyme would be the only cause of the memory impairment observed. Accordingly, there is evidence of a role of Na^+,K^+-ATPase in long-term potentiation (Glushchenko and Izvarina, 1997), in long-term depression (Reich et al., 2004) and in spreading depression - a transient breakdown of neuronal function concomitant with a massive failure in ion homeostasis (Kohling et al., 2003). Recent studies have also reported that Na^+,K^+-ATPase inhibition can lead to memory impairment in the inhibitory avoidance and in the Water Maze tasks (dos Reis et al., 2002; Sato et al., 2004; Wyse et al., 2004), and to cognitive deficits in degenerative diseases, such as Alzheimer disease (Hattori et al., 1998; Lehotsky et al., 1999). Therefore, it is tempting to suggest that the possible mechanism of action of $(PhTe)_2$ in impair memory of recognition is related to Na^+,K^+-ATPase activity inhibition. In addition, the inhibitory effect of $(PhTe)_2$ on cerebral Na^+,K^+-ATPase activity has been reported by Borges and collaborators (2005).

Although the data observed in this study indicate that action of $(PhTe)_2$ in Na^+,K^+-ATPase activity seems not to be connected directly to glutamate release and uptake processes.

In conclusion, the present study establishes that pups presented cognitive impairment after maternal exposure to $(PhTe)_2$, demonstrated by the performance of animals in object recognition memory task. The Inhibition in cerebral Na^+,K^+-ATPase activity could be involved in $(PhTe)_2$-induced cognitive impairment. Additional investigations in specific cerebral structures, such as hippocampus, are necessary to determine the neurochemical mechanisms involved in the effect of $(PhTe)_2$ on learning/memory.

Acknowledgements

The financial support by FAPERGS, CAPES and CNPq is gratefully acknowledged. J.B.T.R. and C.W.N. are the recipients of CNPq fellowships.

References

Annau, Z., Cuomo V., 1988. Mechanisms of neurotoxicity and their relationship to behavioral changes. Toxicology 49, 219-225.

Bliss, T.V.P., Errington, M.L., Lynch, M.A., Williams, J.H., 1990. Presynaptic mechanisms in hippocampal long-term potentiation. Cold Spring Harbor Symposium on Quantitative Biology 55, 119-129.

Boles, J.O., Lebioda, L., Dunlap, R.B., Odum, J.D., 1995. Telluromethionine in structural biochemistry. Biochemistry And Biotechnology, Southern Association Of Agricultural Scientists 8, 29-34.

Borges, V.C., Rocha, J.B.T., Nogueira, C.W., 2005. Effect of diphenyl diselenide, diphenyl ditelluride and ebselen on cerebral Na^+, K^+-ATPase activity in rats. Toxicology 215, 191–197.

Budisa, N., Steipe, B., Demange, P., Eckerskorn, C., Kellernman, J., Huber, R., 1995. High level biosynthetic substitution of methionine in proteins by its analogues 2-aminohexanoic acid, selenomethionine, telluromethionine and ethionine in Escherichia coli. European Journal of Biochemistry 230, 788-796.

Comasseto, J.V., Ling, L.W., Petragnani, N., Stefani, H.A., 1997. Vinylic selenides and tellurides - Preparation, reactivity and synthetic applications. Synthesis 4, 373-403.

Davis, M., Rainnie, D., Cassel, M., 1994. Neurotransmission in the rat amygdale related to fear and anxiety. Trends in Neurosciences 17, 208-214.

Daisley, J.N., Gruss, M., Rose, S.P.R., Braun, K., 1998. Passive avoidance training and recall are associated with increased glutamate levels in the intermediate medial hyperstriatum ventrale of the day-old chick. Neural Plasticity 6, 53-61.

Dolphin, A.C., Errington, M.L. and Bliss, T.V.P., 1982. Long-term potentiation of the perforant path in vivo is associated with increased glutamate release. Nature 297, 496-498.

dos Reis, E. A., de Oliveira, L. S., Lamers, M. L., Netto, C. A., & Wyse, A. T. S., 2002. Arginine administration inhibits hippocampal Na(+),K(+)-ATPase activity and impairs retention of an inhibitory avoidance task in rats. Brain Research 951, 151–157.

Dru, D., Agnew, W.F. Greene, E., 1972. Effects of tellurium ingestion on learning capacity of the rat. Psychopharmacology 24, 508-515.

Dunkley, P. R., Heath, J., Harrison, S. M., Jarvie, P. E., Glenfield, P. Y., Rostas, J. A. P. 1988. A rapid gradient procedure for isolation of synaptosomes directly from an S-1 fraction-homogeneity and morphology of subcellular fractions. Brain Research 441, 59–71.

Erecinska, M., Silver, I., 1994. Ions And Energy In Mammalian Brain. Progress In Neurobiology 43, 37-71.

Fiske, C.H., Subbarow, Y.J., 1925. The calorimetric determination of phosphorus. Biological Chemistry 66, 375–381.

Glushchenko, T.S., Izvarina, N.L., 1997. Na + ,K + -ATPase activity in neurons and glial cells of the olfactory cortex of rat brain during the development of long-term potentiation. Neuroscience and Behavioral Physiology 27, 49– 52.

Goodrum, J.F., 1998. Role of organotellurium species in tellurium neuropathy. Neurochemical Research 23, 1313-1319.

Graeff, F.G., Netto, F.C., Zangrossi, H., 1998. The elevated T-maze as an experimental model of anxiety. Neuroscience & Biobehavioral Reviews 23, 237-246.

Green, M., Harwood, H., Barrowman, C., Rahman, P., Eggeman, A., Festry, F., Dobsonb, P., Ng, T.,2007. A facile route to CdTe nanoparticles and their use in bio-labelling. Journal of Materials Chemistry 17, 1989–1994.

Hattori, N., Kitagawa, K., Higashida, T., Yagyu, K., Shimohama, S., Wataya, T., Perry, G., Smith, M.A., Inagaki, C., 1998. CI-ATPase and Na+/K(+)-ATPase activities in Alzheimer's disease brains. Neuroscience Letters 2, 141–144.

Izquierdo, I. and Medina, J.H., 1997. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiology of Learning and Memory 68, 285–316.

Kaczmarek, L., Kossut, M., Skangielkramska, J., 1997. Glutamate receptors in cortical plasticity: molecular and cellular biology. Physiological Reviews 77, 217-255.

Kohling, R., Koch, U. R., Hagemann, G., Redecker, C., Straub, H., & Speckmann, E. J., 2003. DiVerential sensitivity to induction of spreading depression by partial disinhibition in chronically epileptic human and rat as compared to native rat neocortical tissue. Brain Research 975, 129–134.

Lalonde, R, Qian, S, Strazielle, C., 2003. Transgenic mice expressing the PSI-A346E mutation: effects on spatial learning, exploration, anxiety, and motor coordination. Behavioural Brain Research 138, 71-79.

Lampert, P., Garro, F., Pentschew, A., 1970. Tellurium neuropathy. Acta Neuropathologica 15, 308-317.

Lampert, P.W., Garrett, R.S., 1971. Mechanism of demyelination in tellurium neuropathy. Electron microscopic observations. Laboratory Investigation 25, 380-388.

Larner, A.J., 1995a. Biological effects of tellurium: a review. Trace Elements Electrolytes 12, 26-31.

Larner, A.J., 1995b. How does garlic exert its hypocholesterolaemic action? The tellurium hypothesis. Medical Hypothesis 44, 295-297.

LeDoux, J.E., 1994. Emotion, memory and the brain. Scientific American 270, 50-57.

Lehotsky, J., Kaplan, P., Racay, P., Matejovicova, M., Drgova, A., & Mezesova, V., 1999. Membrane ion transport systems during oxidative stress in rodent brain:protective eVect of stobadine and other antioxidants. Life Sciences 65, 1951–1958.

Lhullier, F.L.R., Nicolaidis, R., Riera, N.G., Cipriani, F., Junqueira, D., Dahm, K.C.S., Brusque, A.M., Souza, D.O., 2004. Dehydroepiandrosterone increases synaptosomal glutamate release and improves the performance in inhibitory avoidance task. Pharmacology Biochemistry and Behavior 77, 601–606.

Lisman, J.E., Talamini, L.M., Raffone, A., 2005. Recall of memory sequences by interaction of the dentate and CA3: A revised model of the phase precession. Neural Networks 18, 1191–1201.

Lowry, O.H., Rosemburg, N.J., Farr, A.L., Roudall, R., 1951. Protein measurement with Folin-Phenol reagent. Journal of Biological Chemistry 193, 265-275.

Mameli, M., Zamudio, P.A., Carta, M., Valenzuela, C.F., 2005. Developmentally regulated actions of alcohol on hippocampal glutamatergic transmission. Journal of Neuroscience 25, 8027.

Maren, S., 1996. Synaptic transmission and plasticity in the amygdala. Molecular Neurobiology 13, 1-22.

McGahon, B., Holscher, C., McGlinchey, L., Rowan, M.J., Lynch, M.A., 1996. Training in the Morris water maze occludes the synergism between ACPD and arachidonic acid on glutamate release in synaptosomes prepared from rat hippocampus. Learning & Memory 3, 296-304.

Meotti, F.C., Borges, V.C., Zeni, G., Rocha, J.B.T., Nogueira, C.W., 2003. Potential renal and hepatic toxicity of diphenyl diselenide, diphenyl ditelluride and Ebselen for rats and mice. Toxicology Letters 143, 9-16.

Migues, P. V., Leal, R. B., Mantovani, M., Nicolau, M., Gabilan, N. H., 1999. Synaptosomal glutamate release induced by the fraction Bc2 from the venon of the sea anemone Bunodosoma caissarum. NeuroReport 10, 67–70.

Morell, P., Toews, A.D., Wagner, M., Goodrum, J.F., 1994. Gene expression during tellurium-induced primary demyelination. Neurotoxicology 15, 171-180.

Muller, R., Zschiesche, W., Steffen, H., Schaller, K., 1989. Tellurium-intoxication. Klin Wochenschr 67, 1152–1155.

Myhrer, T., 2003. Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis based on studies of four behavioral tasks. Brain Research Reviews 41, 268–287.

Newman, R.A., Osborn, S., Siddik, Z.H., 1989. Determination of tellurium in biological fluids by means of electrothermal vapourization-inductively coupled to plasma mass spectrometry (ETV-ICP-MS). Clinica Chimica Acta 179, 191-196.

Nishimura, Y., Sahoo, S. K., Kim, H.-S., Homma-Takeda, S., Watanabe, Y., Inaba, J., 2003. Biokinetics of radiotellurium in rats. Radiation Protection Dosimetry 105, 285–290.

Nogueira, C.W., Zeni, G., Rocha, J.B.T., 2004. Organoselenium and organotellurium compounds: Toxicology and Pharmacology. Chemical Reviews 104, 6255-6286.

Nogueira, C.W., Rotta, L.N., Perry, M.L., Souza, D.O., Rocha, J.B.T., 2001. Diphenyl diselenide and diphenyl ditelluride affect the rat glutamatergic system in vitro and in vivo. Brain Research 906, 157– 163.

Petragnani, N., 1994. Preparation of the Principal Classes of Organic Tellurium compounds. in: Tellurium in Organic Synthesis (A.R. Katritzky, O. Meth-Cohn, C.W. Rees), Academic Press, London, pp. 9-88.

Puma, C., Deschaux, O., Molimard, R., Bizot, J.C., 1999. Nicotine improves memory in an object recognition task in rats. European Neuropsychopharmacology 9, 323–327.

Ramadan, S.E., Razak, A.A., Ragab, A.M., el –Meleigy, M., 1989. Incorporation of tellurium into amino acids and proteins in a tellurium-tolerant fungi. Biological Trace Element Research 20, 225-232.

Rampon, C., Tang, Y.P., Goodhouse, J., Shimizu, E., Kyin, M., Tsien, J.Z., 2000. Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. Nature Neuroscience 3, 238–244.

Rosa, R.M., Flores, D.G., Appelt, H.R., Braga, A.L., Henriques, J.A.P., Roesler, R., 2003. Facilitation of long-term object recognition memory by pretraining administration of diphenyl diselenide in mice. Neuroscience Letters 341, 217–220.

Rosenzweig, E.S., Barnes, C.A., 2003. Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. Progress in Neurobiology 69,143–179.

Sato, T., Tanaka, K., Ohnishi, Y., Teramoto, T., Irifune, M., Nishikawa, T., 2004. Effects of steroid hormones on (Na+,K+)-ATPase activity inhibitioninduced amnesia on the step-through passive avoidance task in gonadectomized mice. Pharmacological Research 49, 151–159.

Schroeder, H.A., Buckman, J., Balassa., J.J., 1967. Abnormal trace elements in man: tellurium. Journal of Chronic Disease 20, 147-161.

Siddik, Z.H., Newman, R.A., 1988. Use of platinum as a modifer in the sensitive detection o€ tellurium in biological samples. Analytical Biochemistry 172, 190-196.

Stangherlin, E.C., Favero, A.M., Zeni, G., Rocha, J.B.T., Nogueira, C.W., 2005. Teratogenic vulnerability of rat fetuses to diphenyl ditelluride: prenatal assessment. Toxicology 207, 231-239.

Stangherlin, E.C., Favero, A.M., Zeni, G., Rocha, J.B.T., Nogueira, C.W., 2006. Exposure of mothers to diphenyl ditelluride during the suckling period changes behavioral tendencies in their offspring. Brain Research Bulletin 69, 311-317.

Vizi, E.S., Vyskocil, F., 1979. Changes in total and quantal release of acetylcholine in the mouse diaphragm during activation and inhibition of membrane ATPase. The Journal of Physiology 286, 1–14.

Wagner-Recio, M., Toews, A.D., Morell, P., 1994. Tellurium blocks cholesterol synthesis by inhibiting squalen metabolism: preferential vulnerability to this metabolic block leads to peripheral nervous system demyelination. Journal of Neurochemistry 57, 1891-1901.

