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Tese de Doutorado

PAPEL DA RECAPTAÇÃO E DE METABÓLITOS DA DOPAMINA NA DISCINESIA OROFACIAL INDUZIDA POR NEUROLÉPTICOS EM RATOS

Roselei Fachinetto

PPGBT

Santa Maria, RS, Brasil

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por

Roselei Fachinetto

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

Santa Maria, RS, Brasil

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Universidade Federal de Santa Maria
Centro de Ciências Naturais e Exatas
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica

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

PAPEL DA RECAPTAÇÃO E DE METABÓLITOS DA DOPAMINA NA DISCINESIA OROFACIAL INDUZIDA POR NEUROLÉPTICOS EM RATOS

elaborada por

Roselei Fachinetto

como requisito parcial para a obtenção de grau de
Doutor em Bioquímica Toxicológica.

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Santa Maria, 12 de maio de 2008.

"A MENTE QUE SE ABRE A UMA NOVA IDÉIA JAMAIS VOLTARÁ AO SEU TAMANHO ORIGINAL."

(Albert Einstein)

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SUMÁRIO

LISTA DE ABREVIATURAS

COMT - catecol-0-metiltransferase

DA – dopamina

DCFH-DA – diacetato de diclorofluoresceína

DO - discinesia orofacial

DOPAC – ácido 3,4-diidroxifenilacético

DHPG – Diidroxifenilglicol

DOPA - diidroxifenilalanina

DT – discinesia tardia

ERO – espécies reativas de oxigênio

GPx – glutationa peroxidase

H_2O_2 – peróxido de hidrogênio

5-HIAA – ácido 5-hidroxiindolacético

HL - Hiperlipídica

5-HT – serotonina

HVA – ácido homovanílico

MAO – enzima monoaminoxidase

MMV – movimentos de mascar no vazio

MnSOD – Superóxido dismutase dependente de manganês

MPTP – neurotoxina 1-metil-4-fenil-1,2,3,6-tetraidropiridina

NA – noradrenalina

NL – Normolipídica

NMDA – N-Metil-D-Aspartato

$OH^{\bullet}$ – radical hidroxila

SNC – sistema nervoso central

SOD – superóxido dismutase

TBARS – espécies reativas ao ácido tiobarbitúrico

TF – tremor facial

LISTA DE FIGURAS E TABELAS

APRESENTAÇÃO

No item **INTRODUÇÃO**, está descrita uma sucinta revisão bibliográfica sobre os temas trabalhados nesta tese.

Os resultados que fazem parte desta tese estão apresentados sob a forma de artigos, os quais encontram-se no item **ARTIGOS 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** encontradas no final desta dissertação, apresentam interpretações e comentários gerais sobre os artigos científicos contidos neste trabalho.

As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente às citações que aparecem nos itens **INTRODUÇÃO**, **DISCUSSÃO e CONCLUSÕES** desta dissertação.

Tendo em vista que esta tese é a continuação do trabalho do mestrado em função do processo de migração obtido por mim junto a CAPES, encontram-se em **ANEXO (I e II)** os artigos que fizeram parte da Dissertação de Mestrado.

RESUMO

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

PAPEL DA RECAPTAÇÃO E DE METABÓLITOS DA DOPAMINA NA DISCINESIA OROFACIAL INDUZIDA POR NEUROLÉPTICOS EM RATOS

AUTORA: Roselei Fachinetto
ORIENTADOR: Juliano Ferreira
CO-ORIENTADOR: João B.T. Rocha
LOCAL E DATA DA DEFESA: Santa Maria, maio de 2008.

A discinesia orofacial (DO) induzida por flufenazina consiste num modelo de discinesia tardia (DT) cuja patofisiologia tem sido relacionada à hipersensibilidade dopaminérgica e ao estresse oxidativo. Dados da literatura demonstraram que pacientes com DT apresentam reduzida expressão do transportador de dopamina (TDA). Em um estudo prévio, nós demonstramos que animais experimentais que apresentam alta intensidade de movimentos de mascar no vazio (MMV) induzidos por tratamento crônico com haloperidol também apresentaram uma redução na captação de dopamina (DA) no estriado. Tendo em vista que uma das maneiras de reduzir a atividade dos TDA é via modulação redox, um primeiro objetivo deste estudo foi determinar se o tratamento crônico com flufenazina poderia induzir um aumento nos índices de estresse oxidativo em regiões cerebrais (estriado e *substantia nigra*) e quais os efeitos deste tratamento nos níveis de captação de DA no estriado de ratos tratados aguda e cronicamente com flufenazina (Artigo 1). O tratamento com flufenazina produziu MMV na maioria dos ratos tratados (87% após 24 semanas). O tratamento concomitante com disseleneto de difenila diminuiu a prevalência dos MMV para 50%. Além disso, separamos os animais que desenvolveram (+MMV) ou não desenvolveram (-MMV) MMV. Não encontramos nenhuma diferença estatística entre os grupos quando comparados parâmetros de estresse oxidativo. O tratamento crônico, mas não agudo, com flufenazina diminuiu significativamente a captação de DA nos animais que apresentaram MMV. O tratamento concomitante com disseleneto de difenila não foi capaz de prevenir esta redução naqueles ratos que desenvolveram MMV. Um outro objetivo deste trabalho foi avaliar a participação da DA, de outras monoaminas e de seus metabólitos no modelo agudo e crônico de DO induzida por flufenazina em ratos (manuscrito em preparação 1). O tratamento com flufenazina produziu MMV na maioria dos animais tratados (50% após 3 semanas e cerca de 85% após 24 semanas. Não houve diferença estatisticamente significativa entre os grupos controle e tratado com flufenazina agudamente com relação aos níveis de monoaminas e seus metabólitos no estriado de ratos apresentando MMV+. Observamos uma tendência a um aumento nos níveis dos metabólitos da DA, HVA ($p=0.05$) e DOPAC ($p=0.06$), após tratamento crônico com flufenazina. Em conjunto, estes resultados indicam que a redução no transporte de DA pode ser um possível mecanismo relacionado à manutenção da DO crônica em ratos. O metabolismo da DA parece ter participação na manutenção da DO, mas

não no desenvolvimento. Além disso, o uso do disseleneto de difenila parece ser terapia farmacológica promissora para a redução da prevalência da DO.

Palavras-chave: flufenazina, discinesia orofacial, discinesia tardia, radicais livres, neurolépticos, dopamina, estresse oxidativo.

ABSTRACT

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

ROLE OF DOPAMINE UPTAKE AND THEIR METABOLITES IN THE OROFACIAL DYSKINESIA INDUCED BY NEUROLEPTICS IN RATS

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PLACE AND DATE OF THE DEFENSE: Santa Maria, 2008

Fluphenazine-induced orofacial dyskinesia (OD) is a putative animal model of tardive dyskinesia (TD) whose pathophysiology has been related to an increase in dopamine hypersensitivity and oxidative stress. Data from literature have shown that patients with TD present a decrease in dopamine transporter (DAT) expression. In a previously study, we have demonstrated that experimental animals presenting high intensity of vacuous chewing movements (VCM) induced by chronic treatment with haloperidol also presented a reduced dopamine uptake into striatum. Considering that one way to regulate DAT is through redox modulation, the first objective of the present study to determine if the chronic treatment with fluphenazine could induce an increase in oxidative stress index in brain regions (striatum and *substantia nigra*) and an alteration in levels of dopamine uptake in the striatum of rats treated acute and chronically with fluphenazine (Article 1). The fluphenazine treatment produced VCMs in the majority of the treated rats (87% after 24 weeks). Concomitant treatment with diphenyl diselenide decreased the prevalence of VCMs to 50%. Additionally, we separated the rats that developed (+VCM) or did not develop (-VCM) VCMs. We did not find any statistical differences among the groups when oxidative stress parameters were evaluated. Chronic fluphenazine treatment significantly decreased dopamine uptake. Concomitant treatment with diphenyl diselenide was not able to prevent this decrease in those rats that developed VCMs. Another objective of this work was to evaluate the role of dopamine (DA) and other monoamines and their metabolites on acute and chronic of OD induced by fluphenazine in rats (manuscript in preparation 1). The vacuous chewing movements (VCMs) or the levels of monoamines and its metabolites were quantified after 3 (acute) or 24 (chronic) weeks after beginning of treatment. The fluphenazine treatment produced VCMs in part of treated rats (50% after 3 weeks and about 85% after 24 weeks). There were not significant differences between the groups in monoamines levels neither in their metabolites in the striatum under acute fluphenazine treatment in +VCMs rats. However, we observed a trend to increase the levels of the DA metabolites, HVA ($p=0.05$) and DOPAC ($p=0.06$), after chronic treatment with fluphenazine. Our data suggest that an increase in DA metabolism could contribute to the maintenance of VCMs in rats. Moreover, development of VCMs seems not to be dependent of DA metabolism. Moreover, the use of diphenyl disselenide seems to be a promissory pharmacological therapy in the reduction of OD prevalence.

Key-words: fluphenazine, orofacial dyskinesia, tardive dyskinesia, free radicals, neuroleptics, dopamine, oxidative stress.

1. INTRODUÇÃO

1.1. Esquizofrenia

A esquizofrenia consiste numa importante desordem mental, afetando cerca de 1% da população em todo o mundo (Mahadik e cols., 2001), independente da cultura, país ou grupo racial (Bromet e Fenning, 1999). As manifestações da esquizofrenia surgem, geralmente, entre o final da segunda década de vida e início da terceira.

Em 1908, Eugene Bleuler definiu esquizofrenia como sendo a falta de interação entre o processo do pensamento e da percepção. Bleuler também classificou os sintomas da esquizofrenia, conforme suas características, em sintomas positivos, os quais incluem desilusão e alucinação, e negativos, como a perda de motivação e oscilação emocional (Lewis e Lieberman, 2000; Stotz-Ingenlath, 2000).

A etiologia da esquizofrenia continua ainda não completamente esclarecida. A teoria mais aceita para explicar as bases neuroquímicas da esquizofrenia é a de que existe uma hiperatividade da neurotransmissão dopaminérgica das projeções mesencefálicas para o estriado límbico, baseado no fato de que os neurolépticos que possuem maior eficácia terapêutica, principalmente para os sintomas positivos da esquizofrenia, são aqueles com maior afinidade por bloquear receptores dopaminérgicos D_2 (Snyder, 1976).

1.2 Neurolépticos

Os neurolépticos são fármacos utilizados no tratamento de psicoses, em particular a esquizofrenia. A clorpromazina, uma fenotiazina, foi o primeiro neuroléptico descrito, em 1952, por Delay e Deniker. A flufenazina consiste num potente neuroléptico pertencente também à classe das fenotiazinas, que foi introduzida na prática clínica no final da década de 50 (Darling, 1959; Taylor, 1959). Naquela época, a flufenazina foi considerada uma descoberta importante em relação às fenotiazinas já existentes (por exemplo, a clorpromazina) principalmente porque não produzia o efeito colateral da acatisia, comum a

esta classe de medicamentos (Darling, 1959). Em 1957, Paul Janssen descobriu a atividade neuroléptica das butirofenonas. Nesta classe, encontra-se o haloperidol, um neuroléptico que se destaca por sua potência, especificidade e longa ação (Niemegeers, 1983). A principal ação farmacológica dos neurolépticos clássicos ou típicos (haloperidol e flufenazina) consiste em bloquear receptores dopaminérgicos D_2 (Creese e cols., 1976). No entanto, o tratamento com estes fármacos possui eficácia comprometida por causarem efeitos colaterais extrapiramidais agudos e crônicos como, por exemplo, a Discinesia Tardia (DT) e o Parkinsonismo.

Em 1958, Schmutz e cols. sintetizaram uma série de compostos denominados de dibenzazepinas tricíclicas, sendo que a clozapina, que foi o protótipo dos antipsicóticos atípicos, foi uma delas (Schmutz e Eichenberger, 1982). Estes novos compostos foram efetivos em alguns modelos animais de ação antipsicótica. Contudo, em contraste aos neurolépticos típicos, os neurolépticos atípicos não foram efetivos em modelos de estereotipia induzidos por anfetamina e apomorfina (Healy, 2002; Hippius, 1989).

Mais tarde, observou-se que pacientes resistentes ao tratamento com haloperidol e clorpromazina, principalmente aqueles com sintomas negativos de esquizofrenia, respondiam de maneira satisfatória ao tratamento com clozapina (Kane e cols., 1988). Desta forma, novos neurolépticos atípicos, começaram a ser sintetizados visando minimizar os efeitos colaterais extrapiramidais sem, contudo, diminuir a eficácia terapêutica. Entretanto, os antipsicóticos atípicos, além de não possuírem eficácia satisfatória nos sintomas positivos da esquizofrenia, apresentam uma série de efeitos colaterais, entre eles diabetes *mellitus* tipo 2 e agranulocitose e, em alguns casos, a própria DT (Henderson, 2000). Além disso, os antipsicóticos atípicos possuem custo muito elevado se comparados aos típicos. Desta forma os neurolépticos clássicos continuam sendo largamente empregados no tratamento das psicoses.

1.3. Discinesia Tardia

A DT consiste num distúrbio do movimento decorrente do uso prolongado de neurolépticos, sendo considerada o principal efeito colateral destes fármacos. As primeiras

descrições desta síndrome foram publicadas entre 1956 e 1957. Inicialmente, a DT foi denominada de “discinesia persistente” sendo também referida como “síndrome buco-língüo-mastigatória” ou “síndrome da insuficiência extrapiramidal terminal” (Crane, 1968; Kane, 1995). O termo discinesia tardia foi proposto em 1964, por Faurbye e colaboradores.