Widy-Tysziewicz, E., Piechal, A., Gajkowska, B., Smialek, M., 2002. Tellurium-induced cognitive deficits in rats are related to neuropathological changes in the central nervous system. Toxicology Letters 131, 203-214.

Wu, Z., and Yamaguchi, Y., 2004. Input-dependent learning rule for the memory of spatiotemporal sequences in hippocampal network with theta phase precession. Biological Cybernetics 90, 113–124.

Wyse, A.T., Bavaresco, C.S., Reis, E.A., Zugno, A.I., Tagliari, B., Calcagnotto, T., Netto, C.A., 2004. Training in inhibitory avoidance causes a reduction of Na+,K+-ATPase activity in rat hippocampus. Physiology & Behavior 80, 475–479.

Xiong, Z. Q., and Stringer, J. L., 2000. Sodium pump activity, not glial spatial buVering, clears potassium after epileptiform activity induced in the dentate gyrus. Journal of Neurophysiology 83, 1443–1451.

Yarema, M.C., Curry, S.C., 2005. Acute tellurium toxicity from ingestion of metal-oxidizing solutions. Pediatrics 116, 319-321.

Yu, L., He, K., Chai, D., Yang, C., Zheng, O., 1993. Evidence for telluroamino acid in biological materials and some rules for assimilation of inorganic tellurium by yeast. Analytical Biochemistry 209, 318-322.

Zhang H., Swihart M.T., 2007. Synthesis of Tellurium Dioxide Nanoparticles by Spray Pyrolysis Chemistry of Materials 19, 1290-1301.

Legends

Figure 1. Behavioral analysis. All animals were given to freely exploring the open field for 5 min for the habituation trial (a); training (b) carried out 24 h after habituation; the short-term memory (STM) test (c) carried out 1.5 h after training; and the long-term memory (LTM) test (d) carried out 24 h after training. A, B and C represent the objects. Exploratory preference in: *Training* = $(A_2/(A_1 + A_2)) * 100$; *STM* = $(B/(A_1 + B)) * 100$; *LTM* = $(C/(A_1 + C)) * 100$.

Figure 2. Evaluation of exploratory preference on object recognition task in young rats during Training (percentage of time exploring any of the two identical objects (A)), STM (percentage of time exploring the novel object (B), test carried out 1.5 h after training) and LTM (percentage of time exploring the novel object (C), test carried out 24 h after training). Exploratory preference in: *Training* = $(A_2/(A_1 + A_2)) * 100$; *STM* = $(B/(A_1 + B)) * 100$; *LTM* = $(C/(A_1 + C)) * 100$. The animals were exposed to $(PhTe)_2$ (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) by subcutaneous (s.c.) injection once daily during the first 14 days of lactational period. Results are expressed as mean ± S.E.M. n = 6-8 litters (4 animals each litter). $^{\#}p<0.05$ compared to the control group during training; $*p<0.05$ compared to the control group during STM.

Figure 3. Evaluation of synaptosomal [^{3}H]glutamate uptake of young rats exposed to $(PhTe)_2$ (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) by subcutaneous (s.c.) injection once daily during the first 14 days of lactational period. Results are expressed as mean ± S.E.M. for 3 independent experiments performed in triplicate. $*p < 0.05$ compared to the control group.

Figure 4. Evaluation of synaptosomal [^{3}H]glutamate release of young rats exposed to $(PhTe)_2$ (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) by subcutaneous (s.c.) injection once daily during the first 14 days of lactational period. Results are expressed as mean ± S.E.M. for 3 independent experiments performed in triplicate.

Figure 5. Determination of Na^+, K^+-ATPase activity in brain of young rats exposed to $(PhTe)_2$ (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) by subcutaneous (s.c.) injection once daily during the first 14 days of lactational period. Results are expressed as mean ± S.E.M. for 6-8 independent experiments performed in duplicate. $*p < 0.05$ compared to the control group.

Figure 1.

Figure 2.

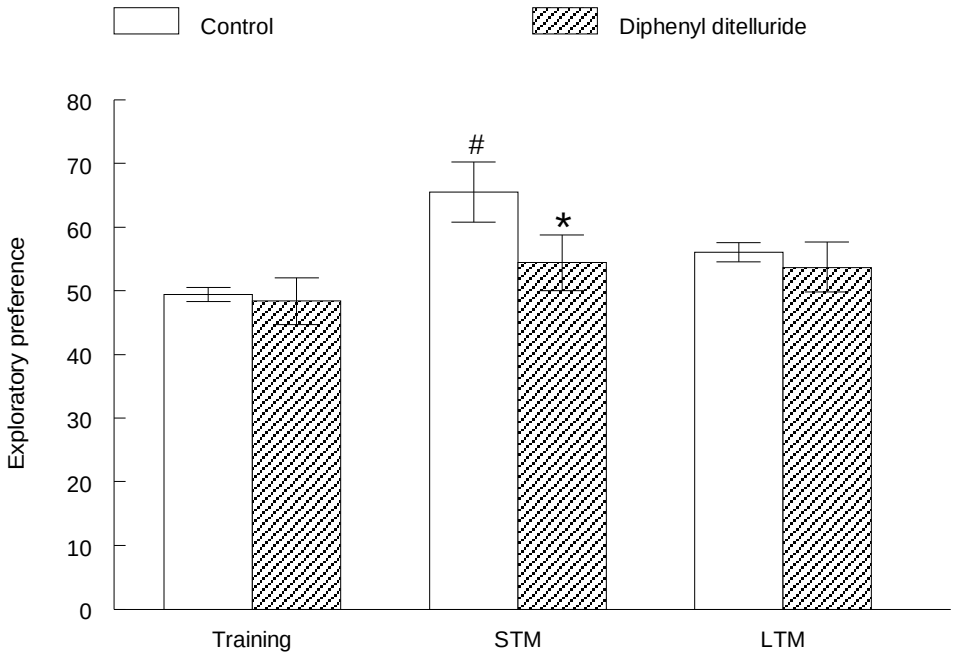


Figure 3.

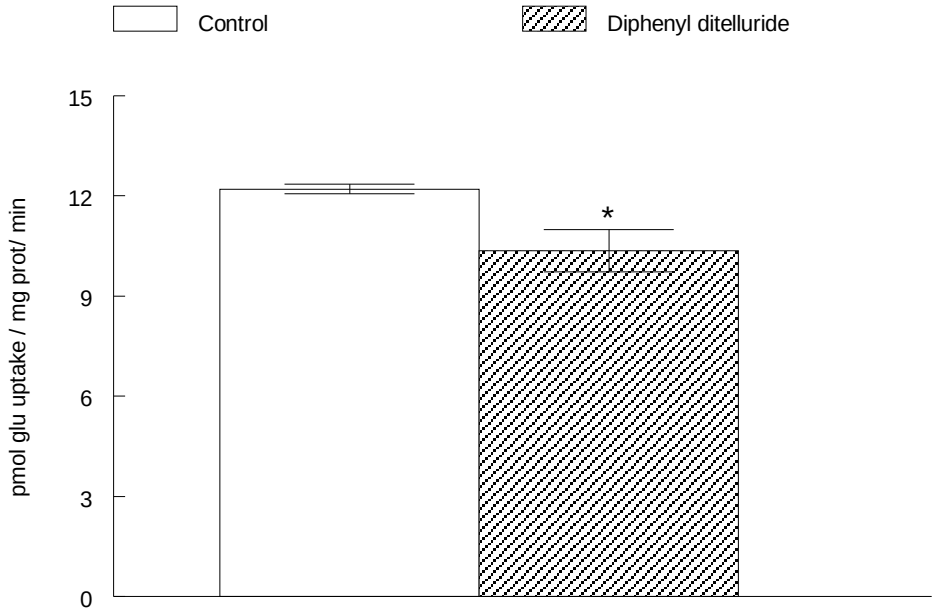


Figure 4.

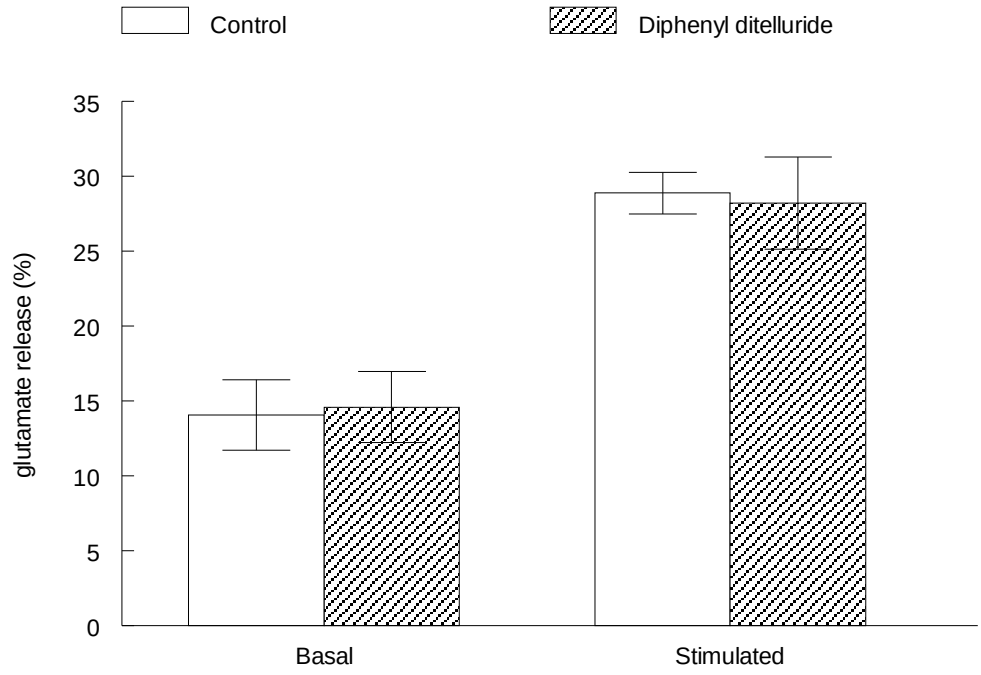
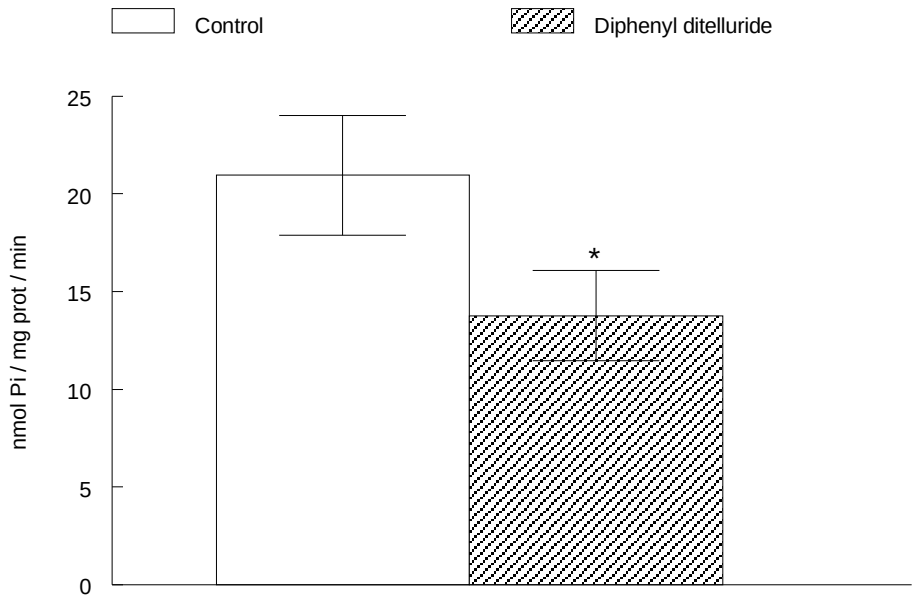


Figure 5.



4.3 – Manuscrito 2:

Exposição ao ditelureto de difenila, via leite materno, causa estresse oxidativo no córtex cerebral, hipocampo e estriado de filhotes de rato

EXPOSURE TO DIPHENYL DITELLURIDE, VIA MATERNAL MILK, CAUSES OXIDATIVE STRESS IN CEREBRAL CORTEX, HIPPOCAMPUS AND STRIATUM OF RAT PUPS

Eluza Curte Stangherlin, Ana Paula Ardais, João Batista Teixeira Rocha, Cristina Wayne Nogueira*

Em fase de redação

Exposure to Diphenyl Ditelluride, via maternal milk, Causes Oxidative Stress in Cerebral Cortex, Hippocampus and Striatum of Rat Pups

Eluza Curte Stangherlin[a], Ana Paula Ardais[a], João Batista Teixeira Rocha[a], Cristina Wayne Nogueira[a]*

[a]Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, CEP 97105-900 Santa Maria, RS, Brazil,

Correspondence should be sent to:
Cristina Wayne Nogueira
Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brasil.
Phone: 55-55-3220-8140
FAX: 55-55-3220-8978
E-mail: criswn@quimica.ufsm.br (Nogueira CW)

Abstract

The purpose of the present study was to evaluate the effect of diphenyl ditelluride [$(PhTe)_2$] exposure to mothers on the cerebral oxidative status of their offspring. The dams received $(PhTe)_2$ (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) via subcutaneous (s.c.) injection once daily during the first 14 days of lactational period (sub-chronic exposure). At post natal day 30, biochemical parameters of oxidative stress (lipid peroxidation, non-protein thiols (NPSH) and ascorbic acid levels, superoxide dismutase (SOD), catalase and δ-aminolevulinic acid dehydratase (δ-ALA-D) activities) in cerebral structures - cerebral cortex, hippocampus and striatum - of pups exposed to $(PhTe)_2$ via maternal milk were evaluated. Exposure to $(PhTe)_2$ increased lipid peroxidation and inhibited δ-ALA-D, catalase and SOD activities in hippocampus and striatum of pups. $(PhTe)_2$ induced changes in the levels of non-enzymatic defenses in cerebral cortex and striatum of pups. In conclusion, these results showed that $(PhTe)_2$ disrupted cerebral prooxidant/antioxidant balance, which can lead to brain injury via oxidative damage to critical biomolecules.