A DT caracteriza-se por movimentos anormais hipercinéticos, sem propósito, repetitivos e involuntários que podem ocorrer durante ou após a interrupção de um tratamento prolongado com neurolépticos. Estes distúrbios do movimento ocorrem, mais freqüentemente, na região orofacial e incluem movimentos de mastigação, protusão da língua, estalido dos lábios, movimentos de franzir a face e piscar os olhos. Em alguns casos, os distúrbios hipercinéticos podem também atingir o pescoço, os membros (principalmente os superiores) e o tronco (Kane, 1995). Também podem desenvolver-se sintomas axiais de movimentos pélvicos para frente e para trás ou movimentos rotatórios, descontínuos, dos quadris. Estes sintomas possuem flutuações no decorrer do tempo, podendo variar em intensidade até mesmo dentro do mesmo dia (Gardos e Cole, 1983; Wolfarth e Ossowska, 1989; Laporta e cols., 1990).

Em 1988, a Associação Psiquiátrica Americana estimou a prevalência da DT em cerca de 10 a 20% dos pacientes que utilizam cronicamente fármacos neurolépticos. Alguns autores estimam que a média de prevalência da DT em pacientes recebendo tratamento com neurolépticos clássicos consiste de um índice em torno de 20–25%, mas este índice aumenta com a idade. De fato, a idade é considerada um dos fatores de risco para a DT, e atinge cerca de 50% dos pacientes, com mais de 50 anos de idade, em tratamento com neuroléptios (Kane e Smith, 1982; Gardos e Cole, 1983; Yassa e Jeste, 1992). O mais sério aspecto da DT consiste na persistência da síndrome por meses ou até anos após a retirada do tratamento, sendo que esta pode ser irreversível (Crane, 1973; Jeste e cols., 1979; Casey, 1985; Glazer e cols., 1990).

1.4. Modelos animais de discinesia tardia

Para o estudo dos mecanismos da DT, que se desenvolve em humanos, existem alguns modelos animais que são utilizados. Nos modelos animais, a discinesia é chamada

de discinesia orofacial (DO). Dentre os modelos animais de DO destacam-se os modelos agudos induzidos por neurolépticos e o modelo de DO induzido por reserpina por serem os mais comumente utilizados.

Contudo, esses modelos agudos em geral têm sido criticados devido a uma série de fatores, sendo que a principal crítica consiste no fato de a síndrome extrapiramidal aguda apresentar mais similaridades com Parkinsonismo do que com a DT propriamente dita (Egan e cols., 1996). Além disso, sabe-se que, em humanos, a retirada do tratamento prolongado com neurolépticos leva a uma exacerbação da síndrome, o que é visto apenas em modelos crônicos de DO (Gunne e cols., 1982; Egan e cols., 1994).

Algumas similaridades entre DO em animais e DT em humanos podem ser observadas. Tanto em humanos como em animais existe um subgrupo que é mais suscetível ao desenvolvimento da síndrome (Tamminga e cols., 1990; Egan e cols., 1994; Shirakawa e Tamminga, 1994). Os movimentos orofaciais induzidos por neurolépticos em animais possuem a mesma freqüência (1-3 Hz) da DT em humanos (See e Ellison, 1990). Como em humanos, o número de movimentos de mascar no vazio (MMV), que consiste no parâmetro mais utilizado para avaliação do desenvolvimento da DO, possui flutuações no decorrer do tempo e pode piorar muito quando existe um fator de estresse envolvido (Waddington, 1990; Kaneda e cols., 1992; Egan e cols., 1994). Em animais também é visto um aumento da DO com o aumento da idade tanto induzida por neurolépticos quanto espontânea (Kaneda e cols., 1992; Egan e cols., 1994; Jorgensen e cols., 1994; Andreassen e cols., 1996, 1998).

1.5. Hipóteses para a DT

Algumas hipóteses neuroquímicas têm sido propostas na tentativa de elucidar o mecanismo de desenvolvimento da DT. No entanto, sua exata patofisiologia permanece não esclarecida. A seguir descrevemos algumas das hipóteses mais aceitas para explicar a gênese da DT.

1.5.1. Hipótese da supersensibilidade dopaminérgica

A dopamina (DA) é um neurotransmissor amplamente distribuído através do sistema nervoso central (SNC). Especialmente, este neurotransmissor é largamente encontrado no estriado (Palkovits e Brownstein, 1989) e é sintetizado por neurônios dopaminérgicos, os quais fazem parte do sistema nigroestriatal. O sistema dopaminérgico nigroestriatal está diretamente relacionado aos sintomas extrapiramidais.

É conhecido que a dopamina é sintetizada a partir do aminoácido tirosina, sendo que a enzima regulatória desta via é a tirosina hidroxilase, que converte tirosina em diidroxifenilalanina (DOPA). Esta por sua vez é convertida até dopamina (DA) por ação da enzima L-aminoácido descarboxilase aromática. A DA é então liberada na fenda sináptica em resposta ao estímulo nervoso que leva a um aumento de cálcio citosólico e despolarização do neurônio pré-sináptico. Na fenda sináptica, a DA por sua vez, interage receptores encontrados em neurônios pré e pós-sinápticos, exercendo assim suas ações celulares. Existem mecanismos de controle para a redução dos níveis extracelulares da DA. Um deles seria via ação da DA em receptores pré-sinápticos que levariam à redução na atividade da tirosina hidroxilase e consequentemente à redução na síntese de dopamina. Outro mecanismo importante seria através da retirada da dopamina extracelular via transportador de dopamina (TDA) (Goodman e Gilman, 2006). A DA é enzimaticamente desaminada pela monoamina oxidase (MAO) para formar 3,4-diidroxifenilacetaldeído. Este composto é então oxidado pela enzima aldeído desidrogenase para produzir o ácido 3,4-diidroxifenilacético (DOPAC), o qual é subsequentemente metilado pela enzima catecol-o-metiltransferase (COMT) para formar o ácido homovanílico (HVA) (Cooper e cols., 2003).

A supersensibilidade dopaminérgica consiste na mais popular hipótese para explicar o desenvolvimento da DT após uso crônico de neurolépticos. Segundo esta hipótese, a DT é resultante de uma resposta do sistema nervoso central (CNS), secundária ao bloqueio crônico dos receptores dopaminérgicos pelos neurolépticos, em locais relacionados ao controle dos movimentos. Em resposta a este bloqueio crônico, há um aumento compensatório no número de receptores dopaminérgicos, receptores estes que provavelmente respondem a menores níveis de DA levando a um estado hiperdopaminérgico e a manifestações clínicas como, por exemplo, a DT (Klawans e

Rubovits, 1972; Burt e cols., 1977; Rubinstein e cols., 1990). No entanto, esta teoria possui algumas contradições. A principal inconsistência da teoria é que o mais importante fator de risco para o desenvolvimento da DT é a idade (Cavallero e Smeraldi, 1995; Kane, 1995; Woerner e cols., 1998). Contudo, foi demonstrado que o envelhecimento faz com que ocorra a redução tanto do número quanto da sensibilidade dos receptores dopaminérgicos (Lohr e Jeste, 1988; Sachdev, 1999).

Apesar de a hipótese da supersensibilidade dos receptores dopaminérgicos possuir algumas inconsistências, ainda assim o sistema dopaminérgico parece estar diretamente relacionado ao desenvolvimento da DT e DO. Dados da literatura demonstram que a administração de neurolépticos, por bloquear receptores dopaminérgicos pré-sinápticos responsáveis pela retroinibição da síntese de DA, acabam por levar a um aumento secundário de sua síntese e, consequentemente, elevação nos níveis extracelulares deste neurotransmissor (Lohr, 1991; Andreasen e Jorgensen, 2000).

Neste contexto, a principal forma de retirada da DA da fenda sináptica e, portanto da redução da neurotransmissão dopaminérgica, ocorre através da recaptação de dopamina via TDA (Beckman e Quick, 1998; Kahlig e Galli, 2003). O TDA (Figura 1) consiste numa proteína integral de membrana que contém 12 domínios transmembrana. É membro da família de transportadores dependente de Na^+/Cl^-, sendo codificado por um único gene (SLC6A3). O TDA é encontrado exclusivamente em neurônios dopaminérgicos, sendo que o estriado é a região do SNC mais rica neste tipo de transportador (Amara e Kuhar, 1993; Giros e Caron, 1993). Dessa forma, TDA tem papel crucial em desordens que alteram a plasticidade neuronal dopaminérgica. Alguns trabalhos têm demonstrado alterações na expressão do TDA em humanos e em animais experimentais que recebem tratamento com neurolépticos (Saldaña e cols., 2006; Yoder e cols., 2004). Recentemente demonstramos que animais experimentais que desenvolvem DO, em resposta ao tratamento crônico com o neuroléptico clássico haloperidol, apresentam níveis reduzidos de recaptação de DA (Fachinetto e cols., 2007).

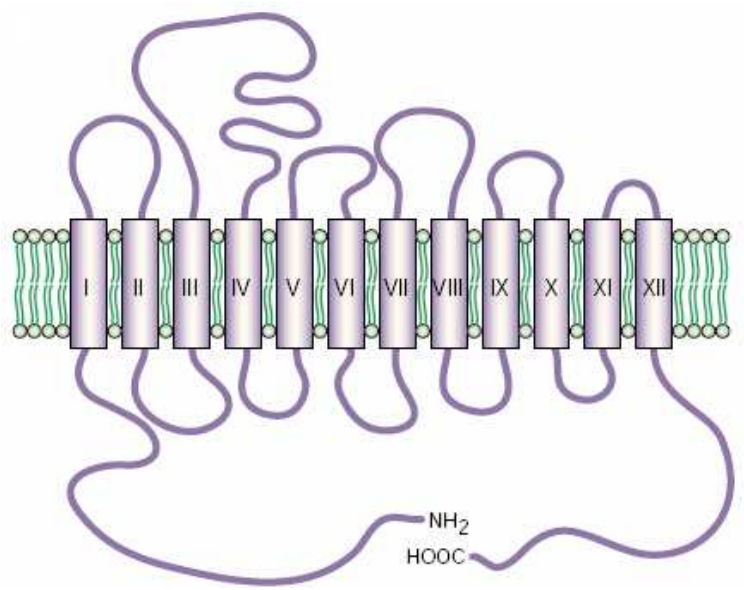

Figura 1: Estrutura do transportador de monoaminas. Ambas terminações N e C terminais são intracelulares e o mesmo possui 12 domínios transmembrana. Fonte: Torres e cols., 2003.

1.5.2. Hipótese dos radicais livres

Uma hipótese que vem ganhando atenção na literatura é a de que os radicais livres possam ter uma importante participação no desenvolvimento da DT (Cadet e cols., 1986, 1987; Lohr e cols., 2003). A hipótese dos radicais livres tem como base para o seu desenvolvimento o sistema dopaminérgico.

De acordo com isto, dados da literatura indicam que a administração de neurolépticos, por bloquear receptores dopaminérgicos, pode causar um aumento secundário na síntese de dopamina e consequentemente um aumento no seu metabolismo via aumento da atividade da MAO (Lohr, 1991; Andreasen e Jorgensen, 2000). Sabe-se que a atividade das oxidases em geral, forma como produto o peróxido de hidrogênio (H_2O_2) que ao reagir com metais de transição via reação de fenton, forma radicais livres, como radicais hidroxila ($OH^{\cdot}$) e superóxido. Além disso, a própria dopamina pode sofrer auto-oxidação formando dopamina quinona que pode agir como espécie reativa de oxigênio (Lohr, 1991; 2003).

Além disso, o bloqueio dos receptores estriatais dopaminérgicos pode produzir um aumento no glutamato extracelular, o que pode levar a um aumento na produção de radicais livres (Coyle e Puttfarcken, 1993; Tsai e cols., 1998; Castilho e cols., 1999) através de mecanismos excitotóxicos.

Concordando com o fato de que os radicais livres estão envolvidos no desenvolvimento da DO, foi demonstrado que a administração de substâncias pró-oxidantes é capaz de potencializar o desenvolvimento de DO em modelos animais (Andreassen e cols., 1998; Calvent e cols., 2002). Além disto, existem evidências na literatura de que pacientes esquizofrênicos ingerem uma maior quantidade de gordura na dieta do que a população em geral (Brown e cols., 1999) e, a ingestão de dietas ricas em gordura está associada a um aumento de estresse oxidativo em roedores (Folmer e cols., 2003). Mais recentemente, foi demonstrado que dietas ricas em gordura aumentam a vulnerabilidade dos neurônios dopaminérgicos à neurotoxina 1-metil-4-fenil-1,2,3,6-tetraidropiridina (MPTP) (Choi e cols., 2005). De particular importância, recentemente demonstramos que animais recebendo uma dieta rica em gordura e, tratados cronicamente com haloperidol, apresentaram maiores intensidades de DO quando comparados ao grupo controle (Fachinetto e cols., 2005).

Sabe-se que o cérebro é particularmente vulnerável à ação tóxica das espécies reativas de oxigênio principalmente pela grande quantidade de energia utilizada que, via metabolismo oxidativo, pode levar à produção de espécies reativas de oxigênio (Lohr, 1991). Além disso, o SNC, por ser um local extremamente rico em ácidos graxos poliinsaturados, propicia a peroxidação lipídica (Lohr e cols., 2003). Com base em trabalhos recentes acerca de drogas capazes de reverter ou mesmo de impedir o desenvolvimento da DT, o uso de substâncias com potencial antioxidante parece ser promissor. Considerando que o sistema antioxidante endógeno, tanto enzimático, representado pelas enzimas glutationa peroxidase (GPx), catalase e superóxido dismutase (SOD), quanto não enzimático, principalmente representado pelas vitaminas E e C, parece ser fundamental neste caso, a estratégia terapêutica ou mesmo preventiva, estaria na administração de substâncias capazes de restabelecer as defesas antioxidantes endógenas ou mesmo atuar complementando-as.