Keywords*:* Diphenyl ditelluride, tellurium, rat pups, oxidative stress, brain, antioxidant.

1. Introduction

The metallic element tellurium (Te) is known to be present in plant material, particularly in members of the Alium family (Larner, 1995). Currently, inorganic Te is used in industry of nanoparticulate semiconductors (Green et al., 2007; Zhang and Swihart, 2007). In this context, Te dioxide based nanoparticles would be particularly interesting for the formation of polymer/nanoparticle nanocomposites with a high refractive index and high optical nonlinearity (Zhang and Swihart, 2007). Moreover, the use of organic Te compounds will increase due to its importance in organic synthesis (Zeni et al., 2006).

A number of studies have shown that trace amounts of tellurium are present in body fluids (Siddik and Newman, 1988; Newman et al., 1989). But to date, no telluroproteins have been identified in animal cells. By contrast, attention has been drawn to the toxicity of tellurium.

Currently, in the literature it was reported two cases of toxicity in young children from ingestion of metal-oxidizing solutions that contained substantial concentrations of tellurium (Yarema and Curry, 2005).

Data of our group of research suggest that diphenyl ditelluride [$(PhTe)_2$], an organochalcogen compound, is a teratogenic agent causing various morphologic abnormalities in rat fetuses in development (Stangherlin et al., 2005). Besides, exposure to mothers to low doses of $(PhTe)_2$ may result in disinhibition behavior on elevated plus maze task (Stangherlin et al., 2006).

Of particular importance, our research group has obtained persuasive evidence indicating that $(PhTe)_2$ causes marked neurotoxic effects in rodents after acute or prolonged exposure either by subcutaneous or intraperitoneal routes (Maciel et al., 2000; Nogueira et al., 2001; Meotti et al., 2003). Although the specific molecular targets that mediate organochalcogens toxicity are not known, these compounds can interact directly with low molecular thiols, oxidizing them to disulfides (Nogueira et al., 2004). In fact, reduced cysteinyl residues from proteins react with these compounds, which may cause, in the case of the enzymes, the loss of their catalytic activity (Park et al., 2000; Gupta and Porter, 2001; Nogueira et al., 2003). $(PhTe)_2$ inhibit the δ-aminolevulinate dehydratase (δ-ALA-D) activity (Barbosa et al., 1998). This sulfhydryl-containing enzyme catalyzes the condensation of two δ-aminolevulinic acid (ALA) molecules with the formation of porphobilinogen, which is a heme precursor (Jaffe, 1995). Consequently, δ-ALA-D inhibition may impair heme biosynthesis (Sassa et al., 1989) and can result in the accumulation of ALA, which may affect the aerobic metabolism and may have some prooxidant activity (Bechara et al., 1993).

The cellular redox status is defined as the balance between intracellular oxidants and antioxidants (Castagne et al., 1999). The brain is considered as a sensitive organ prone to oxidative damage because it has a high rate of oxidative metabolism, a high content of polyunsaturated fatty acids and low levels of protective enzymes to eliminate free radicals (Kodavanti, 1999). The increased free radical levels can alter dramatically the neuronal function (Frantseva et al., 2000). In particular, it has been suggested that free radicals acting via oxidative stress, may be involved in neuronal plasticity and the excessive content of reactive species derivate of oxygen (ROS) can induce neuronal damage in young and adult rats (McCobb et al., 1988). To circumvent oxidative stress organisms have systems that prevent hazardous effects of free radicals such as enzymatic (superoxide dismutase (SOD) and catalase (CAT)) e non-enzymatic (non-protein thiols (NPSH) and ascorbic acid) defenses.

Thus, the purpose of the present study was to evaluate the effect of $(PhTe)_2$ exposure to mothers on the cerebral oxidative status of their offspring. For this end, biochemical parameters of oxidative stress (lipid peroxidation, NPSH and ascorbic acid levels, SOD, catalase and δ-ALA-D activities in cerebral structures (cerebral cortex, hippocampus and striatum) of pups exposed to $(PhTe)_2$ via maternal milk were evaluated.

2. Experimental Procedure

2.1 Materials

Diphenyl ditelluride $(PhTe)_2$ was prepared according to literature method (Petragnani, 1994). Analysis of the ^{1}H NMR and ^{13}CNMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of $(PhTe)_2$ (99.9%) was determined by GC/HPLC. $(PhTe)_2$ is solid compound, very stable and can be stored in the lab, in the simple flasks for long time. $(PhTe)_2$ was diluted in canola oil which was obtained from a standard commercial supplier.

2.2 Animals

Adult female Wistar rats (200-250 g) and their offspring from our own breeding colony were used. The animals were kept on a 12 light/dark cycle, at a controlled temperature (22±2ºC), with free access to water and food (Guabi, RS, Brazil). The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil.

2.3 Exposure to Diphenyl Ditelluride

Sexually naïve female rats were mated with male previously tested as fertile (three females and one male in each cage). The onset of pregnancy was confirmed by the presence of sperm in vaginal smears (day 0 of pregnancy) and pregnant dams were immediately housed in individual cages. At birth, the dams received $(PhTe)_2$ (0.03 mg/kg, experimental group) or canola oil (1 ml/kg, control group) via subcutaneous (s.c.) injection once daily during the first 14 days of lactational period (sub-chronic exposure). The dose of $(PhTe)_2$ used in this study was selected on the basis of LD_{50} study carried out in our laboratory (Meotti et al., 2003). Maternal body weight was recorded during this period. The body weight of rat pups was recorded once daily until 30 postnatal day (PND 30). At birth, all litters were culled to eight pups. Whenever possible, only male rats were kept within the litter and females were kept just to maintain equal litter sizes. On PND 21, pups were weaned and placed on ad libitum standard rat chow diets. After 1-week post-weaning period (in PND 30), the animals were killed by decapitation without anesthesia, the brain was removed and cerebral structures - cerebral cortex, hippocampus and striatum - were separated. The tissues were kept under cooling.

2.4 Biochemical parameters

2.4.1 Lipid peroxidation

Thiobarbituric acid reactive species (TBARS) were determined as described by Ohkawa et al. (1979). An aliquot of cerebral structures tissue was incubated with 0.8 % thiobarbituric acid (TBA), acetic acid buffer pH 3.4 and 8.1 % sodium dodecil sulphate (SDS) at 95°C for 2 hours. The color reaction was measured at 532 nm.

2.4.2 Determination of non-protein thiols (NPSH)

NPSH in cerebral structures were determined by the method of Ellman (1959). Briefly, the homogenate was centrifuged at 4000 x g at 4°C for 10 minutes and the supernatant was mixed (1:1) with 10% trichloroacetic acid. After the centrifugation, the protein pellet was discarded and free -SH groups were determined in the clear supernatant. An aliquot of supernatant was added in potassium phosphate buffer 1 M pH 7.4 and 10 mM 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB). The color reaction was measured at 412 nm.

2.4.3 Ascorbic acid determination

Ascorbic acid determination was performed as described by Jacques-Silva et al. (2001). Protein (cerebral structures) was precipitated in 10 volumes of a cold 4 %

trichloroacetic acid solution. An aliquot of the sample in a final volume of 1 ml of the solution was incubated for 3 hours at 38°C then H_2SO_4 65 % (v/v) was added to the medium. The reaction product was determined using color reagent containing 4.5 mg/ml dinitrophenyl hydrazine and $CuSO_4$ (0.075 mg/ml). The color reaction was measured spectrophotometrically at 520 nm.

2.4.4 Catalase activity

The catalase activity was assayed spectrophotometrically by the method of Aebi et al. (1984), which involves monitoring the disappearance of H_2O_2 in the presence of cerebral structures homogenate at 240 nm. An aliquot of cerebral structures tissue was added in 50mM potassium phosphate buffer pH 7.0 and the enzymatic reaction was initiated by adding H_2O_2. The enzymatic activity was expressed in Units (1U decomposes 1 μmol H_2O_2/min at pH 7 at 25°C).

2.4.5 Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity in cerebral structures homogenate was assayed spectrophotometrically as described by Misra and Fridovich (1972). This method is based on the capacity of SOD in inhibiting autoxidation of adrenaline to adrenochrome. The color reaction was measured at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50 % at 26°C. The cerebral structures tissue was diluted 1:10 (v/v) for determination of SOD activity in test day. Aliquots of cerebral structures were added in a glycine buffer 50 mM pH 10.3. Enzymatic reaction was started by adding of the epinephrine.

2.4.6 δ-Aminolevulinic acid dehydratase (δ-ALA-D) activity

δ-ALA-D activity in the cerebral structures was assayed according to the method of Sassa (1982) by measuring the rate of product (porphobilinogen) formation except that 1 M potassium phosphate buffer pH 6.8 and 12 mM ALA were used. An aliquot of tissue was incubated for 3 hours at 37°C. The reaction was linear in relation to protein and time of incubation. The reaction product was determined using modified Erlich's reagent at 555 nm.

2.4.7 Protein determination

Protein was measured by the Coomassie blue method according to Bradford (1976) using bovine serum albumin as standard. An aliquot of cerebral structures tissue diluted 1:10

(v/v) was added in Comassie blue reactive. The color was measured spectrophotometrically at 595 nm.

2.5 Statistical Analysis

Results are reported as mean ± S.E.M. n = 6-8 litters (4 animals each litter and the litter is an experimental unit). Statistical analysis was performed using a one-way ANOVA followed by the Duncan's test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1 Body weight

There was no difference in the body weight gain between rat pups exposed to diphenyl ditelluride and controls (data not shown).

3.2 Biochemical parameters

3.2.1 Lipid peroxidation

As can be observed in Figure 1, exposure to $(PhTe)_2$ via maternal milk increased lipid peroxidation in the TBARS assay ($p<0.05$ by Duncan's multiple range test). This effect was observed in the hippocampus and striatum of pups. One-way ANOVA demonstrated that exposure to $(PhTe)_2$ via maternal milk did not alter lipid peroxidation in cerebral cortex of pups.

3.2.2 NPSH levels

In cerebral cortex and striatum, one-way ANOVA ($p<0.05$ by Duncan's multiple range test) of NPSH levels yielded a significant increase in pups exposed to $(PhTe)_2$. In hippocampus, NPSH levels were not modified for $(PhTe)_2$ exposure (Figure 2).

3.2.3 Ascorbic acid content

One-way ANOVA ($p<0.05$ by Duncan's multiple range test) showed that ascorbic acid content was increased in striatum of $(PhTe)_2$ exposed pups. In hippocampus and cerebral cortex, the ascorbic acid content was similar to the control group (Figure 3).

3.2.4 Catalase activity

The catalase activity was inhibited in hippocampus and striatum of pups exposed to $(PhTe)_2$ via maternal milk. While, the enzyme activity in cerebral cortex was not altered by $(PhTe)_2$ exposure (Figure 4).

3.2.5 SOD activity

In hippocampus and striatum, SOD activity was inhibited in pups exposed to $(PhTe)_2$. In cerebral cortex, the enzyme activity was similar among groups $(PhTe)_2$ (Figure 5).

3.2.6 δ-ALA-D activity

One-way ANOVA revealed that δ-ALA-D activity was inhibited in hippocampus and striatum of pups exposed to $(PhTe)_2$ via maternal milk ($p<0.05$ by Duncan's multiple range test). Exposure to $(PhTe)_2$ did not alter δ-ALA-D activity in cerebral cortex of pups (Figure 6).

4. Discussion

The findings of the present study demonstrate that sub-chronic exposure to $(PhTe)_2$, via maternal milk, caused different responses in cerebral oxidative stress in rat pups in early postnatal period, a period in which the brain is still in developing. The exposure to $(PhTe)_2$ increased lipid peroxidation and inhibited δ-ALA-D, catalase and SOD activities in hippocampus and striatum of pups. Moreover, exposure to $(PhTe)_2$ induced changes in the levels of non-enzymatic defenses in cerebral cortex and striatum of pups.

Reactive oxygen species (ROS) such as superoxide radical anion, hydroxyl radical and hydrogen peroxide are produced in metabolic and physiological processes and harmful oxidative reactions may occur in organisms (Halliwell, 2006). The oxidative effects of ROS are controlled by non-enzymatic antioxidants, such as ascorbic acid, NPSH and glutathione, and also by enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase). Under some conditions, the increase in oxidants and the decrease in antioxidants cannot be prevented, and the oxidative/antioxidative balance shifts towards the oxidative status. Consequently, oxidative stress, has been implicated in over 100 disorders develop (Halliwell, 2006). In this context, the brain is extremely vulnerable to oxidative stress, in part because it is highly enriched with non-heme iron, which is catalytically involved in the production of oxygen free radicals. In addition, the brain contains a relatively high degree of polyunsaturated fatty acids that are particularly good substrates for peroxidation reactions (Halliwell and Gutteridge, 2000). The results of the current study suggest that $(PhTe)_2$, by

interacting with biological membranes, induced an increase of lipid peroxidation in hippocampus and striatum of pups.

Moreover, exposure to $(PhTe)_2$ produced also an inhibition of δ-ALA-D in hippocampus and striatum of pups. This inhibition of δ-ALA-D activity is probably a consequence of oxidative stress induced by exposure to $(PhTe)_2$. Many cerebral enzymes which contain sulfhydryl groups, such as δ-ALA-D, are sensitive to oxidizing agents (Borges et al., 2005) and to situations associated with oxidative stress (Demasi et al., 1996; Prigol et al., 2007). It is important to point out that ALA accumulation has been reported in tissues of animals and patients which δ-ALA-D activity is inhibited (Juknat et al., 1995). ALA can undergo autooxidation generating reactive oxygen species and the ALA enoyl radical (Bechara et al., 1993). These reactive species increased lipid peroxidation (Emanuelli et al., 2003) and induced oxidative damage (Demasi et al., 1996).

Regarding to catalase and SOD, exposure to $(PhTe)_2$ inhibited the activity of these enzymes in hippocampus and striatum of pups. Antioxidant enzymes are considered to be a primary defense that prevents biological macromolecules from oxidative damage. SOD is mainly located in neurons whereas GPx, the major protective enzyme against the action of H_2O_2, is mostly present in astrocytes. The brain has a much higher SOD to GPx activity ratio than other organs of the rat (Benzi and Moretti, 1995). This, together with lower CAT activity, makes the brain the most vulnerable organ to H_2O_2. Moreover, acute exposure generally enhanced the production of these antioxidant enzymes as a result of adaptive response, which consequently mitigate the damage (Hilbert and Mohsenin, 1996). However, after prolonged exposure, the toxic effects appear to override the adaptive mechanism of the body tissues, as indicated by a decrement in the levels of these enzymes (Hulea et al., 1995). The experimental protocol carried out in this study was performed in two weeks of exposure, an intermediary time, classified as sub-chronical protocol. This time can express alterations observed in acute and/or chronic exposures.

The exposure to $(PhTe)_2$ induced changes in the levels of non-enzymatic defenses in cerebral cortex and striatum of pups. In fact, NPSH levels were found increased in cerebral cortex and striatum, and ascorbic acid content was increased only in striatum of pups exposed to $(PhTe)_2$ via maternal milk. The changes observed in the non-enzymatic antioxidant defenses could be explained by the adaptive response of cerebral tissue. This may in part reflect the participation of these antioxidants in toxicological mechanisms by which $(PhTe)_2$ causes oxidative damage in brain of pups.

Additionally, the data obtained herein suggest that compared to cerebral cortex, hippocampus and striatum were more susceptible to oxidative stress induced by $(PhTe)_2$, since that different parameters of oxidative stress were altered in these structures.

In conclusion, our results show that one possible molecular mechanism involved in the $(PhTe)_2$ disrupted cerebral prooxidant/antioxidant balance, which can lead to brain injury via oxidative damage to critical biomolecules.

Legends

Figure 1 - Effect of exposure to $(PhTe)_2$ via maternal milk on TBARS levels in cerebral structures of pups. Results are reported as mean ± S.E.M. n = 6-8 litters (4 animals each litter). Data are expressed as nmol MDA (malondialdehyde)/g tissue. (*) Denoted $p < 0.05$ as compared to the respective control group.

Figure 2 - Effect of exposure to $(PhTe)_2$ via maternal milk on NPSH levels in cerebral structures of pups. Results are reported as mean ± S.E.M. n = 6-8 litters (4 animals each litter). Data are expressed as μmol NPSH/g tissue. (*) Denoted $p < 0.05$ as compared to the respective control group.

Figure 3 - Effect of exposure to $(PhTe)_2$ via maternal milk on ascorbic acid levels in cerebral structures of pups. Results are reported as mean ± S.E.M. n = 6-8 litters (4 animals each litter). Data are expressed as μmol ascorbic acid/g tissue. (*) Denoted $p < 0.05$ as compared to the respective control group.

Figure 4 - Effect of exposure to $(PhTe)_2$ via maternal milk on catalase activity in cerebral structures of pups. Results are reported as mean ± S.E.M. n = 6-8 litters (4 animals each litter). Data are expressed as U/mg protein. (*) Denoted $p < 0.05$ as compared to the respective control group.

Figure 5 - Effect of exposure to $(PhTe)_2$ via maternal milk on SOD activity in cerebral structures of pups. Results are reported as mean ± S.E.M. n = 6-8 litters (4 animals each litter). Data are expressed as U SOD/mg protein. (*) Denoted $p < 0.05$ as compared to the respective control group.

Figure 6 - Effect of exposure to $(PhTe)_2$ via maternal milk on δ-ALA-D activity in cerebral structures of pups. Results are reported as mean ± S.E.M. n = 6-8 litters (4 animals each litter). Data are expressed as nmol PBG (porphobilinogen)/mg protein/hour. (*) Denoted $p < 0.05$ as compared to the respective control group.

Acknowledgements

The financial support by FAPERGS, CAPES and CNPq is gratefully acknowledged C.W.N. is the recipient of CNPq fellowships.

References

Aebi, H. (1984). Catalase in vitro. Method. Enzymol. 105, 121-126.

Barbosa N. B. V., Rocha J. B. T., Zeni G., Emanuelli T., Beque M. C., Braga A. L. Effect of Organic Forms of Selenium on d-Aminolevulinate Dehydratase from Liver, Kidney, and Brain of Adult Rats. Toxicology and applied pharmacology 149, 243–253 (1998)

Bechara, E. J. H., Medeiros, M. H. G., Monteiro, H. P., Hermes-Lima, M., Pereira, B., Demasi, M., Costa, C. A., Abdall, D. S. P., Onuki, J., Wendel, C. M. A., and Masci, P. D. (1993). A free radical hypothesis of lead poisoning and inborn porphyrias associated with 5-aminolevulinic acid overload. Quimica Nova 16, 385–392.

Benzi, G., Moretti, A., 1995. Age- and peroxidative stress-related modifications of the cerebral enzymatic activities linked to mitochondria and glutathione system. Free Radic. Biol. Med. 19, 77–101.

Borges, V.C., Rocha, J.B.T., Nogueira, C.W., 2005. Effect of diphenyl diselenide, diphenyl ditelluride and Ebselen on cerebral Na+ K+-ATPase activity in rats. Toxicology 215, 191–197.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Anal. Biochem. 72, 248-254.

Castagne, V., Gautschi, M., Lefevre, K., Posada, A., Clarke, P.G., 1999. Relationships between neuronal death and the cellular redox status. Prog. Neurobiol. 59, 397–423.

Demasi, M., Penatti, C.A.A., De Lucia, R., Bechara, E.J.H., 1996. The prooxidant effect of 5-aminolevulinic acid in the brain tissue of rats: implications in neuropsychiatric manifestations in porphyrias. Free Radical Biol. Med. 20, 291–299.

Ellman, G.L. (1959). Tissue sulfhydryl groups. Arch. Biochem. 82, 70-77.

Emanuelli, T., Pagel, F.W., Porciúncula, L.O., Souza, D.O., 2003.Effects of 5-aminolevulinic acid in the glutamatergic neurotransmission. Neurochem. Int. 42, 115–121.

Farina, M., Folmer, V., Bolzan, R.C., Andrade, L.H., Zeni, G., Braga, A.L., Rocha, J.B.T., 2001. Selenoxides inhibit δ-aminolevulinic acid dehydratase. Toxicol. Lett. 119, 27-37.

Frantseva M.V., V.J.L. Perez, P.A. Hwang, P.L. Carlen, Free radical production correlates with cell death in an vitro model of epilepsy, Eur. J. Neurosci. 12 (2000) 1431–1439.

Green, M., Harwood, H., Barrowman, C., Rahman, P., Eggeman, A., Festry, F., Dobsonb, P., Ng, T.,2007. A facile route to CdTe nanoparticles and their use in bio-labelling. Journal of Materials Chemistry 17, 1989–1994.

Gupta, N., Porter, T.D., 2001. Inhibition of human squalene monooxigenase by selenium compounds. J. Biochem. Mol. Toxicol. 16, 18–23

Halliwell, B. Oxidative stress and neurodegeneration: where arewe now? Journal of Neurochemistry, 2006, 97, 1634–1658

Halliwell B. and Gutteridge, J.M.C., editors (2000). Free radicals in biology and medicine, 3rd ed. Oxford: Oxford Science Publications.

Hilbert, J., Mohsenin, V., 1996. Adaptation of lung antioxidants to cigarette smoking in humans. Chest 110, 916–920.

Hulea, S.A., Olinescu, R., Nita, S., Crocnan, D., Kummerow, A., 1995. Cigarette smoking causes biochemical changes in blood that are suggestive of oxidative stress: a case control study. Journal of Environmental Pathology Toxicology and Oncology 14, 173–180.

Jacques-Silva, M.C., Nogueira, C.W., Broch, L.C., Rocha, J.B.T. (2001) Diphenyl diselenide and acorbic acid changes deposition of selenium and ascorbic acid in brain of mice, Pharmacol. Toxicol. 88, 119-125.

Jaffe, E. K. (1995). Porphobilinogen synthase, the first source of heme's asymmetry. J. Bioenerg. Biomembr. 27, 169–179.

Juknat, A.A., Kotler, M.L., Batlle, A.M.C., 1995. High δ-aminolevulinic acid uptake in rat cerebral cortex: effect on porphyrin biosynthesis.Comp. Biochem. Physiol. 111C, 143–150.

Kodavanti PRS. Reactive oxygen species and antioxidant homeostasis in neurotoxicology. In: Tilson HA, Harry GJ, editors. Neurotoxicology. USA: Taylor & Francis; 1999.

Larner, A.J., 1995. How does garlic exert its hypocholesterolaemic action? The tellurium hypothesis. Medical Hypothesis 44, 295-297.

Maciel, E.N., Bolzan, R.C., Braga, A.L., Rocha, J.B.T., 2000. Diphenyl diselenide and diphenyl ditelluride differentially affect d-Aminolevulinate dehydratase from liver, kidney, and brain of mice. Journal of Biochemical and Molecular Toxicology 14, 310–319.

McCobb D.P., Cohan C.S., Connor J.A., Kater S.B., (1988). Interactive effects of serotonin and acetylcholine on neurite elongation. Neuron 1 377–385.

Meotti, F.C., Borges, V.C., Zeni, G., Rocha, J.B.T., Nogueira, C.W., 2003. Potential renal and hepatic toxicity of diphenyl diselenide, diphenyl ditelluride and Ebselen for rats and mice. Toxicology Letters 143, 9-16.

Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170-3175.

Newman, R.A., Osborn, S., Siddik, Z.H., 1989. Determination of tellurium in biological fluids by means of electrothermal vapourization-inductively coupled to plasma mass spectrometry (ETV-ICP-MS). Clinica Chimica Acta 179, 191-196.

Nogueira, C.W., Zeni, G., Rocha, J.B.T., 2004. Organoselenium and organotellurium compounds: Toxicology and Pharmacology. Chemical Reviews 104, 6255-6286.

Nogueira, C.W., Quinhones, E.B., Jung, E.A.C., Zeni, G., Rocha, J.B.T., 2003. Anti-inflammatory and antinociceptive activity of diphenyl diselenide. Inflamm. Res. 52, 56–63.

Nogueira, C.W., Rotta, L.N., Perry, M.L., Souza, D.O., Rocha, J.B.T., 2001. Diphenyl diselenide and diphenyl ditelluride affect the rat glutamatergic system in vitro and in vivo. Brain Research 906, 157– 163.

Ohkawa, H., Ohishi, N., and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351-358.

Park, H.S., Park, E., Kim, M.S., Ahn, K., Kim, I.Y., Choi, E.J., 2000. Selenite inhibits the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) through a thiol redox mechanism. J. Biol. Chem. 275, 2527–2531.

Petragnani, N., 1994. Preparation of the principal classes of organic tellurium compounds. In: Tellurium in Organic Synthesis. Academic Press, London, pp. 9–88.

Prigol M., Wilhelm E.A., Schneider C.C., Rocha J.B.T., Nogueira C.W., Zeni G. (2007). Involvement of oxidative stress in seizures induced by diphenyl diselenide in rat pups. Brain Research 1147, 226 – 232.

Sassa, S. 1982. Delta-aminolevulinic acid dehydratase assay. Enzyme 28: 133-145.

Sassa, S., Fujita, H., and Kappas, A. (1989). Genetic and chemical influences on heme biosynthesis. In Highlights of Modern Biochemistry (A. Kotyk, J. Skoda, V. Paces and V. Kostka, eds.), Vol.1, pp. 329–338. VSP, Utrecht.

Siddik, Z.H., Newman, R.A., 1988. Use of platinum as a modifer in the sensitive detection of tellurium in biological samples. Analytical Biochemistry 172, 190-196.

Stangherlin, E.C., Favero, A.M., Zeni, G., Rocha, J.B.T., Nogueira, C.W., 2005. Teratogenic vulnerability of rat fetuses to diphenyl ditelluride: prenatal assessment. Toxicology 207, 231-239.

Stangherlin, E.C., Favero, A.M., Zeni, G., Rocha, J.B.T., Nogueira, C.W., 2006. Exposure of mothers to diphenyl ditelluride during the suckling period changes behavioral tendencies in their offspring. Brain Research Bulletin 69, 311-317.

Yarema, M.C., Curry, S.C., 2005. Acute tellurium toxicity from ingestion of metal-oxidizing solutions. Pediatrics 116, 319-321.

Zeni G., Lüdtke D.S., Panatieri R.B., Braga A.L., 2006. Vinylic Tellurides: From Preparation to Their Applicability in Organic Synthesis. Chem. Rev. 106, 1032-1076.

Zhang H., Swihart M.T., 2007. Synthesis of Tellurium Dioxide Nanoparticles by Spray Pyrolysis Chemistry of Materials 19, 1290-1301.