Dados da literatura têm demonstrado que, em animais tratados com neurolépticos, existe aumento nos níveis de peroxidação lipídica e de carbonilação de proteínas, redução na atividade de enzimas antioxidantes, como a SOD, a catalase e a GPx, e também redução da glutationa reduzida e conseqüente aumento da glutationa oxidada (Post e cols., 2002; Naidu e cols., 2003a; Abílio e cols., 2004; Burger e cols., 2004; 2005 a; b; Faria e cols., 2005; Sadan e cols., 2005; Pillai e cols., 2006). Neste contexto, a enzima SOD tem recebido atenção na literatura. Foi demonstrada uma significativa redução no alelo polimórfico (alta atividade) para a MnSOD em pacientes com esquizofrenia e DT em comparação com aqueles sem DT, sugerindo que a alta atividade do alelo MnSOD pode proteger contra o desenvolvimento da DT (revisado por Lohr e cols., 2003). Além disso, o uso de substâncias com potencial antioxidante como é o caso da Vitamina E, melatonina, quercetina, diseleneto de difenila e ebselen foi capaz de atenuar ou mesmo reverter completamente a DO bem como restaurar os parâmetros bioquímicos que se apresentavam alterados (Sachdev e cols., 1999; Naidu e cols., 2003 a, b; Burger e cols., 2004; 2005a; 2006).

Mais importante, em humanos foi demonstrado que existe aumento de parâmetros oxidativos no fluído cérebro espinhal e plasma de pacientes com DT (Pall e cols., 1987; Tsai e cols., 1998; Lohr e cols., 1990). Foi relatado que o uso de substância antioxidantes como a vitamina E pode prevenir o aparecimento da discinesia em pacientes (Egan e cols., 1992; Dabiri e cols., 1994). No entanto, seu uso como tratamento após a síndrome já instalada, parece não ter eficácia.

De particular importância, recentes dados de nosso laboratório têm apontado para o papel protetor de substâncias orgânicas contento selênio e com atividade anti-oxidante (disseleneto de difenila e ebselen) contra a DO induzida por haloperidol ou reserpina em ratos (Burger e cols., 2004; 2005a; 2006). O Selênio (Se) é um micronutriente presente em alguns alimentos que fazem parte da dieta. Na forma de selenocisteína, forma uma parte vital de várias seleno-enzimas, incluindo a glutationa peroxidase (GPx) (Klotz e cols., 2003; Brenneisen e cols., 2005; Steinbrenner e cols., 2006). GPx juntamente com a catalase e superóxido dismutase (SOD) são os principais sistemas antioxidantes contra a formação de radicais livres. Desta forma, os níveis de Se poderiam modular desordens relacionadas ao estresse oxidativo que envolvem o sistema dopaminérgico, como, por exemplo, a DT. Estudos epidemiológicos têm demonstrado que a deficiência de selênio na dieta está

relacionada com a gênese e ou progressão de diversas patologias como desordens neurológicas, cardiovasculares, câncer e diabetes (Wilber, 1980; Salonen e cols., 1982; Clark e cols., 1991; El-Bayoumy, 1991; Combs e Gray, 1998; Armstrong e cols., 1996). Contudo, é conhecido que compostos inorgânicos de Se podem apresentar toxicidade (Brandão e cols., 2006; para revisão, ver Nogueira e cols., 2004). Por outro lado, compostos orgânicos de Se possuem um bom perfil farmacológico desde que estes compostos possuem baixo potencial de toxicidade (Commandeur e cols., 2001; Nogueira e cols., 2003; 2004). Além disso, nosso grupo tem demonstrado que compostos orgânicos de Se possuem propriedades anti-inflamatória, antioxidante, antitumoral e neuroprotetora (Borges e cols., 2005; Nogueira e cols., 2004; Santos e cols., 2005). Entretanto, a participação do estresse oxidativo tem sido extensivamente estudada em modelos agudos de DO, o que faz com que seja necessário o estudo mais detalhado em modelos crônicos, tendo em vista as críticas que são impostas ao modelo agudo principalmente no que diz respeito à sua maior semelhança ao Parkinsonismo do que à DT, conforme comentado anteriormente (Para revisão ver Salamone e cols., 1998). Além disso, existem dados controversos na literatura a respeito da efetividade de terapias antioxidantes para o tratamento de pacientes discinéticos. Estudos realizados demonstram que o tratamento tem maior eficácia se administrado desde o início da terapia com neurolépticos, sendo pouco efetivo na reversão da síndrome já instalada (Egan e cols., 1994).

O sistema dopaminérgico tem envolvimento direto no desenvolvimento da DT e muitos trabalhos têm focado a participação da DA neste processo. Apesar de este ser o foco de estudos de muitos grupos, pouco se conhece a respeito da participação do TDA nesta patologia. Diante do exposto, fica claro que estudos envolvendo modelos agudos e crônicos devem ser realizados para elucidar o papel do transporte e do metabolismo de DA, bem como a real participação do estresse oxidativo no desenvolvimento e manutenção da DO.

2. OBJETIVOS

Objetivo geral

O objetivo geral deste estudo consiste em avaliar o papel da recaptação de dopamina, bem como a participação deste neurotransmissor e seus metabólitos no desenvolvimento e manutenção da discinesia orofacial induzida por flufenazina em ratos.

Objetivos específicos

→ Determinar a intensidade e prevalência da DO em ratos tratados aguda e cronicamente com flufenazina;

→ Verificar o efeito do tratamento crônico com o antioxidante disseleneto de difenila no modelo de DO induzido por flufenazina em ratos;

→ Avaliar a recaptação de DA no estriado de ratos tratados aguda e cronicamente com flufenazina;

→ Quantificar os níveis de DA, serotonina, noradrenalina e seus metabólitos no estriado de ratos tratados aguda e cronicamente com flufenazina;

→ Investigar a presença de estresse oxidativo no estriado de ratos tratados aguda e cronicamente com flufenazina.

3- ARTIGOS CIENTÍFICOS

Os resultados que fazem parte desta tese estão apresentados sob a forma de artigos científicos, os quais se encontram aqui organizados. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se nos próprios artigos. O **artigo 1** está disposto na forma que foi publicado na edição da revista científica **Psychopharmacology.** O **manuscrito em preparação 1** está disposto na forma em que normalmente se submete para publicação.

3.1 – EFEITO DO DISSELENETO DE DIFENILA SOBRE DISCINESIA OROFACIAL INDUZIDA POR FLUFENAZINA EM RATOS

Artigo 1

DIPHENYL DISELENIDE DECREASES THE PREVALENCE OF VACUOUS CHEWING MOVEMENTS INDUCED BY FLUPHENAZINE IN RATS

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ORIGINAL INVESTIGATION

Diphenyl diselenide decreases the prevalence of vacuous chewing movements induced by fluphenazine in rats

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Abstract
Rationale Chronic treatment with neuroleptics causes, as a side effect, tardive dyskinesia in humans; however, the mechanisms involved in its pathophysiology remain unclear.
Objectives The purpose of this study was to examine the effects of diphenyl diselenide, an organoselenium compound with antioxidant properties, in an animal model of vacuous chewing movements (VCMs) induced by long-term treatment with fluphenazine.
Results Adult male rats were treated during 24 weeks with fluphenazine (25 mg/kg, intramuscularly [i.m.], once every 21 days) and diphenyl diselenide (1 mg/kg, subcutaneously, three times a week). VCMs and body weight gain were quantified every 3 weeks. The fluphenazine treatment produced VCMs in the majority of the treated rats (87% after 24 weeks). Concomitant treatment with diphenyl diselenide decreased the prevalence of VCMs to 50%. Additionally, we separated the rats that developed or did not develop VCMs. We did not find any statistical differences among the groups when oxidative stress parameters were evaluated. Chronic fluphenazine treatment significantly decreased [^{3}H]-dopamine uptake. Concomitant treatment with diphenyl diselenide was not able to prevent this decrease in those rats that developed VCMs.
Conclusions Our data suggest that the reduction in dopamine transport can be a possible mechanism related to the maintenance of VCMs in rats. Moreover, diphenyl diselenide seems to be a promising pharmacological agent in the reduction in the prevalence of VCMs in rats.

Keywords Tardive dyskinesia · Orofacial dyskinesia · Dopamine uptake · Fluphenazine · Diphenyl diselenide · Oxidative stress

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Introduction

The use of classical neuroleptics is the most effective treatment for schizophrenia. However, these drugs cause, as side effects, a syndrome characterized by involuntary movements of the orofacial region and, sometimes, musculature of the members and trunk, known as tardive dyskinesia (TD; Kane 1995). The mean of prevalence of TD is 20–25% in subjects receiving classical neuroleptic treatment, and the rate increases significantly with age (Kane and Smith 1982; Woerner et al. 1991). The most serious aspect of TD is that it may persist for months or years after drug withdrawal, and in some patients, it is irreversible (Casey 1985; Crane 1973; Jeste et al. 1979).

The molecular mechanisms responsible for TD are still not completely understood, and several hypotheses have been postulated (Andreassen and Jorgensen 2000; Ebadi and Srinivasan 1995; Lohr et al. 2003). TD has been attributed to the supersensitivity of dopamine receptors, but this mechanism is not consistent with a number of factors

documented in TD patients (Andreassen and Jorgensen 2000; Lohr et al. 2003). One hypothesis that has gained clinical experimental support in the literature is that free radicals may play an important role in TD development (Cadet et al. 1986, 1987; Lohr et al. 2003). In fact, clinical studies have shown that the chronic use of neuroleptic drugs is capable of inducing oxidative stress (Lohr et al. 1990; Pall et al. 1987; Tsai et al. 1998) and that treatment with antioxidant substances (such as vitamin E) can attenuate the development of TD (Dabiri et al. 1994; Egan et al. 1992). In animal models of orofacial dyskinesia (OD), the participation of oxidative stress in the appearance of involuntary movements in the orofacial region has been demonstrated (Abílio et al. 2004; Burger et al. 2004, 2005a, b; Faria et al. 2005; Naidu et al. 2003; Post et al. 2002; Sadan et al. 2005).

Selenium (Se) is a micronutrient present in high proportion in some foods of the mammalian diet, and it forms a vital part of several Se-dependent enzymes, including glutathione peroxidase (GPx; Klotz et al. 2003; Brenneisen et al. 2005; Steinbrenner et al. 2006). GPx together with catalase and superoxide dismutase (SOD) are the main cellular antioxidant systems against free radical formation. Thus, Se levels could modulate oxidative stress-related disorders that involve the dopaminergic system, such as TD. However, it is known that inorganic Se compounds can be toxic (Brandão et al. 2005; for review, see Nogueira et al. 2004). On the other hand, organoselenium compounds have a better pharmacological profile because they possess low toxic potential (Commandeur et al. 2001; Nogueira et al. 2003, 2004). Moreover, our group has demonstrated that organoselenium compounds possess anti-inflammatory, antioxidant, antitumoral, and neuroprotective properties (Borges et al. 2005; Nogueira et al. 2004; Santos et al. 2005). Of particular importance, recent data from our laboratory have pointed to a protective role of diphenyl diselenide and ebselen, two organochalcogens, against haloperidol or reserpine-induced orofacial movements in rats (Burger et al. 2004, 2005a, 2006).

Thus, the aim of the present study was to investigate the action of the organoselenium compound diphenyl diselenide, a thiol peroxidase mimetic, on a chronic model of vacuous chewing movements (VCMs) induced by long-term treatment with fluphenazine.

Materials and methods

Animals

Male Wistar rats weighing 270–320 g and with age from 3 to 3.5 months from our own breeding colony were kept in cages of three or four animals each, with continuous access to food and water in a room with controlled temperature (22±3°C) and on a 12-h light/dark cycle with lights on at 7:00 A.M. The animals were maintained and used in accordance to the guidelines of the Brazilian Association for Laboratory Animal Science.

Drugs and treatments

Fluphenazine enantate (Flufenan®) was a gift from Cristália (Brazil). Diphenyl diselenide was synthesized by the previously described method (Palmier 1986). Firstly, to select the dose of fluphenazine used in chronic treatment, we carried out acute experiment using different doses of fluphenazine. Rats were divided into four groups with six animals each. The control group received an administration of soy oil intramuscularly (2 mL/kg, i.m.) at first day of experiment. The others three groups received fluphenazine enantate (12.5, 25, or 50 mg/kg, i.m.). Based in a previous study (Van Kampen and Stoessl 2000), behavioral testing was conduced 3 weeks after injection.

For chronic experiment, rats were divided into control, diphenyl diselenide, fluphenazine, and fluphenazine plus diphenyl diselenide groups. Fluphenazine enantate was administered i.m. every 21 days (25 mg/kg, i.m.). Diphenyl diselenide was dissolved in soy oil and administered subcutaneously (s.c.) three times per week in nonconsecutive days (1 mg/kg, s.c.; Burger et al. 2006). The control group received soy oil (1 mL/kg) in the same way as the diphenyl diselenide group. The treatment was carried out over the course of 6 months.

Behavioral testing

Behavior was assessed before the treatment with vehicles, fluphenazine, or diphenyl diselenide (basal evaluation), with nine animals per group. Animals that presented more than 40 VCMs (used by us as a parameter of OD) were excluded of the study. Thus, the number of animals in each group that received treatment was six, six, eight, and eight for the control, diphenyl diselenide, fluphenazine, and fluphenazine plus diphenyl diselenide groups, respectively. The effect of drugs on behavior was examined every 21 days beginning on the 21st day after the first fluphenazine injection (that occurred on the same day of the basal behavior) and was carried out over the course of 6 months. To quantify the occurrence of VCMs, rats were placed individually in cages (20×20×19 cm), and hand-operated counters were employed to quantify VCMs frequency. VCMs are defined as single-mouth openings in the vertical plane not directed toward physical material. If VCMs occurred during a period of grooming, they were not taken into account. VCMs were measured continuously for 6 min after a period of 6-min adaptation. During the

observation sessions, a mirror was placed under the floor of the experimental cage to permit observation when the animal was faced away from the observer. Experimenters were always blind with regard to the treatment conditions.

In a preliminary study of interater reliability, we found that the use of this method of observation and the definition of the parameter evaluated usually result in greater than 93% agreement between three different observers for VCMs. The calculated α value was significant for $p<0.05$.