Figure 1

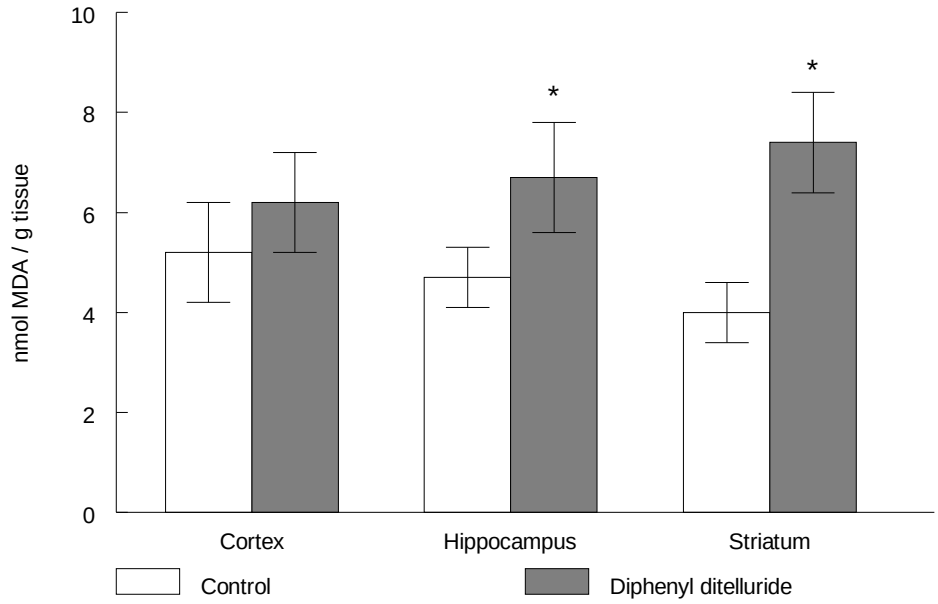


Figure 2

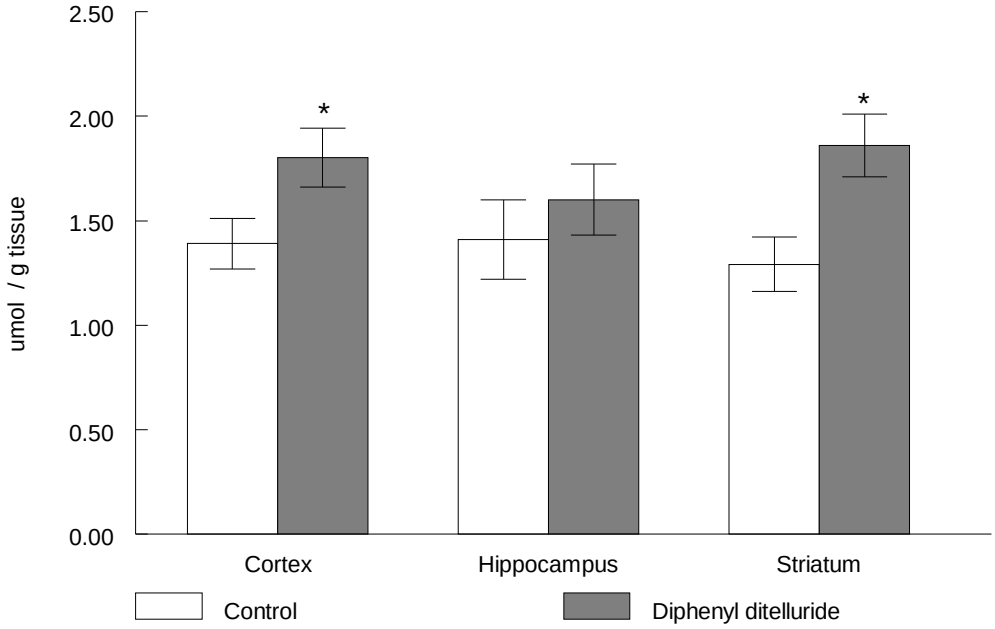


Figure 3

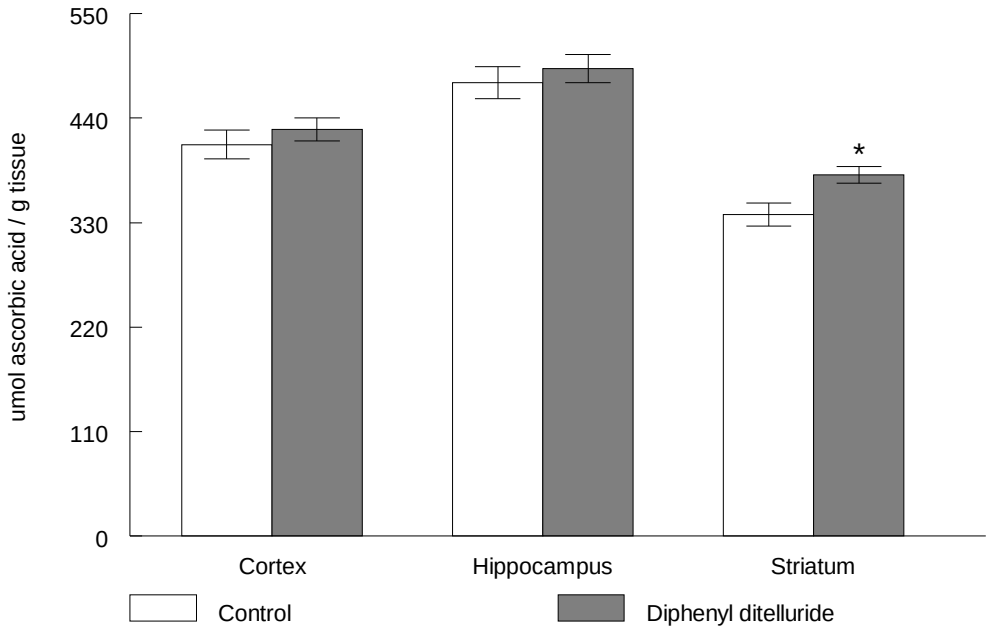


Figure 4

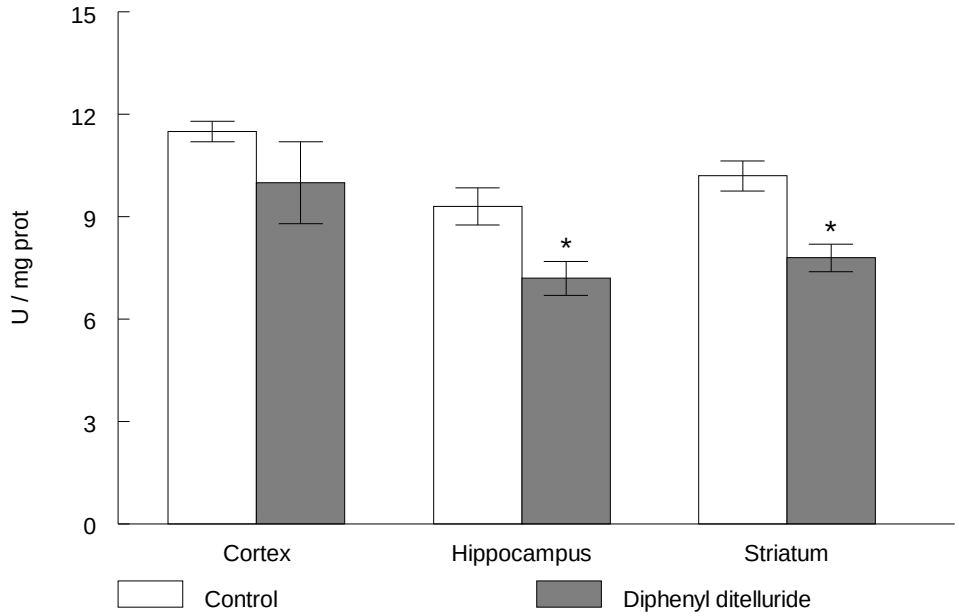


Figure 5

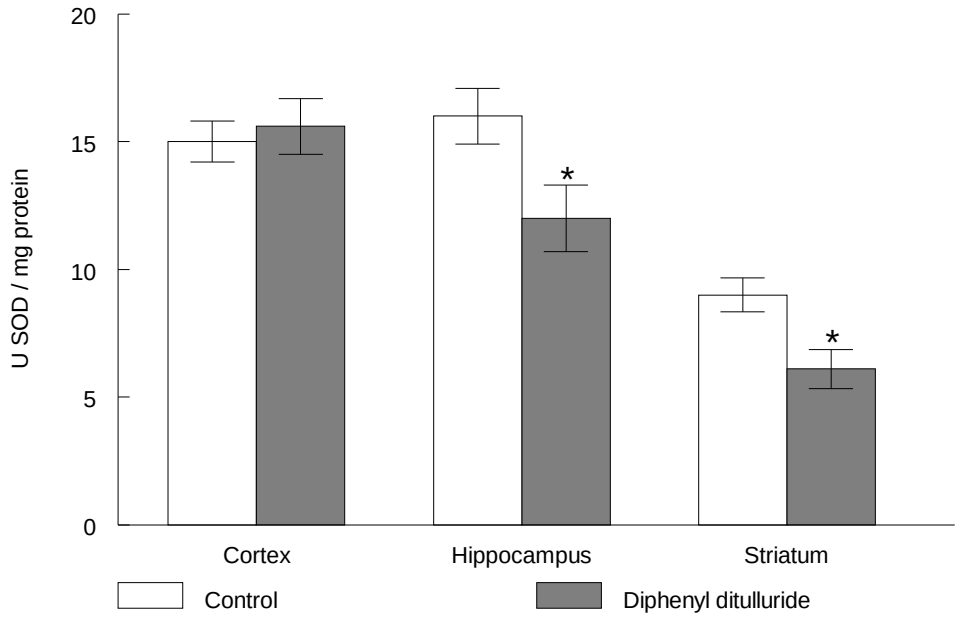
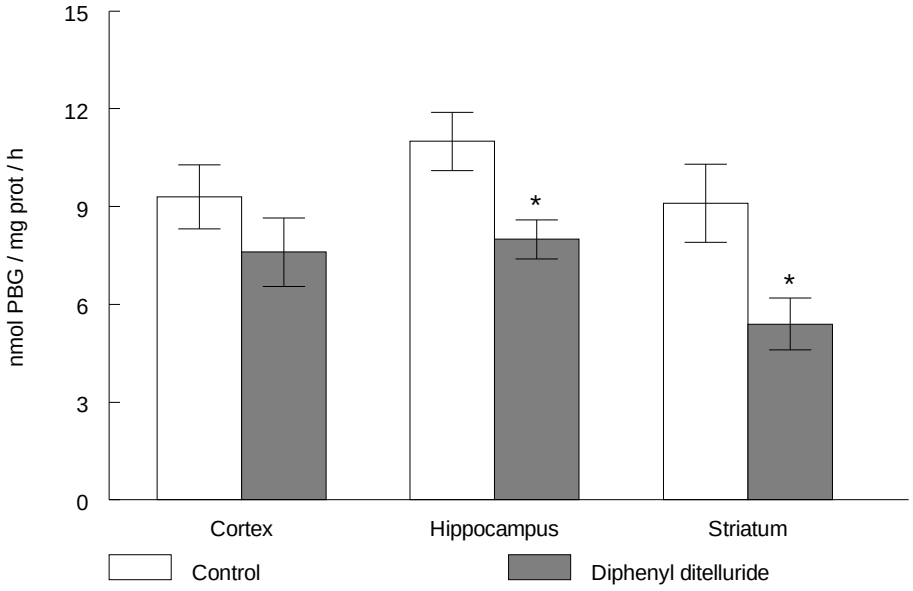


Figure 6.



5. DISCUSSÃO

Esse estudo avaliou o efeito da exposição materna a baixas doses de ditelureto de difenila, durante o período de amamentação, sob aspectos comportamentais e bioquímicos, em filhotes de rato.

Os resultados obtidos após a avaliação comportamental revelaram, num primeiro momento, tendências desinibitórias, determinadas pelo desempenho dos animais expostos ao ditelureto de difenila via leite materno, no labirinto em cruz-elevado (artigo 1). O teste do campo aberto também poderia sugerir alterações da mesma natureza. Porém, no campo aberto, os animais expostos ao ditelureto de difenila demonstraram um comportamento normal, comparável ao comportamento dos animais controle. Esse fato sugere que as alterações observadas são específicas para uma determinada tarefa, e ocorrem em um ambiente mais complexo. Ainda, para roedores, essas alterações podem não ser adaptativas e podem potencialmente expor os animais afetados a situações de perigo. Outras observações importantes revelaram que a exposição ao ditelureto de difenila não alterou a atividade exploratória ou a coordenação motora dos animais (artigo 1).

Num segundo momento, os resultados obtidos após a avaliação comportamental revelaram que os animais expostos ao ditelureto de difenila via leite materno tiveram um prejuízo de memória, revelado no teste do reconhecimento do objeto (manuscrito 1). Esse resultado está de acordo com um estudo que também revelou deficiência cognitiva induzida por telúrio inorgânico em ratos (Widy-Tyszkiewicz et al., 2002).

Sendo assim, as observações desses estudos sugerem que o ditelureto de difenila (ou metabólito dele) consegue passar para os filhotes através do leite materno, provavelmente por esse composto ter uma natureza lipídica. Uma vez nos tecidos do filhote, ele tem a capacidade de injuriar o tecido cerebral, a ponto de causar alterações que se revelam nas mudanças comportamentais observadas.

A investigação dos possíveis mecanismos pelos quais o ditelureto de difenila atua revelou que ele causou uma inibição da captação de glutamato em sinaptossomas de cérebro total e não interferiu no processo de liberação de glutamato, no mesmo ensaio (manuscrito 1). Esses eventos poderiam promover um aumento de glutamato na fenda sináptica, pela inibição da sua captação. Porém, o favorecimento da neurotransmissão glutamatérgica parece estar mais relacionado com eventos ansiogênicos/inibitórios (LeDoux, 1994; Maren, 1996) ou ainda, de facilitação dos processos relacionados com a cognição e a memória (Daisley et al., 1998; Lhullier et al., 2004; Mameli et al., 2005; McGahon et al., 1996), ou seja,

comportamentos contrários aos observados nesse estudo. Dessa forma, nesse protocolo experimental, a alteração da homeostase do sistema glutamatérgico ocasionada pelo ditelureto de difenila parece não estar diretamente relacionada com as alterações comportamentais observadas nos filhotes. Entretanto, não se pode descartar o envolvimento da via glutamatérgica em estruturas cerebrais específicas no que diz respeito à ansiedade ou à memória (Izquierdo e Medina, 1997; Lisman et al., 2005; Rosenzweig e Barnes, 2003; Wu e Yamaguchi, 2004; LeDoux, 1994; Maren, 1996).

No protocolo experimental utilizado nesse estudo, foi observada uma inibição na atividade da enzima Na^+,K^+-ATPase cerebral (manuscrito 1). Essa importante enzima reguladora do potencial de membrana é responsável pelo transporte ativo dos íons sódio e potássio no sistema nervoso (Doucet, 1988; Jorgensen, 1986). Além disso, a inibição da atividade dessa enzima está relacionada com o aumento da liberação de neurotransmissores excitatórios (Vizi e Vyskocil, 1979). Então, como no presente estudo a liberação de glutamato não foi alterada pela exposição ao ditelureto de difenila, provavelmente esses dois eventos (transmissão glutamatérgica e atividade da Na^+,K^+-ATPase) não estão relacionados. Porém, vários estudos relacionam a inibição da atividade da Na^+,K^+-ATPase com o prejuízo da memória (Wyse et al., 2004; Xiong e Stringer, 2000; dos Reis et al., 2002; Sato et al., 2004). Sendo assim, a inibição da atividade dessa enzima provavelmente é um dos mecanismos relacionados com o prejuízo de memória dos animais testados nesse estudo.

Num terceiro momento, foi constatado que a exposição ao ditelureto de difenila via leite materno causou uma série de alterações no status oxidativo cerebral dos filhotes (manuscrito 2). As estruturas cerebrais mais afetadas foram o hipocampo e o estriado. Nessas regiões, foi observado um aumento da peroxidação lipídica e uma inibição da atividade das enzimas superóxido dismutase, catalase e δ-ALA-D. O aumento do estresse oxidativo no hipocampo e no estriado pode ser explicado pelo fato de que o ditelureto de difenila pode interagir com as membranas biológicas, induzindo um aumento da peroxidação lipídica. Em algumas situações, o aumento de substâncias oxidantes pode ser tão intenso que a diminuição das defesas antioxidantes não consegue ser prevenida (Halliwell, 2006). E assim, o tecido fica vulnerável ao dano. Provavelmente a inibição da atividade das enzimas foi uma conseqüência do estresse oxidativo. E mais, a inibição da atividade da Na^+,K^+-ATPase também pode ter sido ocasionada pelo aumento do estresse oxidativo. Ainda, no estriado houve um aumento dos níveis de ácido ascórbico e de grupos tióis não-protéicos. No córtex, por sua vez, houve um aumento somente dos níveis de grupos tióis não-protéicos. O aumento dos níveis desses

dois antioxidantes não-enzimáticos pode ter sido uma resposta adaptativa dos tecidos cerebrais ao estresse.

Sendo assim, parece que a exposição ao ditelureto de difenila via leite materno alterou o status oxidativo nas estruturas cerebrais dos filhotes, interferindo, consequentemente, na homeostase funcional dessas regiões. Essa alteração oxidativa localizada provavelmente é um dos principais mecanismos envolvidos nas mudanças comportamentais observadas nesse estudo. Isso porque o hipocampo e o estriado, as duas regiões mais afetadas pelo estresse oxidativo induzido pela exposição ao ditelureto de difenila, são as regiões mais relacionadas com ansiedade/desinibição e cognição/memória.

Vários estudos demonstram que os sintomas da depressão e da ansiedade envolvem estruturas cerebrais como o hipocampo (Cheeta et al., 2000; Kempermann, 2002), o córtex pré-frontal (Zhong e Yan et al., 2004; Shah et al., 2004), e o córtex cerebral (Setnik e Nobrega, 2004; Talpalar e Grossman, 2004). Além disso, lesões pré-frontais estão relacionadas com a desinibição, a deficiência da memória de trabalho e as disfunções de atenção. Lesões experimentais no estriado estão associadas com a etiologia da desordem de hiperatividade com deficiência de atenção (Lou, 1996). Lesões estriatais em animais produzem hiperatividade e desempenho insatisfatório em testes de memória de trabalho (Alexander et al., 1986). Além disso, o envolvimento do hipocampo em muitas, se não em todas as formas de memória é conhecido há muito tempo (Barnes, 1979, 1996; Squire, 1992).

6. CONCLUSÕES

De acordo com os resultados apresentados nesta tese podemos inferir o seguinte: a exposição materna ao ditelureto de difenila, durante as duas primeiras semanas do período lactacional, causou, nos filhotes:

- Alterações comportamentais, sendo elas:

(a) tendências comportamentais desinibitórias no teste do labirinto em cruz-elevado, e

(b) prejuízo de memória no teste do reconhecimento ao objeto.

As demais tarefas comportamentais avaliadas não foram alteradas pela exposição.

- Em sinaptossomas de cérebro, inibição da captação de [^{3}H]glutamato, enquanto que a liberação de [^{3}H]glutamato não foi afetada.

- Inibição da atividade da enzima Na^+,K^+ATPase cerebral.

- Alteração do status oxidativo em estruturas cerebrais:

(a) hipocampo e estriado: aumento dos níveis de peroxidação lipídica, inibição da atividade das enzimas superóxido dismutase, catalase e δ-ALA-D;

(b) estriado: além das alterações acima citadas, verificou-se ainda, um aumento nos níveis das defesas antioxidantes não-enzimáticas, ácido ascórbico e grupos tióis não-protéicos;

(c) córtex cerebral: aumento dos níveis de grupos tióis não-protéicos.

A exposição ao ditelureto de difenila via leite materno alterou o status oxidativo nas estruturas cerebrais dos filhotes, e consequentemente, alterou a homeostase funcional dessas regiões. Essa alteração oxidativa localizada provavelmente é um dos principais mecanismos envolvidos nas alterações comportamentais observadas nesse estudo. Isso porque o hipocampo e o estriado, as duas regiões mais afetadas pelo estresse oxidativo induzido pela exposição ao ditelureto de difenila, são as regiões mais relacionadas com os comportamentos referentes à ansiedade/desinibição e à cognição/memória.

7. REFERÊNCIAS BIBLIOGRÁFICAS

AGNEW, W.F.; CURRY, E. Period of teratogenic vulnerability of rat embryo to induction of hydrocephalus by tellurium. **Experientia,** v. 28, p. 1444-1445, 1972.

AGNEW, W.F., FAUVRE, F.M., PUDENZ, P.H. Tellurium hydrocephalus: Distribution of tellurium-127m between maternal, fetal and neonatal tissues of the rat. **Exp. Neurol.,** v. 21, p. 120-131, 1968.

ALEXANDER, G.E.; DELONG, M.R.; STRICK, P.L. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. **Annu. Rev. Neurosci.**, v. 9, p. 357–381, 1986.

ALMEIDA, S.S.; TONKISS, J.; GALLER, J.R. Prenatal protein malnutrition affects exploratory behavior of female rats in the elevated plus-maze test. **Physiol. Behav**., v. 60, p. 675–680, 1996.

ALTMAN. J.; DAS, G.D. Autoradiographic and histological studies of postnatal neurogenesis. I. A longitudinal investigation of kinetics migration and transformation of cells incorporating tritiated thymidine in neonate rats with special reference to postnatal neurogenesis in some brain regions. **J. Comp. Neurol.,** v. 126, p. 337, 1966.

ANDERSSON, C.-M.; BRATTSAND, R.; HALLBERG, A. Diaryl tellurides as inhibitors of lipid peroxidation in biological and chemical systems. **Free Radical Res.**, v. 20, p. 401-410, 1994.

ANDERSSON, C.-M. et al. Glutathione Peroxidase-Like activity of diaryl tellurides. **Bioorg. Med. Chem. Lett.**, v. 3, p. 2553-2558, 1993.

ANNAU, Z.; CUOMO, V. Mechanisms of neurotoxicity and their relationship to behavioral changes. **Toxicology**, v. 49, p. 219–225, 1988.

BANHEGYI, G. et al. Ascorbate metabolism and its regulation in animals. **Free Radical Bio. Med.**, v. 23, p. 793-803, 1997.

BARBOSA, N.B.V. et al. Effect of organic forms of selenium on δ-aminolevulinate dehydratase from liver, kidney, and brain of adult rats. **Toxicol. Appl. Pharmacol**., v. 149, p. 243-253, 1998.

BARNES, C. A. Involvement of LTP in memory: Are we ‘‘searching under the street light?’’ **Neuron**, v. 15, p. 751–754, 1996.

BARNES, C. A. Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. **J. Comp. Physiol. Psychol.**, v. 93, p. 74–104, 1979.

BEAL, M.F.; HYMAN, B.T.; KOROSHETZ,W. Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases. **Trends Neurosci**., v. 16, p. 125–131, 1993.

BECHARA, E.J.H. et al. A free radical hypothesis of lead poisoning and inborn porphyrias associated with 5-aminolevulinic acid overload. **Química Nova**, v. 16, p. 385-392, 1993.

BECKER, D., VILJOEN, D., KRAMER, S. Inhibition of red cell and brain atpase by delta-aminolaevulinic acid. **Biochim. Biophys. Acta**, v. 225, p. 26, 1971.

BENNET, M.R.; BALCAR, V.J. Forty years of amino acid transmission in the brain. **Neurochem. Int.,** v. 35, p. 269-280, 1999.

BERK, M.; PLEIN, H.; BELSHAM, B. The specificity of platelet glutamate receptor supersensitivity in psychotic disorders. **Life Sci**., v. 66, p. 2427-2432, 2000.

BERTORELLO, A.M.; KATS, A.L. Regulation of Na^+-K^+- pump activity: pathways between receptors and effectors. **NIPS**, v. 10, p. 253–259, 1995.

BLAIS, F. X.; ONISCHUK, R. T.; DE MEIO, R. H. Hemolysis by tellurite: I: The tellurite test for hemolysis. **J. AOA,** p. 73, 1972.

BLINZINGER, K.; HAGER, H. Uber die zellulare Speicherung von Tellur und ihre Beziehung zu den unter dem Lysosomenbegriff zusammengefassten intrazytolplasmatischen Korpern. **Verh. Deut. Ges. Pathol.,** v. 49, p. 357-362, 1965.

BLISS, T.V.P.; COLLINBRIDGE, G.L.A. A synaptic model of memory long-term potentiation in the hippocampus. **Nature**, v. 361, p. 31-39, 1993.

BOLES, J.O. et al. Telluromethionine in structural biochemistry. **SAAS Bull. Biochem. Biotechnol.,** v. 8, p. 29-45, 1995.

BORGES, V.C. et al. Organochalcogens affect the glutamatergic neurotransmission in human platelets. **Neurochem. Res.,** v. 29, p. 1505-1509, 2004.

BOVERIS, A.; CADENAS, E. Cellular sources and steady-state levels of reactive oxygen species. In : CLERCH, L.; MASSARO, D. **Oxygen, gene expression and cellular function.** Marcel Decker: New York, v. 105, p.1-25, 1997.

BRAGA, A. L. et al. Stereoconservative formation and reativity of α-chalcogen-functionalized vinylithium compounds from bromo-vinylic chalcogens. **Synlett,** v. 5, p. 595-596, 1997.

BRAGA, A. L. et al. Synthesis of selenocetais from enol ethers. **J. Chem. Res.,** p. 206-207, 1996.

BUDISA, N. et al. High level biosynthetic substitution of methionine in proteins by its analogues 2-aminohexanoic acid, selenomethionine, telluromethionine and ethionine in *Escherichia coli*. **Eur. J. Biochem.,** v. 230, p. 788-796, 1995.

CARFAGNA, M.A.; PONSLER, G.D.; MUHOBERAC, B.B. Inhibition of ATPase activity in rat synaptic plasma membranes by simultaneous exposure to metals. **Chem. Biol. Interact.,** v. 100, p. 53–65, 1996.

CARLTON, W.W.; KELLY, W.A. Tellurium toxicosis in Pekin ducks. **Toxicol. Appl. Pharmacol.,** v. 2, p. 203-214, 1967.

CHAKRABORTY, D. et al. Studies on ascorbic acid metabolism in rats under chronic toxicity due to organophosphorus inseticides: effects of supplementation of ascorbic acid in high doses. **J. Nutr**., v. 108, p. 973-980, 1978.

CHATTERJEE, G.C.; RUDRA PAL, D. Metabolism of L-ascorbic acid in rats under *in vivo* administration of mercury: effects of L-ascorbic acid supplementation. **Int. J. Vit. Nutr. Res**., v. 45, p. 284-292, 1975.

CHEETA, S.; KENNY, P.J.; FILE, S.E. Hippocampal and septal injections of nicotine and 8-OH-DPAT distinguish among different animal tests of anxiety. **Prog. NeuroPsychopharmacol**. **Biol. Psychiatry,** v. 24, p. 1053–1067, 2000.

COLLINGRIDGE, G.L.; AND LESTER, R.A.J. Excitatory amino acid receptors in the vertebrate central nervous system. **Pharmacol. Rev.**, v. 40, p. 143-210, 1989.

COMASSETO, J. V. et al. Vinylic selenides and tellurides – preparations, reactivity and synthetic applications. **Synthesis,** p. 373, 1997.

COMASSETO, J. V. Vinylic selenides. **J. Organomet. Chem.,** v. 253, p. 131-181, 1983.

CONN, P.J.; PINN, J.P. Pharmacology and function of metabotropic glutamate receptors. **Annu. Rev. Pharmacol. Toxicol.**, v. 37, p. 205-237, 1997.

COTMANN, C.W. et al. **Excitatory amino acid neurotransmission**. Pharmacology: The fourth generation of Progress, Floyd E, Bloom and David J. Kupfer, eds. Raven Press, New York, 1995.

CROFT, K.D. The chemistry and biological effects of flavonoides and phenolic acids. **Towards Prolongation of the Healthy Life Span.**, v. 854, p. 435-443, 1998.

CROSKERRY, P.G., et al. Perinatal brain DNA in normal and growth hormone-treated rat. **Brain Res.**, v. 52, p. 413-418, 1973.

CUTLER, M.G.; MOORE, M.R.; EWART, F.G. effects of delta-aminolevulinic-acid administration on social-behavior in the laboratory mouse. **Psychopharmacol.,** v. 61, p. 131-135, 1979.

DAISLEY, J.N. et al. Passive avoidance training and recall are associated with increased glutamate levels in the intermediate medial hyperstriatum ventrale of the day-old chick. **Neural Plasticity**, v. 6, p. 53-61, 1998.