It was previously reported that the treatment with neuroleptic drugs does not result in the development of VCMs in all treated rats (Kane and Smith 1982; Shirakawa and Tamminga 1994). In the present study, we have also verified the prevalence of neuroleptic-induced VCMs. In our laboratory, control rats present maximally 40 VCMs during a period of 6 min. Thus, in this study, we analyzed the rats that developed neuroleptic-induced VCMs (+VCM, more than 40 VCMs) separately from those that did not develop neuroleptic-induced VCMs (−VCMs, less than 40 VCMs), as described by Andreassen et al. (2003), Egan et al. (1994), and Shirakawa and Tamminga (1994).

Tissue preparations

Rats were killed about 24 h after the last session of behavioral quantification (on the 21st day after the last administration of fluphenazine and 48 h after of the last administration of diphenyl diselenide). The brains were immediately excised and put on ice. The striatum and region containing the substantia nigra were separated, weighed, and homogenized in 10 vol (w/v) of 10 mM Tris–HCl, pH 7.4. A portion of the striatum was dissected for slices used for the [^{3}H]-dopamine uptake assay.

[^{3}H]-dopamine uptake

[^{3}H]-dopamine uptake was carried out as described by Holz and Coyle (1974) with some modifications. The following three solutions were used in this experiment: (1) a buffered solution consisting of 127 mM NaCl, 1.2 mM Na_2HPO_4, 5.36 mM KCl, 0.44 mM KH_2PO_4, 0.95 mM $MgCl_2$, 0.70 mM $CaCl_2$, 10 mM glucose, 1 mM Tris–HCl, and Selegiline 1 μM, pH 7.4, (2) a solution containing solution 1 and 100 μM cocaine, and (3) a solution with the same composition of solution 1 but without selegiline. To measure [^{3}H]-dopamine uptake, the striatum was cut into 400-μm slices, which were washed with buffer (solution 3). The slices (0.2–0.3 mg protein) were preincubated in 96-well polycarbonate plates for 15 min at 35°C in solution 1. [^{3}H]-dopamine was added to the incubation medium, and the uptake was carried out for 10 min at 35°C, after which, the reaction was stopped by five washes of 30 s each with 1-mL ice-cold cocaine solution (solution 2). Immediately after washing, 0.25 mL of 0.5 M NaOH and 0.2% sodium dodecyl sulfate was added to the slices that were digested by 10 min of incubation at 60°C. Aliquots of the lysates were taken for protein content measurement by the Lowry et al. (1951) method. For determination of the intracellular amount of dopamine, liquid scintillation counting was used. [^{3}H]-Dopamine uptake was calculated by a difference between total and blocked with cocaine (incubated with solution 2). Results were expressed as cocaine-sensitive [^{3}H]-dopamine uptake per milligram of protein.

Oxidative stress parameters

To evaluate the levels of reactive oxygen species (ROS), the homogenates were centrifuged for 10 min at 1,500×g. Just after the centrifugation, an aliquot of supernatant was used for 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) oxidation. DCFH-DA oxidation was determined spectrofluorimetrically using the membrane-permeable fluorescent dye DCFH-DA (7 μM). Fluorescence was determined at 488 nm for excitation and 520 nm for emission. A standard curve was carried out using increasing concentrations of 2′,7′-dichlorofluorescein (DCF) incubated in parallel (Pérez-Severiano et al. 2004).

To assess lipid peroxidation, we quantified thiobarbituric reactive substances (TBARS). The homogenates were centrifuged for 10 min at 1,500×g. Just after the centrifugation, an aliquot of 200 μL or of supernatant was incubated for 1 h at 37°C and then used for lipid peroxidation quantification as earlier described (Ohkawa et al. 1979). MDA values were determined at 532 nm.

To verify SOD activity, the striatum or substantia nigra were adequately diluted to 40 volumes with Tris–HCl 10 mM (pH 7.5), and the assay was performed according to the method of Misra and Fridovich (1972). Briefly, epinephrine rapidly autooxidizes at pH 10.5 producing adrenochrome, a pink-colored product that can be detected at 480 nm. The addition of samples (10, 25, 50 μL) containing SOD inhibits the autooxidation of epinephrine. The rate of inhibition was monitored during 180 s at intervals of 30 s. The amount of enzyme required to produce 50% inhibition at 25°C was defined as one unit of enzyme activity. The SOD activity was expressed as units per gram of tissue.

Statistical analysis

Data from body weight and behavioral parameter (VCMs) were analyzed by three- (s.c. treatment, i.m. treatment, or time as factors) and two-way analysis of variance (ANOVA; treatment or time as factors), respectively. F values are presented in the text only if the p value associated with it was less than 0.05. Prevalence data were

analyzed by the Chi-squared test. Data from TBARS and ROS quantification, [^{3}H]-dopamine uptake, and SOD activity were analyzed by one-way ANOVA, followed by Duncan's post-hoc tests when appropriate. Significance was considered when $p<0.05$.

Results

Effects of different doses of fluphenazine on VCMs 3 weeks after treatment

Treatment with fluphenazine induced a high prevalence of VCMs in rats, with 100% of treated rats presenting more than 40 VCMs at a dose of 50 mg/kg compared with its vehicle (Chi-squared=12 and $p<0.05$). At doses of 12.5 and 25 mg/kg, fluphenazine treatment caused 50% prevalence of animals presenting more than 40 VCMs (Fig. 1a).

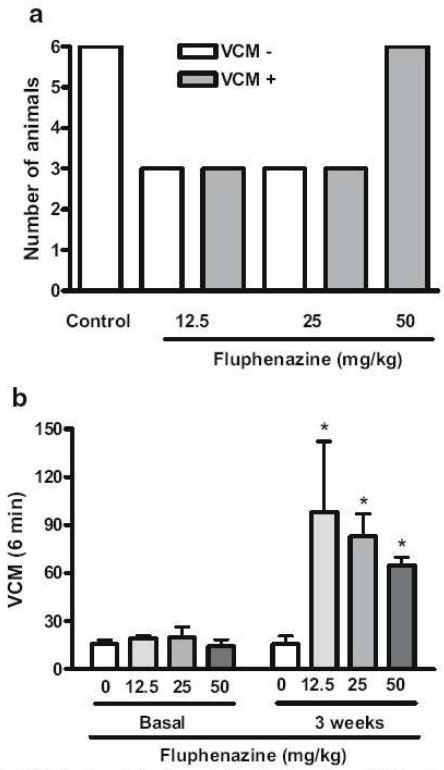

Fig. 1 Effects of acute treatment with fluphenazine at different doses (12.5, 25, and 50 mg/kg) or vehicle solution on prevalence (**a**) and intensity (**b**) of vacuous chewing movements. Values are express as means±SEM. Control group and group treated with 50 mg/kg of fluphenazine ($n=6$) and groups treated with 12.5 and 50 mg/kg of fluphenazine ($n=3$)

Considering the intensity of VCMs induced by acute treatment with different doses of fluphenazine, it was observed a marked increase on VCMs after 3 weeks of treatment at all doses when compared with its vehicle (Fig. 1b). However, any statistical difference was found in the VCMs intensity among different doses of fluphenazine. Thus, we have chosen the submaximal dose of 25 mg/kg to perform chronic treatments.

Effects of diphenyl diselenide on VCMs induced by long-term treatment with fluphenazine

Fluphenazine caused a marked increase on VCM when compared with its vehicle (F (8, 152)=9.11 and $p<0.001$). In fact, a significant interaction between fluphenazine and VCM measures (F (8, 152)=4.34, $p<0.001$) was observed. Treatment with fluphenazine induced a high prevalence of VCMs in rats, with 87.5% of treated rats presenting more than 40 VCMs compared with its vehicle (Chi-squared=10.50 and $p<0.001$). The treatment with diphenyl diselenide was able to reduce the prevalence of VCMs to 50% (Chi-squared=2.618 and $p=0.05$) in fluphenazine-administered rats (Fig. 2b).

However, diphenyl diselenide was not able to alter the number of VCMs in those animals that developed VCMs (Fig. 2a). Furthermore, the administration of diphenyl diselenide alone did not cause any alteration in animal behavior (Fig. 2a).

Effects of diphenyl diselenide on body weight during long-term treatment with fluphenazine

Fluphenazine but not its vehicle caused a significant body weight gain decrease that was time dependent (Fig. 2c). In fact, a significant interaction between fluphenazine and body weight measures (F (8, 176)=4.21, $p<0.05$) was observed. We did not observe any significant difference in body weight reduction between rats that developed and did not develop VCMs (data not shown). Moreover, the administration of diphenyl diselenide alone or with fluphenazine did not cause any alteration on body weight gain (Fig. 2c).

Effects of acute and chronic treatment with fluphenazine on [^{3}H]-dopamine uptake

Three weeks of treatment with fluphenazine (25 mg/kg) did not cause any significant effect on [^{3}H]-dopamine uptake in striatal slices when compared to its vehicle (Fig. 3a). However, the treatment with fluphenazine for 24 weeks caused a significant decrease in [^{3}H]-dopamine uptake in striatal slices when compared to its vehicle (Fig. 3b). Diphenyl diselenide cotreatment did not protect from fluphenazine-induced [^{3}H]-dopamine uptake reduction

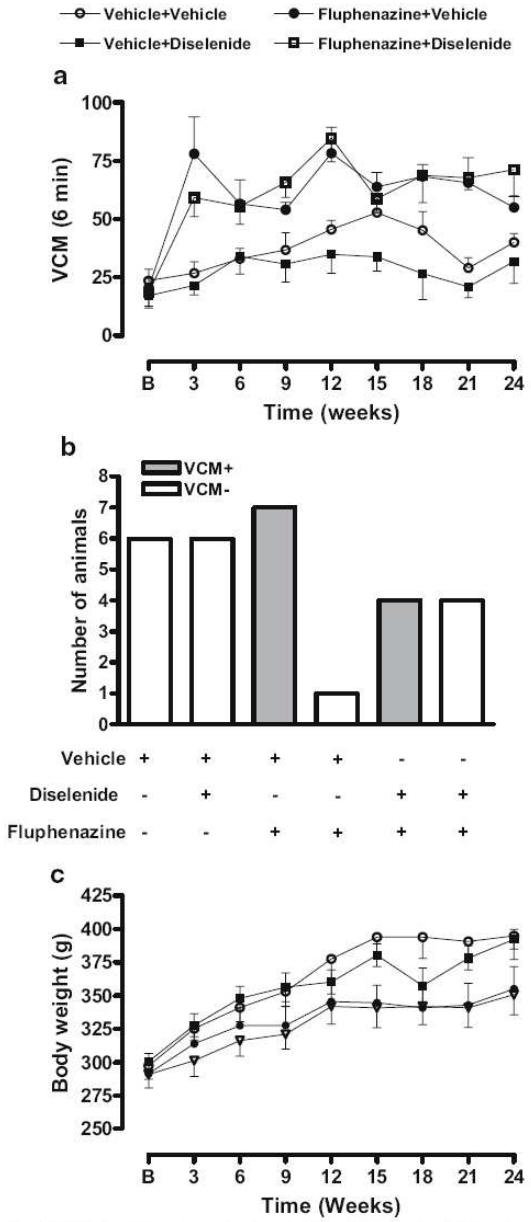

Fig. 2 Effects of long-term treatment with fluphenazine (25 mg/kg once every 21 days), diphenyl diselenide (1 mg/kg, three times a week), fluphenazine plus diphenyl diselenide, or vehicle solutions on intensity (**a**) or prevalence (**b**) of vacuous chewing movements and body weight gain (**c**). Values are expressed as means±SEM

(Fig. 3b). Of note, in rats protected from fluphenazine-induced VCMs by the diphenyl diselenide cotreatment, the level of [^{3}H]-dopamine uptake was similar to vehicle levels (data not shown). Diphenyl diselenide administration alone did not alter [^{3}H]-dopamine uptake in rats treated with vehicle.

Effects of fluphenazine and diphenyl diselenide on oxidative stress parameters

There was not a significant difference among the groups in DCFH-DA–acetate oxidation levels. However, there was a tendency for the levels DCFH-DA oxidation to decrease in

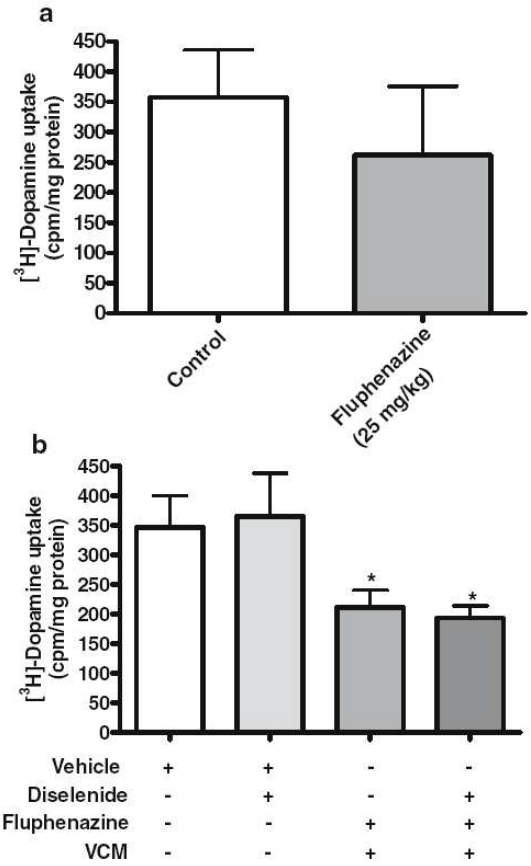

Fig. 3 Effect of fluphenazine treatment on [^{3}H]-dopamine uptake. **a** Effect of acute treatment with fluphenazine (25 mg/kg) on [^{3}H]-dopamine uptake (cpm/mg protein) in striatum of rats. **b** Effect of long-term treatment with fluphenazine (25 mg/kg, once every 21 days), diphenyl diselenide (1 mg/kg, three times a week), fluphenazine plus diphenyl diselenide, or control solution on specific [^{3}H]-dopamine uptake (cpm/mg protein) in striatum of rats. Data (mean±SEM) were analyzed by one-way ANOVA followed by Duncan's multiple range tests. *Asterisks* represent differences from the control group

the striatum and substantia nigra of rats with +VCM for both the group treated with fluphenazine alone and the group cotreated with diphenyl diselenide (Table 1).