DAVISON, A.N.; DOBBING, J. Myelination as a vulnerable period in brain development. **Brit. Med. Bull.**, v. 22, p. 40, 1966.

DAVIES, K.J.A. **Oxidative damage and repair: Chemical, biological and medical aspects**. Oxford: Pergamon, p. 910, 1991.

DEUTICKE, B.; LÜTKEMEIER, P.; POSE, B. Tellurite-induced damage of the erythrocyte membrane. Manifestations and mechanisms. **Biochem. Biophys. Acta,** v. 1109, p. 97-107, 1992.

DICHTER, M.A.; WILCOX, K.S. **Excitatory synaptic transmission.** Epilepsy: A comprehensive Textbook. J. Engel, Jr. and T.A. Pedley. Eds. Lippincott-Raven Publishers, Philadelphia, 1997.

DOBBING J, SANDS J. Vulnerability of developing brain. IX. The effect of nutritional growth retardation on the timing of the brain growthspurt. **Biol. Neonate,** v. 19, p. 363–378, 1971.

DOS REIS, E. A. et al. Arginine administration inhibits hippocampal Na^+,K^+-ATPase activity and impairs retention of an inhibitory avoidance task in rats. **Brain Res**., v. 951, p. 151–157, 2002.

DOUCET, A. Function and control of Na^+-K^+-ATPase in single nephron segments of the mammalian kidney. **Kidney Int.**, v. 34, p. 749–760, 1988.

DRESEL, E.I.B.; FALK, J.E. Conversion of delta-aminolevulinic acid to porphobilinogen in a tissue system. **Nature**, p. 1172-1185, 1953.

DUCKETT, S. et al. Tellurium-induced neuropathy. Correlative physiological, morphological and electron microprobe studies. **Neuropath. Appl. Neuro.,** v. 5, p. 265-278, 1979.

DUCKETT, S.; WHITE, R. Cerebral lipofucsinosis induced with tellurium: electron dispersive x-ray spectrophotomrtry analysis. **Brain Res.,** v. 73, p. 205-214, 1974.

DUCKETT, S. Teratogenesis caused by tellurium. **Ann. N.Y. Acad. Sci.,** v. 192, p. 220-226, 1972.

DUCKETT, S.; ELLEM, K.A.O. The location of tellurium in fetal tissues, particularly the brain. **Exp. Neurol.,** v. 32, p. 49-71, 1971.

DUCKETT, S.; SCOTT, T. The target period during fetal life for the production of tellurium hydrocephalus. **Experientia,** v. 27, p. 1064-1065, 1971.

DUCKETT, S. The morphology of tellurium-induced hydrocephalus. **Exp. Neural.,** v. 31, p. 1-16, 1971.

EGGER, G.J.; LIVESEY, P.J.; DAWSON, R.G. Ontogenic aspects of central cholinergic involvement in spontaneous alternation behavior. **Dev. Psychobiol.**, v. 6, p. 289–299, 1973.

ENGMAN, L. et al. Organotellurium compounds as efficient retarders of lipid peroxidation in methanol. **Free Radical Bio. Med.,** v. 19, p. 441-452, 1995.

ENGMAN, L. et al. Thiol peroxidase activity of diaryl ditellurides as determined by a ^1H NMR method. **J. Am. Chem. Soc.,** v. 114, p. 9737-9743, 1992.

ERECINSKA, M.; SILVER, I.A. Ions and energy in mammalian brain. **Progress in Neurobiology**, v. 43, p. 37-71, 1994.

EULER, G.V.; LIU, Y. Glutamate and glycine decrease the affinity of [^3H] MK-801 binding in prsence of Mg^{2+}. **Eur. J. Pharmacol.**, v. 245, p. 233-239, 1993.

FAIRHILL, L.T. Tellurium. In: **Industrial Toxicology**, pp. 120. Hafner Publishing Co, New York & London, 1969.

FARBER, J.L.; KYLE, M.E.; COLEMANN, J.B. Biology of disease. Mechanisms of cell injury by activated oxygen species. **Lab. Invest.**, v. 62, p. 670-678, 1990.

FARINA, M. et al. Selenoxides inhibit δ-aminolevulinic acid dehydratase. **Toxicol. Lett**., v. 119, p. 27-37, 2001.

FERRARESE, C. et al. Decreased platelet glutamate uptake in patients with amyotrophic lateral sclerosis. **Neurology**, v. 56, p. 270-272, 2001.

FERRARESE, C. et al. Glutamate uptake is decreased in platelets from Alzheimer's disease patients. **Ann. Neurol.,** v. 47, p. 641-643, 2000.

FOLMER, V. et al. High sucrose consumption potentiates the sub-acute cadmium effect on Na^+-K^+-ATPase but not on and δ- aminolevulinate dehydratase in mice. **Toxicol. Lett**., v. 153, p. 333–341, 2004.

GENN, R.F., et al. Age associated sex differences in response to food deprivation in two animal tests of anxiety. **Neurosci. Biobehav. Rev.**, v. 27, p. 155–161, 2003.

GMELIN, C. H. R. Versuche über die Wirkungem des Baryts, Strontians, u.s.w auf den thierischen organismus. Tübingen 1824, 43. (Citated by Challenger, Frederick: Biological methylation). **Chem. Rev.**, v. 36, p. 315, 1945, 1824.

GOERING, P.L. Lead protein interactions as a basis for lead toxicity. **Neurotoxicology**, v. 14, p. 45-60, 1993.

GOZLAN, H.; BEM-ARI, Y. NMDA receptor redox sites: are they targets for selective neuronal protection? **TiPS,** v. 16, p. 368-375, 1995.

GOTTLIEB A.; KEYDAR I.; EPSTEIN H.T. Rodent brain growth stages: an analytical review. **Neonate,** v. 32, p. 166–176, 1977.

GRAEFF, F.G.; NETTO, F.C.; ZANGROSSI, H. The elevated T-maze as an experimental model of anxiety. **Neurosci. Biobehav. Rev**., v. 23, p. 237–246, 1998.

GREEN, M. et al. A facile route to CdTe nanoparticles and their use in bio-labelling. **Journal of Materials Chemistry**, v. 17, p. 1989–1994, 2007.

HALLIWELL, B. Oxidative stress and neurodegeneration: where are we now? **J. Neurochem**., v. 97, p. 1634–1658, 2006.

HALLIWELL, B.; GUTTERIDGE, J. M. C. Role of free radicals and catalytic metal ions in human disease: an overview. **Met. Enzimol.**, v. 186, p. 1-5, 1990.

HARRY, G.J. et al. Tellurium-induced neuropathy: metabolic alterations associated with demyelination and remyelination in rat sciatic nerve. **J. Neurochem.**, v. 52, p. 938-945, 1989.

IZQUIERDO, I.; MEDINA, J.H. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiology of **Learning and Memory**, v. 68, p. 285–316, 1997.

JAFFE, E.K. et al. Characterization of the role of the stimulatory magnesium of *Escherichia coli* porphobilinogen synthase. **Biochem.**, v. 34, p. 244-251, 1995.

JORGENSEN, P.L. Structure, function and regulation of Na^+-K^+-ATPase in the kidney. **Kidney Int.,** v. 29, p. 10–20, 1986.

KANDA, T. et al. G. Novel water-soluble diorganyl tellurides with thiol peroxidase and antioxidant activity. **J. Org. Chem**., v. 64, p. 8161-8169, 1999.

KEMPERMANN, G. Regulation of adult hippocampal neurogenesis-Implications for novel theories of major depression. **Bipolar Disord.,** v. 4, p. 17–33, 2002.

KVAMME, E. Synthesis of glutamate and its regulation. **Prog. Brain Res.,** v. 116, p. 73-85, 1998.

LADEN, B.; PORTER, T. Inhibition of human squalene monooxigenase by tellurium compounds. Evidence of interaction with vicinal sulfhydryls. **J. Lipid. Res.**, v. 42, p. 235-240, 2001.

LAMPERT, P.W.; GARRETT, R.S. Mechanism of demyelination in tellurium neuropathy. Electron microscopic observations. **Lab. Invest.,** v. 25, p. 380-388, 1971.

LALONDE, R.; KIM, H.D.; FUKUCHI, K. Exploratory activity, anxiety, and motor coordination in bigenic APPswe + PS1/Delta E9 mice. **Neurosci. Lett.**, v. 369, p. 156–161, 2004.

LALONDE, R.; QIAN, S.; STRAZIELLE, C. Transgenic mice expressing the PSIA346E mutation: effects on spatial learning, exploration, anxiety, and motor coordination. **Behav. Brain Res.,** v. 138, p. 71–79, 2003.

LALONDE, R.; STRAZIELLE, C. **Motor performance of spontaneous murine mutations with cerebellar atrophy**, in: CRUSIO, W.; GERLAI, E. (Eds.), Handbook of Molecular-Genetic Techniques for Brain and Behavior Research (Techniques in the Behavioral and Neural Sciences, vol. 13), Elsevier, Amsterdam, p. 627–637, 1999.

LARNER, A.J. How does garlic exert its hypocholesterolaemic action? The tellurium hypothesis. **Med. Hypothesis**, v. 44, p. 295-297, 1995.

LEDOUX, J.E. Emotion, memory and the brain. **Sci. Am.**, v. 270, p. 50-57, 1994.

LHULLIER, F.L.R. et al. Dehydroepiandrosterone increases synaptosomal glutamate release and improves the performance in inhibitory avoidance task. **Pharmacol. Biochem. Behav.**, v. 77, p. 601–606, 2004.

LIPTON, S.A.; ROSENBERG, P.A. Excitatory amino acids as a final common pathway for neurological disorders. **New. Eng. J. Med.,** v. 330, p. 613-622, 1994.

LISMAN, J.E.; TALAMINI, L.M.; RAFFONE, A. Recall of memory sequences by interaction of the dentate and CA3: A revised model of the phase precession. **Neural Networks**, v. 18, p. 1191–1201, 2005.

LOU, H. Etiology and pathogenesis of attention-deficit hyperactivity disorder (ADHD); significance of prematurity and perinatal hypoxic-haemodynamic encephalopathy. **Acta Paediatr., v.** 85, p. 1266–1271, 1996.

MACIEL, N. et al. Diphenyl diselenide and diphenyl ditelluride differentially affects aminolevulinate dehydratase from liver, kidney and brain of mice. **J. Biochem. Mol. Toxic., v.** 14, p. 310-319, 2000.

MAMELI, M. et al. Developmentally regulated actions of alcohol on hippocampal glutamatergic transmission. **J Neurosci**., 25, p. 8027, 2005.

MAREN, S. Synaptic transmission and plasticity in the amygdala. **Mol. Neurobiol.,** v. 13, p. 1-22, 1996.

McCORD, J.M.; FRIDOVICH, I. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). **J. Biol. Chem.**, v. 244, p. 6049-6055, 1969.

MCGAHON, B. et al. Training in the Morris water maze occludes the synergism between ACPD and arachidonic acid on glutamate release in synaptosomes prepared from rat hippocampus. **Learning & Memory,** v. 3, p. 296-304, 1996.

MELDRUM, B.S.; AKBAR, M.T.; CHAPMAN, A.G. Glutamate receptors and transporters in genetic an acquired modles of epilepsy. **Epilepsy Res.,** v. 36, p. 189-204, 1999.

MEOTTI, F.C. et al. Potential renal and hepatic toxicity of diphenyl diselenide, diphenyl ditelluride and Ebselen for rats and mice. **Toxicol. Lett.**, v. 143, p. 9-16, 2003.

MIZUNO, R. Electron microscopic study on the cerebral cortex of rabbits intoxicated with tellurium. **Yokohama Med. J.**, v. 20, p. 101-121, 1969.

MORELL, P. et al. Gene expression during tellurium-induced primary demyelination. **Neurotoxicology,** v. 15, p. 171-180, 1994.

MORETTO, M.B. et al. Ebselen and diorganochalcogenides inhibition of $^{45}Ca^{2+}$ influx into brain synaptosomes is voltage-dependent. **J. Biochem. Mol. Toxic.**, v. 17, p. 154-160, 2003.

MORGANE, P.J.; MOKLER, D.J.; GALLER, J.R. Effects of prenatal protein malnutrition on the hippocampal formation. **Neurosci. Biobehav. Rev.**, v. 26, p. 471-483, 2002.

MÜLLER, R. et al. Tellurium intoxication. **Kiln. Wocherschr.**, v. 67, p. 1152-1155, 1989.

NICOLETTI, F. et al. Metabotropic glutamate receptors: a new target for the therapy of neurodegenerative disorders? **Trends Neurosci**., 19, p. 267-272, 1996.

NEWMAN, R.A.; OSBORN, S.; SIDDIK, Z.H. Determination of tellurium in biological fluids by means of electrothermal vapourization-inductively coupled to plasma mass spectrometry (ETV-ICP-MS). **Clin. Chim. Acta**, v. 179, p. 191-196, 1989.

NOGUEIRA, C.W. et al. Anti-inflammatory and antinociceptive activity of diphenyl diselenide. **Inflamm. Res.,** v. 52, p. 56-63, 2003a.

NOGUEIRA, C.W. et al. Organochalcogens effects on δ-aminolevulinic acid dehydratase activity from human erythrocytic cells in vitro. **Toxicology,** v. 191, p. 169-178, 2003b.

NOGUEIRA, C.W. et al. Exposure to ebselen changes glutamate uptake and release by rat brain synaptosomes. **Neurochem. Res.,** v. 27, p. 283-288, 2002.

NOGUEIRA, C.W. et al. Diphenyl diselenide and diphenyl ditelluride affect the rat glutamatergic system in vitro and in vivo. **Brain Res.,** v. 906, p. 157-163, 2001.

NYSKA, A. et al. Toxicity study in rats of a tellurium based immunomodulating drug, AS-101: a potencial drug for AIDS and cancer patients. **Arch. Toxicol.,** v. 63, p. 386-393, 1989.