Neither treatment with fluphenazine alone nor with diphenyl diselenide alone caused any effect on striatal and nigral SOD activity when compared with its vehicle (Table 1). In rats cotreated with diphenyl diselenide and fluphenazine, a significant increase in SOD activity was observed in the striatum but not in the substantia nigra when compared to fluphenazine alone or to its vehicle (Table 1).

There were no significant differences among the groups in TBARS levels in the striatum and substantia nigra of rats treated with diphenyl diselenide and fluphenazine (Table 1).

Discussion

TD has been a major problem associated with long-term neuroleptic treatment in humans; however, the mechanisms involved in its pathophysiology remain unclear. Our current results show that chronic fluphenazine treatment induces VCMs, an effect related with a reduction in dopamine uptake. Moreover, the organoselenium compound diphenyl diselenide reduced the prevalence of fluphenazine-induced VCMs.

It has been demonstrated that the level of Se in the brain is an important factor in the etiology of several neurodegenerative diseases (Chang 1983; Zafar et al. 2003). In addition, studies from our group have previously shown that the organoselenium compounds demonstrate protective effects on neurotoxicity models (for review, see Nogueira et al. 2004). For example, we have previously demonstrated that the organoselenium compound diphenyl diselenide attenuated acute parameters of OD produced by haloperidol or reserpine in rats (Burger et al. 2004, 2006). However, there are some criticisms about acute models of OD. In acute models of OD, the drug withdrawal decreases and/or inhibits the behavioral disturbances, while in chronic models of OD and in TD, drug withdrawal exacerbates this syndrome. For instance, acute models of OD have been classified as Parkinson's disease or extrapyramidal syndrome, where a reversal is possible by administering anticholinergic drugs (see, for review, Andreassen and Jorgensen 2000; Jenner and Marsden 1983; Egan et al. 1996). Therefore, we investigated the effects of diphenyl diselenide on a chronic model of VCMs as well as its possible mechanism of action. Diphenyl diselenide reduced the prevalence of VCMs but not the intensity of VCMs in fluphenazine-treated rats. Because the rats were cotreated with fluphenazine and diphenyl diselenide that did not develop VCMs showed values of dopamine uptake close to those of the control group, the reduction in VCMs in this group seems to be related to the maintenance of dopamine uptake at control levels.

The precise mechanisms that contribute to the development of TD remain elusive but appear to involve multiple neurotransmitter systems and receptor types (see, for review, Andreassen and Jorgensen 2000; Gunne et al. 1984; Lee et al. 1997; Lohr et al. 2003). Initially, the supersensitivity of dopamine D_2 receptors was proposed as the principal basis for TD supported by findings of increased dopamine D_2 receptor density in the rat striatum after an acute (about 2–3 weeks) administration of antipsychotics, including fluphenazine (Burt et al. 1977; Boyson et al. 1988). However, more recent studies have found high levels of parameters related to OD without an alteration in striatal D_2 receptors after chronic (18 weeks) treatment of rats with fluphenazine (Van Kampen and Stoessl 2000). Moreover, there is a poor temporal and spatial correlation between the development of TD and dopamine receptor supersensitivity (see, for review, Gerlach and Casey 1988; Gunne et al. 1984). However, other dopamine neurotransmission disturbances could to be important to TD development. For

Table 1 Effects of fluphenazine and diphenyl diselenide treatment on oxidative stress parameters (mean±SEM)

Brain regions	TBARS (nmol of MDA/g tissue)	H_2-DCF oxidation (nmol of DCF/g tissue)	SOD activity (units/g tissue)
Striatum			
Control	15.03±0.49	17.18±2.27	765.6±61.2
Diselenide	16.74±4.99	20.44±2.73	766.4±88.8
Fluphenazine	14.66±1.36	14.58±1.52	789.6±92.4
Dis+Flu−VCM	15.82±5.00	16.58±2.39	948.4±107.2*
Dis+Flu+VCM	13.86±0.28	16.05±2.22	1056±54.4*
Substantia nigra			
Control	15.44±1.05	21.66±2.92	756±100
Diselenide	17.44±1.27	22.69±3.69	816.8±120
Fluphenazine	14.92±1.20	20.07±1.77	478.2±86.4
Dis+Flu−VCM	17.09±2.60	23.23±6.55	595.2±76.8
Dis+Flu+VCM	12.87±0.64	16.07±2.41	618±78

*Represents differences from control group

example, an intrastriatal antisense treatment against dopamine D_{1A} receptor was capable of reducing chronic fluphenazine-induced VCMs in rats (Van Kampen and Stoessl 2000).

Dopamine is a neurotransmitter involved in several conditions (Mehler-Wex et al. 2006; Miczek et al. 2002; See 1993; van Erp and Miczek 2007). Besides the major regulator of dopamine neurotransmission seems to be its reuptake from the synaptic cleft by dopamine transporters (Horn 1990; Iversen 1971), dopamine uptake modulation has not been systematically studied in animal models of TD. In this study, we have found for the first time that the chronic treatment of rats with fluphenazine reduces dopamine uptake in striatal slices. However, the reduction in dopamine uptake seems not to be involved in VCM increase produced by acute treatment with fluphenazine in rats. These results imply that chronic treatment with fluphenazine could be causing an overflow of dopamine into the synaptic cleft of extrapyramidal dopaminergic neurons by inhibiting high-affinity dopamine uptake, which may be one of the possible mechanisms of typical neuroleptic-induced TD. Our results are in accordance with literature data that have demonstrated that long-term fluphenazine treatment increases dopamine levels in the nucleus accumbens and brainstem (Jackson-Lewis et al. 1991). Moreover, our findings may explain, at least in part, why typical neuroleptic drugs (including fluphenazine) can cause an increase in dopamine turnover (See 1993), as catecholamine turnover is minimized by the high efficiencies of neuronal reuptake (Iversen 1971; Horn 1990).

Several factors might explain the reduction in dopamine uptake in the striatum of rats presenting VCMs, including altered dopamine transport function and neurodegeneration of dopamine uptake cells. Interestingly, the chronic treatment of rats with fluphenazine (for 8 months) presented a significantly lower density of the large neurons in the central part of the striatum (Jeste et al. 1992). In addition, it has been shown that some neuroleptics can directly interact with and inhibit the dopamine transporter (Lee et al. 1997). One study has demonstrated that a high concentration of fluphenazine can reduce dopamine uptake by rat striatal synaptosomes in vitro (Westfall et al. 1976). In addition to these putative mechanisms, literature data have shown that oxidative stress decreases the activity of dopamine transporters (Huang et al. 2003; Hashimoto et al. 2004). In view of the antioxidant property of diphenyl diselenide, we have also investigated the role of oxidative stress in fluphenazine-induced VCMs.

Oxidative stress has been proposed as an important pathogenetic mechanism in TD. It is suggested that the increased dopamine turnover caused by neuroleptics stimulates the production of reactive substances (such as hydrogen peroxide and dopamine quinones) as products of dopamine metabolism by monoamine oxidase activity (Andreassen and Jorgensen 2000). However, there are many contradictory results in studies that investigate oxidative stress and TD. In fact, the long-term treatment of TD with antioxidant vitamin E had limited success (Adler et al. 1999; Egan et al. 1992; Lohr et al. 1987). In addition, vitamin E treatment produced contradictory results in parameters of OD caused by chronic treatment with fluphenazine in rats (Lohr et al. 2000; Sachdev et al. 1999). We have found that the treatment with fluphenazine or diphenyl diselenide did not alter the levels of lipid peroxidation when compared with the control group of animals. This result contrasts with an early clinical study that demonstrated an increase in cerebrospinal fluid (CSF) lipid peroxidation in patients treated with phenothiazine drugs (Pall et al. 1987). However, other clinical studies did not find any differences in CSF or blood lipid peroxidation of dyskinetic patients when compared to healthy or schizophrenic controls (Brown et al. 1998; Tsai et al. 1998).

Regarding ROS production, we did not detect any significant difference in DCFH-DA oxidation in the striatum and substantia nigra of the rats treated only with fluphenazine. It has been shown that the acute (3–4 weeks) treatment with haloperidol caused an increase in VCMs and in striatal oxidative stress parameters in rats (Naidu et al. 2003; Burger et al. 2005a). The discrepancy between these results and our own may be explained by the differing capacities of neuroleptic drugs to cause oxidative stress. In fact, haloperidol seems to possess a pro-oxidant effect, increasing lipid peroxidation in vitro (Jeding et al. 1995). This pro-oxidant effect can be indirectly mediated by its toxic metabolites, which can cause oxidative stress by a direct mitochondrial dysfunction (Wright et al. 1998; Avent et al. 1996). Preliminary experiments carried out by our group have shown that fluphenazine per se did not produce pro-oxidant or antioxidant effect in vitro (data not shown). Moreover, another important point to consider is the relationship between the duration of oxidative stress and parameters of OD. In fact, Shivakumar and Ravindratnath (1993) have shown that the treatment with haloperidol induced oxidative stress as late as a month after the administration, an event that is generally no longer observed after 3 months of treatment in mice. Furthermore, we have detected increased parameters of OD but not oxidative stress in several brain regions 7 months after haloperidol treatment in rats feeding on a normal diet (Fachinetto et al. 2005). Interestingly, we did not find alterations in lipid peroxidation, ROS levels, or SOD activity in the striatum of rats at a week after fluphenazine treatment (unpublished results). It has been reported that the effectiveness of antioxidants is reduced in cases of long-standing TD (Egan et al. 1992; Dabiri et al. 1994) suggesting their clinical use for the prevention of TD but

not for the treatment of established dyskinesia. Thus, oxidative stress seems to be related with the development of haloperidol-induced behavioral related to OD but possibly not with the maintenance of TD. If the initial dysfunction in the nigrostriatal system occurs because of an early increase in oxidative stress in fluphenazine-treated animals, we were unable to demonstrate this.

Thus, there must be mechanisms other than oxidative stress involved in TD development. Enhancement of glutamatergic transmission caused by a blockade of presynaptic dopamine receptors seems to participate in the genesis of TD (Tsai et al. 1998). Of note, diphenyl diselenide is able to inhibit glutamate receptor binding in rat brain membranes (Nogueira et al. 2001).

Taken together, our data suggest a mechanism involving the reduction in dopamine transport related with the maintenance of chronic VCMs in rats. Its mechanism of action probably involves neuroprotection via modulation in the levels of dopamine in the synaptic cleft and antiglutamatergic properties. Considering that the development of new drugs are of crucial importance for the treatment of the side effects of neuroleptics, we hope that our paper may contribute to further investigations about mechanisms underlying the development of TD and its possible prevention or protection.

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References

Abílio VC, Silva RH, Carvalho RC, Grassl C, Calzavara MB, Registro S, D'Almeida V, Ribeiro RA, Frussa-Filho R (2004) Important role of striatal catalase in aging- and reserpine-induced oral dyskinesia. Neuropharmacology 47:263–272

Adler LA, Rotrosen J, Edson R, Lavori P, Lohr J, Hitzemann R, Raisch D, Caliguri M, Tracy K (1999) Vitamin E treatment for tardive dyskinesia. Arch Gen Psychiatry 56:836–841

Andreassen OA, Jorgensen HA (2000) Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats. Implications for tardive dyskinesia? Prog Neurobiol 61:525–541

Andreassen OA, Ferrante RJ, Aamo TO, Beal MF, Jorgensen HA (2003) Oral dyskinesias and histopathological alterations in substantia nigra after long-term haloperidol treatment of old rats. Neuroscience 122:717–725

Avent KM, Usuki E, Eyles DW, Keeve R, Van der Schyf CJ, Castagnoli N Jr, Pond SM (1996) Haloperidol and its tetrahydropyridine derivative (HPTP) are metabolized to potentially neurotoxic pyridinium species in the baboon. Life Sci 59:1473–1482

Borges LP, Borges VC, Moro AV, Nogueira CW, Rocha JB, Zeni G (2005) Protective effect of diphenyl diselenide on acute liver damage induced by 2-nitropropane in rats. Toxicology 210:1–8

Boyson SJ, McGonigle P, Luthin GR, Wolfe BB, Molinoff PB (1988) Effects of chronic administration of neuroleptic and anticholinergic agents on densities of D2 dopamine and muscarinic cholinergic receptors in rat striatum. J Pharmacol Exp Ther 244:987–993

Brandão R, Lara FS, Pagliosa LB, Soares FA, Rocha JB, Nogueira CW, Farina M (2005) Hemolytic effects of sodium selenite and mercuric chloride in human blood. Drug Chem Toxicol 28:397–407

Brenneisen P, Steinbrenner H, Sies H (2005) Selenium, oxidative stress, and health aspects. Mol Aspects Med 26:256–267

Brown K, Reid A, White T, Henderson T, Hukin S, Johnstone C, Glen A (1998) Vitamin E, lipids, and lipid peroxidation products in tardive dyskinesia. Biol Psychiatry 43:863–867

Burger ME, Fachinetto R, Calegari L, Paixão MW, Braga AL, Rocha JBT (2004) Effects on age on orofacial dyskinesia reserpine-induced and possible protection of diphenyl-diselenide. Brain Res Bull 64:339–345

Burger ME, Fachinetto R, Zeni G, Rocha JBT (2005a) Ebselen attenuates haloperidol-induced orofacial dyskinesia and oxidative stress in rat brain. Pharmacol Biochem Behav 81:608–615

Burger ME, Fachinetto R, Alves A, Calegari L, Rocha JBT (2005b) Acute reserpine and subchronic haloperidol treatments change synaptosomal brain glutamate uptake and elicit orofacial dyskinesia in rats. Brain Res 1031:202–210

Burger MB, Fachinetto R, Wagner C, Perottoni J, Pereira RP, Zeni G, Rocha JBT (2006) Effects of diphenyl-diselenide on orofacial dyskinesia model in rats. Brain Res Bull 70:165–170

Burt D, Creese I, Snyder SH (1977) Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 196:326–327

Cadet JL, Lohr JB, Jeste DV (1986) Free radical and tardive dyskinesia. Trends Neurosci 9:107–108

Cadet JL, Lohr JB, Jeste DV (1987) Tardive dyskinesia and Schizophrenic burnout: the possible involvement of cytotoxic free radicals. In: Henn FA, DeLisi LE (eds) Handbook of schizophrenia: the neurochemistry and pharmacology of schizophrenia. Elsevier, Amsterdam, pp 425–438

Casey DE (1985) Tardive dyskinesia: reversible and irreversible. Psychopharmacology 2:88–97

Chang LW (1983) Protective effects of selenium against methylmercury neurotoxicity: a morphological and biochemical study. Exp Pathol 23:143–156

Commandeur JNM, Rooseboom M, Vermeulen NPE (2001) Chemistry and biological activity of novel selenium-containing compounds. Biological reactive intermediates VI. Adv Exp Med Biol 500:105

Crane GE (1973) Persistent dyskinesia. Br J Psychiatry 122:395–405

Dabiri LM, Pasta D, Darby JK, Mosbacher D (1994) Effectiveness of vitamin E for treatment of long-term tardive dyskinesia. Am J Psychiatry 151:925–926

Ebadi M, Srinivasan SK (1995) Pathogenesis, prevention and treatment of neuroleptic-induced movement disorders. Pharmacol Rev 47:575–604

Egan MF, Hyde TM, Albers GW, Elkashef A, Alexander RC, Reeve A, Blum A, Saenz RE, Wyatt RJ (1992) Treatment of tardive dyskinesia with vitamin E. Am J Psychiatry 149:773–777

Egan MF, Hurd Y, Hyde TM, Weinberger DR, Wyatt RJ, Kleinman JE (1994) Alterations in mRNA levels of D2 receptors and neuropeptides in striatonigral and striatopallidal neurons of rats with neuroleptic-induced dyskinesias. Synapse 18:178–189

Egan MF, Hurd Y, Ferguson J, Bachus SE, Hamid EH, Hyde TM (1996) Pharmacological and neurochemical differences between acute and tardive vacuous chewing movements induced by haloperidol. Psychopharmacology 127:337–345

Fachinetto R, Burger MB, Wagner C, Wondracek DC, Brito VB, Nogueira CW, Ferreira J, Rocha JBT (2005) High fat diet increases the incidence of orofacial dyskinesia and oxidative stress in specific brain regions of rats. Pharmacol Biochem Behav 81:585–592

Faria RR, Abilio VC, Grassl C, Chinen CC, Negrao LT, de Castro JP, Fukushiro DF, Rodrigues MS, Gomes PH, Registro S, de Carvalho RC, D'Almeida V, Silva RH, Ribeiro RA, Frussa-Filho R (2005) Beneficial effects of vitamin C and vitamin E on reserpine-induced oral dyskinesia in rats: critical role of striatal catalase activity. Neuropharmacology 48:993–1001

Gerlach J, Casey D (1988) Tardive dyskinesia. Acta Psychiatr Scand 77:369–378

Gunne L, Häggström J, Sjöquist B (1984) Association with persistent neuroleptic-induced dyskinesia of regional changes in brain GABA synthesis. Nature 309:347–349

Hashimoto K, Tsukada H, Nishiyama S, Fukumoto D, Kakiuchi T, Shimizu E, Iyo M (2004) Protective effects of N-acetyl-L-cysteine on the reduction of dopamine transporters in the striatum of monkeys treated with methamphetamine. Neuropsychopharmacology 29:2018–2023

Holz RW, Coyle JT (1974) The effects of various salts, temperature and the alkaloids veratridine and batrachotoxin on the uptake of ^{3}H-dopamine into synaptosomes from rat striatum. Mol Pharmacol 10:746–758

Horn AS (1990) Dopamine uptake: a review of progress in the last decade. Prog Neurobiol 34:387–400

Huang CL, Huang NK, Shyue SK, Chern Y (2003) Hydrogen peroxide induces loss of dopamine transporter activity: a calcium-dependent oxidative mechanism. J Neurochem 86: 1247–1259

Iversen LL (1971) Role of transmitter uptake mechanisms in synaptic neurotransmission. Br J Pharmacol 41:571–591

Jackson-Lewis V, Przedborski S, Kostic V, Suber F, Fahn S, Cadet JL (1991) Partial attenuation of chronic fluphenazine-induced changes in regional monoamine metabolism by D-alpha-tocopherol in rat brain. Brain Res Bull 26:251–258

Jeding I, Evans PJ, Akanmu D, Dexter D, Spencer JD, Aruoma OI, Jenner P, Halliwell B (1995) Characterization of the potential antioxidant and pro-oxidant actions of some neuroleptic drugs. Biochem Pharmacol 49:359–365

Jenner P, Marsden CD (1983) Neuroleptics and tardive dyskinesia. In: Coyle JT, Enna SJ (eds) Neuroleptics: neurochemical, behavioral and, clinical perspectives. Raven Press, New York, pp 223–253

Jeste DV, Potkin SG, Sinhá S, Feder S, Wyatt RJ (1979) Tardive dyskinesia—reversible and persistent. Arch Gen Psychiatry 36:585–590

Jeste DV, Lohr JB, Manley M (1992) Study of neuropathologic changes in the striatum following 4, 8 and 12 months of treatment with fluphenazine in rats. Psychopharmacology 106:154–160

Kane JM (1995) Tardive dyskinesia: epidemiology and clinical presentation. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology: the fourth generation of progress, vol. 39. Raven Press, New York 1485–1495

Kane JM, Smith JM (1982) Tardive dyskinesia: prevalence and risk factors, 1959 to 1979. Arch Gen Psychiatry 39:473–481

Klotz LO, Kroncke KD, Buchczyk DP, Sies H (2003) Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. J Nutr 133:1448

Lee S, Oh D, Jung S, Kim Y, Cho H, Koh J, Lee Y (1997) Neuroleptic drugs alter the dopamine transporter-mediated uptake and release of dopamine: a possible mechanism for drug-induced tardive dyskinesia. Pharmacol Res 35:446–450

Lohr JB, Cadet JL, Lohr MA, Jeste DV, Wyatt RJ (1987) Alpha-tocopherol in tardive dyskinesia. Lancet 1:913–934

Lohr JB, Kuczenski R, Bracha HS, Moir M, Jeste DV (1990) Increased indices of free radical activity in the cerebrospinal fluid of patients with tardive dyskinesia. Biol Psychiatry 28:535–539

Lohr JB, Caligiuri MP, Manley MS, Browning JA (2000) Neuroleptic-induced striatal damage in rats: a study of antioxidant treatment using accelerometric and immunocytochemical methods. Psychopharmacology 148:171–179

Lohr JB, Kuczenski R, Niculescu AB (2003) Oxidative mechanisms and tardive dyskinesia. CNS Drugs 17:47–62

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265

Mehler-Wex C, Riederer P, Gerlach M (2006) Dopaminergic dysbalance in distinct basal ganglia neurocircuits: implications for the pathophysiology of Parkinson's disease, schizophrenia and attention deficit hyperactivity disorder. Neurotox Res 10:167–179

Miczek KA, Fish EW, De Bold JF, De Almeida RM (2002) Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and gamma-aminobutyric acid systems. Psychopharmacology 163:434–458

Misra HP, 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

Naidu PS, Singh A, Kaur P, Kulkarni SK (2003) Quercetin, a bioflavonoid, attenuates haloperidol-induced orofacial dyskinesia. Neuropharmacology 44:1100–1106

Nogueira CW, Rotta LN, Perry ML, Souza DO, Rocha JBT (2001) Diphenyl diselenide and diphenyl ditelluride affect the rat glutamatergic system in vitro and in vivo. Brain Res 906:157–163

Nogueira CW, Meotti FC, Curte E, Pilissão C, Zeni G, Rocha JBT (2003) Investigations into the potencial neurotoxicity induced by diselenides in mice and rats. Toxicology 183:29–37

Nogueira CW, Zeni G, Rocha JBT (2004) Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem Rev 104:6255–6286

Ohkawa H, Ohishi H, Yagi K (1979) Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358

Pall HS, Williams AC, Blake DR, Lunec J (1987) Evidence of enhanced lipid peroxidation in the cerebrospinal fluid of patients taking phenothiazines. Lancet 2:596–599

Palmier C (1986) Selenium reagents and intermediates. In: Organic synthesis. Pergamon, Oxford

Pérez-Severiano P, Rodríguez-Pérez M, Pedraza-Chaverrí J, Maldonado PD, Medina-Campos ON, Ortíz-Plata A, Sánchez-García A, Villeda-Hernández J, Galván-Arzate S, Aguilera P, Santamaría A (2004) *S*-Allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats. Neurochem Int 45:1175–1183

Post A, Rucker M, Ohl F, Uhr M, Holsboer F, Almeida OFX, Michaelidis TM (2002) Mechanisms underlying the protective potential of alpha-tocopherol (vitamin E) against haloperidol-associated neurotoxicity. Neuropsychopharmacology 26:397–407

Sachdev P, Saharov T, Cathcart S (1999) The preventative role of antioxidants (selegiline and vitamin E) in a rat model of tardive dyskinesia. Biol Psychiatry 46:1672–1681

Sadan O, Bahat-Stromza M, Gilgun-Sherki Y, Atlas D, Melamed E, Offen D (2005) A novel brain-targeted antioxidant (AD4) attenuates haloperidol-induced abnormal movement in rats: implications for tardive dyskinesia. Clin Neuropharmacol 28:285–288

Santos FW, Zeni G, Rocha JB, Weis SN, Fachinetto JM, Favero AM, Nogueira CW (2005) Diphenyl diselenide reverses cadmium-induced oxidative damage on mice tissues. Chem Biol Interact 151:159–165

See RE (1993) Assessment of striatal extracellular dopamine and dopamine metabolites by microdialysis in haloperidol-treated rats exhibiting oral dyskinesia. Neuropsychopharmacology 9:101–109

Shirakawa O, Tamminga CA (1994) Basal ganglia $GABA_A$ and dopamine D_1 binding site correlates of haloperidol-induced oral dyskinesias in rat. Exp Neurol 127:62–69

Shivakumar BR, Ravindratnath V (1993) Oxidative stress and thiol modification induced by chronic administration of haloperidol. J Pharmacol Exp Ther 265:1137–1141

Steinbrenner H, Alili L, Bilgic E, Sies H, Brenneisen P (2006) Involvement of selenoprotein P in protection of human astrocytes from oxidative damage. Free Radic Biol Med 40:1513–1523

Tsai G, Goff DC, Chang RW, Flood J, Baer L, Coyle JT (1998) Markers of glutamatergic neurotransmission and oxidative stress associated with tardive dyskinesia. Am J Psychiatry 155:1207–1213

van Erp AM, Miczek KA (2007) Increased accumbal dopamine during daily alcohol consumption and subsequent aggressive behavior in rats. Psychopharmacology 191:679–688

Van Kampen JM, Stoessl AJ (2000) Dopamine D_{1A} receptor function in a rodent model of tardive dyskinesia. Neuroscience 101:629–635

Westfall TC, Besson M, Giorguieff M, Glowinski J (1976) The role of presynaptic receptors in the release and synthesis of ^{3}H-dopamine by slices of rat striatum. Arch Pharmacol 292:279–287

Woerner MG, Kane JM, Lieberman JA, Alvir J, Bergmann KJ, Borenstein M, Schooler NR, Mukherjee S, Rotrosen J, Rubinstein M et al (1991) The prevalence of tardive dyskinesia. J Clin Psychopharmacol 11:34–42

Wright AM, Bempong J, Kirby ML, Barlow RL, Bloomquist JR (1998) Effects of haloperidol metabolites on neurotransmitter uptake and release: possible role in neurotoxicity and tardive dyskinesia. Brain Res 788:215–222

Zafar KS, Siddiqui A, Sayeed I, Ahmad M, Salim S, Islam F (2003) Dose-dependent protective effect of selenium in rat model of Parkinson's disease: neurobehavioral and neurochemical evidences. J Neurochem 84:438–446

3.2 – EFEITO DA ADMINISTRAÇÃO AGUDA E CRÔNICA COM FLUFENAZINA NOS NÍVEIS DE MONOAMINAS E SEUS METABÓLITOS EM RATOS: RELAÇÃO COM A DISCINESIA OROFACIAL

Manuscrito em preparação 1
(dados preliminares)

EFFECT OF ACUTE AND CHRONIC TREATMENT WITH FLUPHENAZINE ON MONOAMINES AND THEIR METABOLITES IN RATS: RELATIONSHIP WITH OROFACIAL DYSKINESIA

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Abstract

TD is a serious side effect caused by long-term treatment with neuroleptic drugs. Despite of innumerous studies concerning about the pathophysiology of TD, this syndrome is not elucidated. Thus, the aim of the present study was to investigate the participation of biogenic amines on acute and chronic model of orofacial dyskinesia induced by fluphenazine in rats. Adult male rats were treated during 3 or 24 weeks with fluphenazine (25 mg/kg, i.m., once every 21 days) and vehicle (1 mL/kg, i.m., once every 21 days). The vacuous chewing movements (VCMs) or the levels of monoamines and its metabolites were quantified after 3 (acute) or 24 (chronic) weeks after beginning of treatment. The fluphenazine treatment produced VCMs in part of treated rats (50% after 3 weeks and about 85% after 24 weeks). Thus, we separated the rats that developed (+VCM) or did not develop (-VCM) VCMs. There were not significant differences between the groups in biogenic amines levels neither in their metabolites in the striatum under acute fluphenazine treatment in +VCMs rats. However, we observed a trend to increase the levels of the DA metabolites, HVA ($p=0.054$) and DOPAC ($p=0.063$) after chronic treatment with fluphenazine. Our data suggest that an increase in dopamine metabolism could contribute to the maintenance of VCMs in rats. Moreover, development of VCMs in seems not to be dependent metabolites.