OZAWA, S.; KAMIYA, H.; TSUZUKI, K. Glutamate receptors in the mammalian central nervous system. **Prog. Neurobiol.**, v. 54, p. 581-618, 1998.

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

PAULMIER, C. Selenium reagents and intermediates. In: **Organic Synthesis**. Oxford: Pergamon, 1986.

PELLOW S. et al. Validation of open:close arm entries in an elevated plus-maze as a measure of anxiety in the rat. **J. Neurosci. Meth.**, v. 14, p. 149–167, 1985.

PENTSCHEW, A.; EBNER, F.; KOVATCH, R. In: **Proceedings of Fourth International Congress of Neuropathology**. H. Jacobs, Ed. 3:300. George Thiem Verlag. Stuttgart, Germany, 1962.

PEREIRA, B. et al. 5-Aminolevulinic acid induces alteration of oxidative metabolism in sedentary and exercise trained rats. **J. Appl. Physiol.**, v. 72, p. 226-230, 1992.

PEREZ-D'GREGORIO, R.E.; MILLER, R.K. Teratogenicity of tellurium dioxide: prenatal assessment. **Teratology,** v. 37, p. 307-316, 1988.

PETRAGNANI, N. In: **comprehensive Organometallic Chemistry II** (Ed. A. Mckillop), vol. LI, Pergamon Press, Exeter, UK, 1995.

PETRAGNANI, N.; RODRIGUES, R.; COMASSETO, J. V. **Organomet. Chem.** p. 114-281, 1976.

PIGGOTT, M.A. et al. Examination of parameters influencing [^{3}H] MK-801 binding in post-mortem human cortex. **J. Neurochem.,** v. 58, p. 1001-1008, 1992.

PUMA C. et al. Nicotine improves memory in an object recognition task in rats. **Eur. Neuropsychopharmacol.**, v. 9, p. 323–327, 1999.

RAMADAN, S.E. et al. Incorporation of tellurium into amino acids and proteins in a tellurium-tolerant fungi. **Biol. Trace Elem. Res.,** v. 20, p. 225-232, 1989.

RAMPON C., et al. Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. **Nat. Neurosci.**, v. 3, p. 238–244, 2000.

RAWLINS, F.A.; SMITH, M.E. Myelin synthesis in vitro: a comparative study of central and peripheral nervous tissue. **J. Neurochem.,** v. 18, p. 1861-1870, 1971.

RICE, D.C. Effect of exposure to 3,3, 4,4, 5-pentachlorobiphenyl (PCB 126) throughout gestation and lactation on developmental and spatial delayed alternation performance in rats. **Neurotoxicol. Teratol.,** v. 21, p. 59–69, 1999.

ROBINSON, M.D.; DOWD, L.A. Heterogeneity and functional subtypes of sodium-dependent glutamate transporters in the mammalian central nervous system. **Adv. Pharmacol**., v. 37, p. 69-115, 1997.

ROCHA, J.B.T.; VENDITE, D. Effects of undernutrition and handling during suckling on shuttle avoidance and footshock escape behavior and on plasma-glucose levels of young-rats. **Dev. Psychobiol**., v. 23, p. 157–168, 1990.

ROCINHOLI, L.F.; ALMEIDA, S.S.; DE-OLIVEIRA, L.M. Response threshold to aversive stimuli in stimulated early protein-malnourished rats. **Braz. J. Med. Biol. Res**., v. 30, p. 407–413, 1997.

RODRIGUES, A.L.S. **Delta-aminolevulinato desidratase (E.C.: 4.2.1.24) em sangue de *Pimelodus Maculatus* (Pisces, Pimelodidae): características bioquímicas e efeito de metais pesados.** 1987. Dissertação (Mestrado em Bioquímica)- UFRGS, Porto Alegre, 1987.

ROSE, R.C. Solubility properties of reduced and oxidized ascorbate as determinants of membrane permeation. **Biochem. Byophys. Acta.**, v. 924, p. 254-256, 1987.

ROSENZWEIG, E.S.; BARNES, C.A. Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. **Progress in Neurobiology**, v. 69, p. 143–179, 2003.

SASSA, S.; FUJITA, H.; KAPPAS, A. Genetic and chemical influences on heme biosynthesis. In: A. Kotyk, J. Skoda; V. Paces and V. Kostka (Eds.), **Highlights of modern biochemistry**, VSP, Utrecht, v.1, p. 329-338, 1989.

SATO, T. et al. Effects of steroid hormones on (Na+,K+)-ATPase activity inhibitioninduced amnesia on the step-through passive avoidance task in gonadectomized mice. **Pharmacol. Res.**, v. 49, p. 151–159, 2004.

SCANSETTI, G. Exposure to metals that have recently come into use. **Science Total Environ.**, v. 120, p. 85-91, 1992.

SCATTON, B. The NMDA receptor complex. **Fundam. Clin. Pharmacol.,** v. 7, p. 389-400, 1993.

SCHOEPP, D.D.; CON, P.J. Metabotropic glutamate receptors in brain function and pathology. **Trends Pharmacol. Sci**., v. 14, p. 13-20, 1993.

SHAH, A.A.; SJOVOLD, T.; TREIT, D. Inactivation of the medial prefrontal cortex with the GABA A receptor agonist muscimol increases open-arm activity in the elevated plus-maze and attenuates shock-probe burying in rats. **Brain Res.,** v. 1028, p. 112–115, 2004.

SIDDIK, Z.H.; NEWMAN, R.A. Use a platinum as a modifier in the sensitive detection off tellurium in biological samples. **Anal. Biochem.,** v. 172, p. 190-196, 1988.

SIES, H. Strategies of antioxidants defenses. **Eur. J. Biochem.**, v. 215, p. 213-219, 1993.

SIES, H. Biochemistry of oxidative stress. **Angew. Chem. Int. Ed. Engl.**, v. 25, p. 1058-1071, 1986.

SREDNI, B. et al. The biological activity and immunotherapeutic properties of AS-101, a synthetic organotellurium compound. **Nat. Immun. Cell Grow.,** v. 7, p. 163-168, 1988.

SREDNI, B. et al. A new immunomodulating compound (AS-101) with potential therapeutic application. **Nature,** v. 330, p. 173-176, 1987.

STANGHERLIN, E.C. et al. Teratogenic vulnerability of rat fetuses to diphenyl ditelluride: prenatal assessment. **Toxicology**, v. 207, p. 231-239, 2005.

SETNIK, B.; NOBREGA, J.N. Long-chain acyl-CoenzymeA synthetase-2mRNA: increased cerebral cortex expression in an animal model of depression. **Prog. NeuroPsychopharmacol. Biol. Psychiatry**, v. 28, p. 577–582, 2004.

SQUIRE, L. R. Memory and the hippocampus: A synthesis from findings with rats, monkeys,and humans. **Psychol. Rev.**, v. 99, p. 195–221, 1992.

STEWART, N. G.; CROOKS, R. N. Long-range travel of the radioactive cloud from the accident at Windscale. **Nature,** v. 182, p. 627-628, 1958.

SUN, X. et al. Anticarcinoma activity of a novel drug, 3-ethyl-3'methyl-thiatelluracarbocyanine iodite (Te) a tellurium-containing cyanine targeted at mitochondria. **Clin. Canc. Res.,** v. 2, p. 1335-1340, 1996.

SUTHERLAND, R.J.; RUDY J.W. Configural association theory: the role of the hippocampal formation in learning, memory and amnesia. **Psychobiology**, v. 17, p. 129-144, 1989.

TALPALAR, A.E.; GROSSMAN, Y. Enhanced excitability compensates for high-pressure-induced depression of cortical inputs to the hippocampus. **J. Neurophysiol.**, v. 92, p. 3309-3319, 2004.

TAYLOR, A. Biochemistry of tellurium. Biol. **Trace. Elem. Res.**, v. 55, p. 231-239, 1996.

TIZZANO, J.P.; GRIFFEY, K.I.; SCHOEPP, D.D. Receptor subtypes linked to metabotropic glutamate receptor agonist-mediated limbic seizures in mice. **Ann. NY. Acad. Sci.**, v. 765, p. 230-235, 1995.

U.S. Bureau of Mines, 1985. Mineral Year Book. **U.S. Government Printing Office**, Vol. I, pp. 1018-1021. Washington D.C., 1985.

VAN VLEET, J. F. V.; FERRANS, V. J. Ultrastructural alterations in skeletal muscle of ducklings fed selenium-vitamin E-deficient diet. **Am. J. Vet. Res.,** v. 38, p. 1399-1405, 1982.

VIZI, E.S.; VYSKOCIL, F. Changes in total and quantal release of acetylcholine in the mouse diaphragm during activation and inhibition of membrane ATPase. **J. Physiol.**, v. 286, p. 1-14, 1979.

WAGNER-RECIO, M.; TOEWS, A.D.; MORELL, P. Tellurium blocks cholesterol syntesis by inhibiting squalene metabolism: Preferential vulnerability to this metabolic block leads to peripheral nervous system demyelination. **J. Neurochem.,** v. 57, p. 1891-1901, 1994.

WIDY-TYSZIEWICZ, E. et al. Tellurium-induced cognitive deficits in rats are related to neuropathological changes in the central nervous system. **Toxicol. Lett.**, v. 131, p. 203-214, 2002.

WINICK, M. Nutrition and nerve cell growth. **Feder Proceed**, v. 29, p. 1510, 1970.

WU, Z.; YAMAGUCHI, Y. Input-dependent learning rule for the memory of spatiotemporal sequences in hippocampal network with theta phase precession. **Biological Cybernetics**, v. 90, p. 113–124, 2004.

WYSE, A.T. et al. Training in inhibitory avoidance causes a reduction of Na+,K+-ATPase activity in rat hippocampus. **Physiol. Behav.**, v. 80, p. 475–479, 2004.

XIONG, Z. Q.; STRINGER, J. L. Sodium pump activity, not glial spatial buVering, clears potassium after epileptiform activity induced in the dentate gyrus. **J. Neurophysiol.**, v. 83, p. 1443-1451, 2000.

YAREMA, M.C.; CURRY, S.C. Acute tellurium toxicity from ingestion of metal-oxidizing solutions. **Pediatrics**, v. 116, p. 319-321, 2005.

YOUNG, V. R.; NAHAPETIAU, A.; JONGHORBONI, M. Selenium bioavailability with reference to human nutrition. **American J. Clin. Nutrion,** v. 35, p. 1076-1088, 1981.

YU, L. et al. Evidence for telluroamino acid in biological materials and some rules for assimilation of inorganic tellurium by yeast. **Anal. Biochem.,** v. 209, p. 318-322, 1993.

ZENI, G., BRAGA, A. L., STEFANI, H. A. Palladium-catalyzed coupling of sp(2)-hybridized tellurides. **Accounts Chem. Res.,** v. 10, p. 731-738, 2003.

ZHANG H.; SWIHART M.T. Synthesis of Tellurium Dioxide Nanoparticles by Spray Pyrolysis. **Chemistry of Materials**, v. 19, p. 1290-1301, 2007.

ZHONG, P.; YAN, Z. Chronic antidepressant treatment alters serotonergic regulation of GABA transmission in prefrontal cortical pyramidal neurons. **Neuroscience**, v. 129, p. 65–73, 2004.

8. APÊNDICE

A- Demais trabalhos realizados durante o Curso de Doutorado

FAVERO, A.M.; WEIS, S.N.; STANGHERLIN, E.C.; ZENI, G.; ROCHA, J.B.T.; NOGUEIRA, C.W. Adult male rats sub-chronically exposed to diphenyl diselenide: Effects on their progeny. **Reproductive Toxicology**, v. 23, 119–123, 2007.

LUCHESE, C.; STANGHERLIN, E.C.; ARDAIS, A.P.; NOGUEIRA, C.W.; SANTOS, F.W. Diphenyl diselenide prevents oxidative damage induced by cigarette smoke exposure in lung of rat pups. **Toxicology**, v. 230, 189–196, 2007.

WEIS, S.N.; FAVERO, A.M.; STANGHERLIN, E.C.; MANARIN, F.G.; ROCHA, J.B.T.; NOGUEIRA, C.W.; ZENI, G. Repeated administration of diphenyl diselenide to pregnant rats induces adverse effects on embryonic/fetal development. Reproductive Toxicology, v. 23, 175–181, 2007.

FAVERO, A.M.; WEIS, S.N.; STANGHERLIN, E.C.; ZENI, G.; ROCHA, J.B.T.; NOGUEIRA, C.W. Sub-chronic exposure of adult male rats to diphenyl ditelluride did not affect the development of their progeny. **Food and Chemical Toxicology**, v. 45, 859–862, 2007.

FAVERO, A.M.; WEIS, S.N.; STANGHERLIN, E.C.; ZENI, G.; ROCHA, J.B.T.; NOGUEIRA C.W. Teratogenic effects of diphenyl diselenide in Wistar rats. **Reproductive Toxicology**, v. 20, 561–568, 2005.

STANGHERLIN, E.C.; FAVERO, A.M.; ROCHA, J.B.T.; NOGUEIRA, C.W. Sub-chronical exposure to diphenyl diselenide improves water-maze performance in adult rats. Submetido a **Brain Research**.

STANGHERLIN, E.C.; FAVERO, A.M.; WEIS, S.N.; ROCHA, J.B.T.; NOGUEIRA, C.W. Effect of diphenyl ditelluride on elevated plus maze in adult rats: possible involvement of glutamatergic system. Submetido a **Neurotoxicology**

ARDAIS, A.P.; STANGHERLIN, E.C.; ROCHA, J.B.T.; NOGUEIRA, C.W. Diphenyl Diselenide and Diphenyl Ditelluride: Neurotoxic Effect in Brain of Young Rats, *in vitro*. Em fase de redação.

STANGHERLIN, E.C.; LUCHESE, C.; ARDAIS, A.P.; SANTOS, F.W.; NOGUEIRA, C.W. Passive Smoke Exposure Induces Oxidative Damage in Brain of Rat Pups: Protective Role of Diphenyl Diselenide. Em fase de redação.