Key words: tardive dyskinesia, orofacial dyskinesia, dopamine, HVA, DOPAC, fluphenazine.

INTRODUCTION

The use of classical neuroleptics is the most effective treatment for schizophrenia. Fluphenazine is potent phenothiazine antipsychotic used in the treatment of schizophrenic patients. Its pharmacological action involves the blockage with high affinity of dopamine D_1 and D_2 receptors (Morgan and Finch, 1986). However, tardive dyskinesia (TD) continues to be one of the main side effects occurring in schizophrenic patients under chronic treatment with neuroleptics. TD is characterized by involuntary movements of the orofacial region and, sometimes, musculature of the members and trunk, known as tardive dyskinesia (TD) (Kane 1995).

Although some hypotheses have been postulated to explain at molecular and cellular levels of this syndrome, the exact mechanisms involved in TD development remain unclear. Literature data have demonstrated that abnormalities in various neurotransmitter systems have been implicated in the pathophysiology of TD, especially dopaminergic, serotonergic and noradrenergic systems (Andreassen and Jorgensen, 2000; Boyson et al. 1988; Burt et al. 1977; Kulkarni and Naidu, 2001; Morgan and Finch, 1986).

It was described changes in the noradrenergic and serotonergic function in patients with TD and in animal models of orofacial dyskinesia (OD) (Ohmori et al., 2003; Kaufmann et al., 1986; Naidu et al., 2001; Segman et al., 2003). However, there are some contradictions mainly to the noradrenergic hypothesis (Glazer et al., 1987).

Another hypothesis is that there may be functional excess in the activity of dopamine as a synaptic neurotransmitter in the central nervous system (Klawans and Rubovits, 1972; Burt et al., 1977; Rubinstein et al., 1990). Moreover, TD has been related to dopamine receptor hypersensitivity, which is induced by chronic receptor blockade by treatment with neuroleptic drugs (Cavallero and Smeraldi, 1995; Kane, 1995; Woerner et al., 1998). Since dopamine receptor hypersensitivity hypothesis has limitations (Lohr and Jeste, 1988; Sachdev, 1999), other dopamine neurotransmission disturbances could to be important to TD development.

Of particular importance, we have recently demonstrated the reduction of dopamine uptake in animals with OD (assessed by vacuous chewing movements) induced by chronic treatment, but not by acute treatment, with fluphenazine or haloperidol (Fachinetto et al., 2007a; 2007b). These results imply that chronic treatment with fluphenazine could be

causing an overflow of dopamine into the synaptic cleft of extrapyramidal dopaminergic neurons by inhibiting high-affinity dopamine uptake, which may be one of the possible mechanisms of typical neuroleptic-induced TD. In fact, it has been demonstrated that long-term fluphenazine treatment increases dopamine levels in the *nucleus accumbens* and brainstem (Jackson-Lewis et al. 1991). Moreover, it has been demonstrated that chronic treatment with haloperidol increases the levels of dopamine metabolites in striatum (See, 1993), an effect that might be related with dopamine uptake, since catecholamine turnover is minimized by a high efficient neuronal reuptake (Iversen, 1971; Horn, 1990). However, there are no studies showing the relationship of dopamine or other monoamines as well as their metabolism in the orofacial dyskinesia induced by fluphenazine. Thus, the aim of the present study was to investigate the role of monoamines and their metabolites on acute and chronic of orofacial dyskinesia induced by fluphenazine in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 270-320 g and with age from 3 to 3.5 months, from our own breeding colony were kept in cages of 3 or 4 animals each, with continuous access to food and water in a room with controlled temperature (22±3 °C) and on a 12-h light/dark cycle with lights on at 7:00 am. The animals were maintained and used in accordance to the guidelines of the Brazilian Association for Laboratory Animal Science (COBEA).

Drugs and treatments

Fluphenazine enantate (Flufenan®) was a gift from Cristália (Brazil). For acute experiment, rats were divided into two groups with 6 animals each. The control group received an administration of soy oil intramuscularly (1 ml/kg, i.m.) at first day of experiment. Another group received fluphenazine enantate (25 mg/kg, i.m.). Based in a previous study (Fachinetto et al, 2007b; Van Kampen and Stoessl 2000), behavioral testing was conduced 3 weeks after injection.

For chronic experiment, rats were also divided into two groups, control and fluphenazine group. Fluphenazine enantate was administered intramuscularly (i.m.) every 21 days (25 mg/Kg, i.m.). The control group received soy oil (1 mL/Kg) in the same way fluphenazine group. The treatment was carried out over the course of 6 months (24 weeks).

Behavioral Testing

Behavior was assessed before the treatment with vehicle or fluphenazine (basal evaluation). Animals that presented more than 40 vacuous chewing movements (VCMs, used by us as a parameter of OD) in basal evaluation were excluded of the study. Thus, the number of animals that received acute treatment was 6 to control and 6 to fluphenazine group. To chronic treatment, the number of animals was 7 to control group and 13 to fluphenazine group. The effect of acute treatment with fluphenazine on behavior was examined before the fluphenazine administration (basal evaluation) and on the 21st day after the fluphenazine injection. The effect of chronic treatment with fluphenazine on

behavior was examined, as in acute treatment, before the first administration of fluphenazine (basal evaluation) and after 6 months of treatment.

To quantify the occurrence of VCMs, rats were placed individually in cages (20x20x19 cm) and hand operated counters were employed to quantify VCMs frequency. VCMs are defined as single mouth openings in the vertical plane not directed towards physical material. If VCMs occurred during a period of grooming they were not taken into account. VCMs were measured continuously for 6 min after a period of 6 min adaptation. During the observation sessions, a mirror was placed under the floor of the experimental cage to permit observation when the animal was faced away from the observer. Experimenters were always blind with regard to the treatment conditions. In a preliminary study of interrater reliability, we found that the use of this method of observation and the definition of the parameter evaluated usually results in >93% agreement between 3 different observers for VCMs. The calculated α value was significant with a p value <0.05.

It was previously reported that the treatment with neuroleptic drugs does not result in the development of VCMs in all treated rats (Kane and Smith 1982; Shirakawa and Tamminga 1994; Fachinetto et al. 2007a, b). We have also verified the prevalence of neuroleptic-induced VCMs. In our laboratory, control rats present maximally 40 VCMs during a period of 6 min. Thus, in this study, we analyzed the rats that developed neuroleptic-induced VCMs (+VCM, more than 40 VCMs) separately from those that did not develop neuroleptic-induced VCMs (-VCMs, less than 40 VCMs), as previously described by different groups (Andreassen et al. 2003; Egan et al. 1994; Shirakawa and Tamminga, 1994; Fachinetto et al., 2007a,b). In this paper, we have showed the results referents of animals treated with fluphenazine that developed more than 40 VCM (+VCM). Thus, the final number of animal per group was: to acute treatment, control group with 6 animals and +VCM group with 3 animals; to chronic treatment, control group with 7 animals and +VCM group with 11 animals.

Tissue preparations and monoamines and metabolites estimation

Rats were killed about 24 hours after the last session of behavioral quantification (on the 21st day after the last administration of fluphenazine). The brains were excised and put on ice. The striatum was immediately separated and stored at -80°C for HPLC analysis.

The endogenous levels of biogenic amines dopamine (DA), serotonin (5-HT) and norepinephrine (NA) and its metabolites (HVA, DOPAC, 5-HIAA and DHPG) were estimated by HPLC with electrochemical detection, as described by Ferraz et al., (2002).

Statistical Analysis

Data were analyzed by One-way ANOVA (VCM intensity), unpaired test t (monoamine levels, monoamine metabolites levels and monoamines turnover). Prevalence of OD was analyzed by Chi-Square test. Linear correlation analysis was used to examine the correlation between two independent factors. Significance was considered when $p < 0.05$.

RESULTS

Acute and chronic effects of fluphenazine on VCM

Acute (3 weeks) treatment with fluphenazine caused a 50% prevalence of animals presenting more than 40 VCMs (+VCM). (Chi-square = 2.5 and p=0.11) With regard to the intensity of VCMs induced by acute treatment with fluphenazine, it was observed a marked increase on VCMs after three weeks of treatment when compared with its vehicle ($p<0.05$; Figure 1A). These results are similar to that previously published by our group (Fachinetto et al., 2007b).

Chronic treatment (24 weeks) with fluphenazine induced a high prevalence of VCMs in rats, with 84.5% of treated animals presenting more than 40 VCMs compared with its vehicle (Chi-square = 13.16 and $p<0.001$). Moreover, chronic treatment (24 weeks) with fluphenazine also caused a marked increase in the intensity of VCMs when compared with its vehicle ($p<0.05$; Figure 1B).

Effects of acute and chronic treatment with fluphenazine on DA, NA, 5-HT and their metabolites

There were not statistically significant differences among the groups in biogenic amines levels neither in its metabolites in the striatum of rats under acute and chronic fluphenazine treatment and +VCMs (Table 1 and 2). In according with this, we did not find differences in the turnover of DA, 5-HT and NA metabolites in acute or chronic treatment (Table 2). However, we observed a trend to increase the levels of NA ($p=0.06$; Table 1) and to negatively correlate DA levels and VCM number ($R=0.53$ and $p=0.07$; Figure 1) that did not reach statistical significance. Moreover, we observed a trend to increase the levels of DA metabolites, HVA ($p=0.054$) and DOPAC ($p=0.06$), and the turnover of DA metabolites (HVA/DA: $p=0.08$) after chronic treatment with fluphenazine (Table 1). We have also observed a positive correlation between HVA and VCM ($R=0.46$ and $p=0.05$), and also a trend to correlate DOPAC and VCM ($R=0.42$ and $p=0.08$).

DISCUSSION

Despite of innumerous studies concerning about the etiology of the exact mechanism responsible by this syndrome is still unknown. TD is a serious side effect caused by long-term treatment with neuroleptic drugs, particularly due to its high prevalence and the lack of effective treatment. Our current study shows that acute and chronic fluphenazine treatment caused a significant increase in VCMs. The levels of biogenic amines and its turnover seem not to be significantly altered in acute or chronic treatment. However, we have detected some trends ($p<0.10$ to $p>0.05$) to find differences between vehicle and fluphenazine groups, but they did not reach statistically significant levels ($p<0.05$). As the number of animals in some groups is low (3 animals for acute fluphenazine group) and the number of animals in the groups is quite different (i.e. 7 animals in control and 11 animals in chronic fluphenazine group), we must correct these faults to confirm the exact role of monoamines and their metabolites in dyskinesia. However, our preliminary results allow us to trace some previous speculation about our hypothesis.

Long-term treatment with neuroleptic drugs is capable of producing OD in rats and TD in humans. In the present study, we found a prevalence of 50% and 85%, respectively to acute and long-term treatment with fluphenazine in rats. The intensity of VCMs in both, acute and chronic, treatment with fluphenazine was significantly different from control groups. These results are in accordance with previously published work of our group (Fachinetto et al., 2007) where we found similar values to prevalence of OD in rats.

The major regulator of dopamine neurotransmission seems to be its reuptake from the synaptic cleft by DAT (Horn, 1990; Iversen, 1971). DAT are found exclusively in dopaminergic neurons, and are densely present in the striatum (Amara and Kuhar, 1993; Giros and Caron, 1993). In our previous findings, we have not detected a reduction in dopamine uptake in striatum of rats treated acutely with fluphenazine (Fachinetto et al., 2007b). In accordance with our idea that dopamine uptake could alter its metabolism, we were not able to detect any significant alteration in the striatum levels of dopamine, its metabolites and turnover after acute treatment with fluphenazine in the present study. On the other hand, we have detected a trend to negatively correlate dopamine levels with VCM number in rats acutely treated with fluphenazine, indicating that reduced dopamine levels in

striatum might increase the intensity of orofacial dyskinesia. Of note, it has been described that reduction in dopamine leads to a cholinergic neuron imbalance that could be responsible for the development of extrapiramidal syndrome, including the parkinsonism (Neale et al., 1984; Rupniak et al., 1986; for review, see Salamone et al., 1998). This finding is in line with the idea that acute neuroleptic treatment produces a parkinsonim-like syndrome (for review see Salamone at al., 1998).

Different from acute treatment, chronic administration with fluphenazine is able to reduce dopamine uptake in striatum of rats with high VCM number. These results imply that chronic treatment with fluphenazine could increase the level of dopamine into the synaptic cleft of extrapyramidal dopaminergic neurons, which may be one of the possible mechanisms of neuroleptic-induced TD. In fact, it has been demonstrated that long-term fluphenazine treatment increases dopamine levels in the *nucleus accumbens* and brainstem (Jackson-Lewis et al. 1991). Moreover, it has been demonstrated that chronic treatment with haloperidol increases the levels of dopamine metabolites in striatum (See, 1993). In the present study, we were not able to demonstrate an increase in the levels of DA in the striatum of chronically treated rats, but we have found a trend to increase the DA metabolites, HVA and DOPAC, in rats presenting high levels of VCMs and to positively correlate dopamine metabolites with VCM intensity. Thus, our findings suggest that there is an increase in dopamine metabolism and turnover after chronic neuroleptic treatment. This increase in dopamine metabolites observed in animal models of orofacial dyskinesia is also observed in cerebrospinal fluid of patients presenting tardive dyskinesia (Hsiao et al., 1993). Besides the alteration in the function of dopamine transporter, possibly dopamine-regulating enzymes that are involved in its synthesis and degradation could also be related with tardive dyskinesia.

Taken together, our data confirm the prevalence of VCMs in rats taking acute and chronic treatment with fluphenazine previously published by our group. Moreover, the results suggest a mechanism involving an increase in dopamine metabolites related with the maintenance of chronic VCMs in rats.

REFERENCES

Amara SG, Kuhar MJ (1993) Neurotransmitter transporters: recent progress. Annu Rev Neurosci. 16:73–93.

Andreassen OA and Jorgensen HA (2000) Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats. Implications for tardive dyskinesia? Prog Neurobiol. 61: 525-541.

Andreassen OA, Ferrante RJ, Aamo TO, Beal MF, Jorgensen HA (2003) Oral dyskinesias and histopathological alterations in substantia nigra after long-term haloperidol treatment of old rats. Neuroscience 122: 717-725.

Boyson SJ, McGonigle P, Luthin GR, Wolfe BB, Molinoff PB (1988) Effects of chronic administration of neuroleptic and anticholinergic agents on densities of D2 dopamine and muscarinic cholinergic receptors in rat striatum. J Pharmacol Exp Ther. 244:987-993.

Burt D, Creese I, Snyder SH (1977) Antischizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. Science 196: 326-327.

Cavallero R, Smeraldi E, (1995). Antipsychotic-induced tardive dyskinesia: recognition, prevention and management. CNS Drugs 4: 278-293.

Egan MF, Hurd Y, Hyde TM, Weinberger DR, Wyatt RJ, Kleinman JE (1994) Alterations in mRNA levels of D2 receptors and neuropeptides in striatonigral and striatopallidal neurons of rats with neuroleptic-induced dyskinesias. Synapse 18: 178-189.

Fachinetto R, Villarinho JG, Wagner C, Pereira RP, Avila DS, Burger ME, Calixto JB, Rocha JB, Ferreira J (2007a) Valeriana officinalis does not alter the orofacial dyskinesia induced by haloperidol in rats: role of dopamine transporter. Prog Neuropsychopharmacol Biol Psychiatry. 31:1478-1486.

Fachinetto R, Villarinho JG, Wagner C, Pereira RP, Puntel RL, Paixão MW, Braga AL, Calixto JB, Rocha JB, Ferreira J (2007b) Diphenyl diselenide decreases the prevalence of vacuous chewing movements induced by fluphenazine in rats. Psychopharmacology (Berl). 194:423-432.

Giros B, Caron MG (1993) Molecular characterization of the dopamine transporter. Trends Pharmachol Sci. 14:43-49.

Glazer WM, Charney DS, Heninger GR (1987) Noradrenergic function in schizophrenia. Arch Gen Psychiatry. 44:898-904.

Horn AS (1990) Dopamine uptake: a review of progress in the last decade. Prog Neurobiol 34: 387-400.

Hsiao JK, Colison J, Bartko JJ, Doran AR, Konicki PE, Potter WZ, Pickar D (1993) Monoamine neurotransmitter interactions in drug-free and neuroleptic-treated schizophrenics. Arch Gen Psychiatry 50:606-614.

Iversen LL (1971) Role of transmitter uptake mechanisms in synaptic neurotransmission. Br J Pharmacol 41: 571-591.

Jackson-Lewis V, Przedborski S, Kostic V, Suber F, Fahn S, Cadet JL (1991) Partial attenuation of chronic fluphenazine-induced changes in regional monoamine metabolism by D-alpha-tocopherol in rat brain. Brain Res Bull. 26: 251-258.

Kane JM and Smith JM (1982) Tardive dyskinesia: prevalence and risk factors, 1959 to 1979. Arch Gen Psychiatry 39: 473-481.

Kane JM (1995) Tardive dyskinesia: epidemiology and clinical presentation. In: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: The Fourth Generation of Progress, vol. 39. Raven Press, New York, pp. 1485-1495.

Kaufmann CA, Jeste DV, Shelton RC, Linnoila M, Kafka MS, Wyatt RJ (1986) Noradrenergic and neuroradiological abnormalities in tardive dyskinesia. Biol Psychiatry. 21:799-812.

Klawans HL Jr, Rubovits R (1972). An experimental model of tardive dyskinesia. J Neural Transm. 33:235-246.

Kulkarni SK, Naidu PS (2001) Tardive dyskinesia: An update. Drugs Today. 37:97.

Lohr JB, Jeste DV (1988) Neuroleptic-induced movement disorders: tardive dyskinesia and other tardive symptoms. In: Cavenar Jr, J.O. editor. Psychiatry. Philadelphia (PA): Lippincott, 1-17.

Morgan DG, Finch CE (1986) [^{3}H]Fluphenazine binding to brain membranes: simultaneous measurement of D-1 and D-2 receptor sites. J Neurochem. 46:1623-1631.

Naidu PS, Kulkarni SK (2001) Effect of 5-HT1A and 5-HT2A/2C receptor modulation on neuroleptic-induced vacuous chewing movements. Eur J Pharmacol. 428:81-86.

Neale R, Gerhardt S, Liebman JM (1984) Effects of dopamine agonists, catecholamine depletors, and cholinergic and GABAergic drugs on acute dyskinesias in squirrel monkeys. Psychopharmacology., 82:20-26.

Ohmori O, Shinkai T, Hori H, Nakamura J (2002) Genetic assiciation analysis of 5-HT(6) receptor gene polymorphism (267C/T) with tardive dyskinesia. Psychiatry Res. 110:97-102.

Rubinstein M, Muschietti JP, Gershanik O, Flawia MM, Stefano FJ (1990) Adaptative mechanisms of striatal D1 and D2 dopamine receptors in response to a prolonged reserpine treated mice. J Pharmacol Exp Ther. 252:810-816.

Rupniak NM, Briggs RS, Petersen MM, Mann S, Reavill C, Jenner P, Marsden CD (1986) Differential alterations in striatal acetylcholine function in rats during 12 months' continuous administration of haloperidol, sulpiride, or clozapine. Clin Neuropharmacol. 9:282-292.

Sachdev P, Saharov T, Cathcart S (1999) The preventative role of antioxidants (selegiline and vitamin E) in a rat model of tardive dyskinesia. Biol Psychiatry. 46: 1672-1681.

Salamone JD, Mayorga AJ, Trevitt JT, Cousins MS, Conlan A, Nawab A (1998) Tremulous jaw movements in rats: a model of parkinsonian tremor. Prog Neurobiol, 56:591-611.

See RE (1993) Assessment of striatal extracellular dopamine and dopamine metabolites by microdialysis in haloperidol-treated rats exhibiting oral dyskinesia. Neuropsychopharmacology 9: 101-109.

Segman RH, Goltser T, Heresco-Levy U, Finkel B, Shalem R, Schlafman M, Yakir A, Greenberg D, Strous R, Lerner A, Shelevoy A, Lerer B (2003) Association of dopaminergic and serotonergic genes with tardive dyskinesia in patients with chronic schizophrenia. Pharmacogenomics J. 3:277-283.

Shirakawa O, Tamminga CA (1994) Basal ganglia $GABA_A$ and dopamine D_1 binding site correlates of haloperidol-induced oral dyskinesias in rat. Exp Neurol. 127: 62-69.

Van Kampen JM and Stoessl AJ (2000) Dopamine D_{1A} receptor function in a rodent model of tardive dyskinesia. Neuroscience 101: 629-635.

Woerner MG, Kane JM, Lieberman JA, Alvir J, Bergmann KJ, Borenstein M, Schooler NR, Mukherjee S, Rotrosen J, Rubinstein M et al (1991). The prevalence of tardive dyskinesia. *J Clin Psychopharmacol*; 11:34-42.

LEGENDS FOR FIGURES

Figure 1: Effects of acute (A) and chronic (B) treatment with fluphenazine at dose of 25 mg/Kg or vehicle solution on intensity of vacuous chewing movements. Values are express as means ± S.E.M. * Represent significant differences from control group at same period of observation.

Figure 2: Linear regression analysis between dopamine levels and VCM+ developed by acute treatment with fluphenazine.

Figure 3: Linear regression analysis between HVA levels and VCM+ (A) and DOPAC levels and VCM+ (B) developed by chronic treatment with fluphenazine.

Table 1: Effects of acute and chronic treatment with fluphenazine on DA, NA, 5-HT and their metabolites. Values (ng/g) are express as means ± S.E.M.

Table 2: Effects of acute and chronic treatment with fluphenazine on DA, NA, 5-HT turnover. Values are express as means ± S.E.M.

Figure 1:

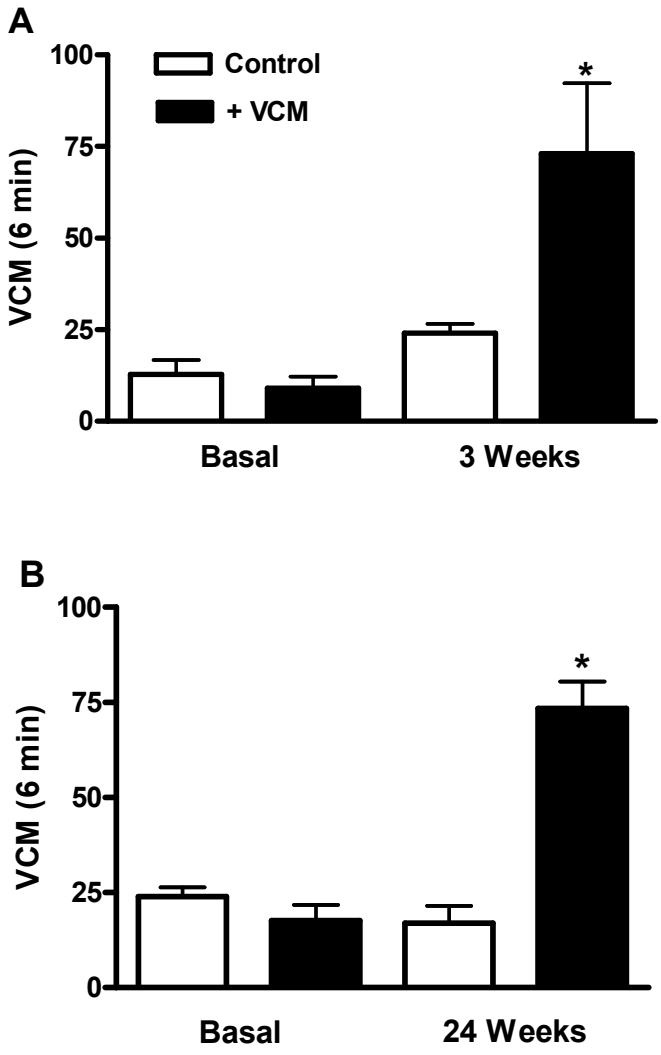

A
100
75
50
25
0
VCM (6 min)
Control
+ VCM
*
Basal
3 Weeks
B
100
75
50
25
0
VCM (6 min)
*
Basal
24 Weeks

Table 1:

	Acute		Chronic	
	Control	Fluphenazine (VCM+)	Control	Fluphenazine (VCM+)
DA	8640.78 ± 1112.19	6007.23 ± 966.25	600.638 ± 104.190	789.327 ± 167.34
HVA	904.95 ± 116.84	824.86 ± 137.09	322.39 ± 20.057	623.16 ± 113.68^{++}
DOPAC	2652.91 ± 242.18	2642.64 ± 357.61	1093.23 ± 141.91	2721.16 ± 637.15*
NA	91.06 ± 11.10	131.65 ± 11.89^{a}	137.13 ± 5.59	208.86 ± 44.85
DHPG	35.93 ± 6.8	33.91 ± 12.86	1053.2 ± 389.66	832.28 ± 129.68
5-HT	1030.80 ± 99.84	1018.66 ± 288.02	180.49 ± 28.13	282.02 ± 42.94
5-HIAA	1099.70 ± 44.63	1237.34 ± 57.50	570.06 ± 68.73	587.02 ± 102.72

*P=0.06, Chronic fluphenazine vs. control
$^{++}$P=0.05, Chronic fluphenazine vs. control
^{a}P=0.06, Acute fluphenazine vs. control

Table 2:

	Acute		Chronic	
	Control	Fluphenazine	Control	Fluphenazine
DOPAC/DA	0.365 ± 0.10	0.487 ± 0.146	2.00 ± 0.311	6.29 ± 2.02
HVA/DA	0.132 ± 0.05	0.146 ± 0.033	0.617 ± 0.08	1.43 ± 0.35*
DHPG/NA	0.413 ± 0.076	0.253 ± 0.095	7.97 ± 3.17	4.78 ± 0.71
5-HT/5-HIAA	1.12 ± 0.13	1.43 ± 0.39	3.56 ± 0.59	2.57 ± 0.43

*P=0.08, Chronic fluphenazine vs. control

Figure 2:

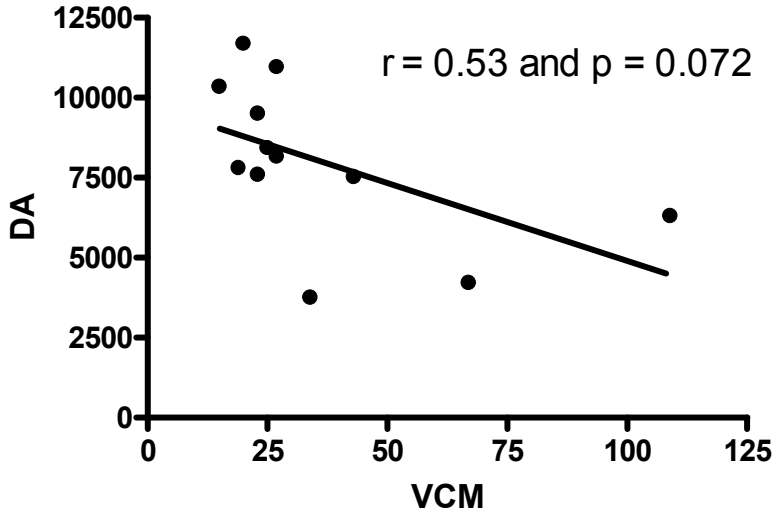
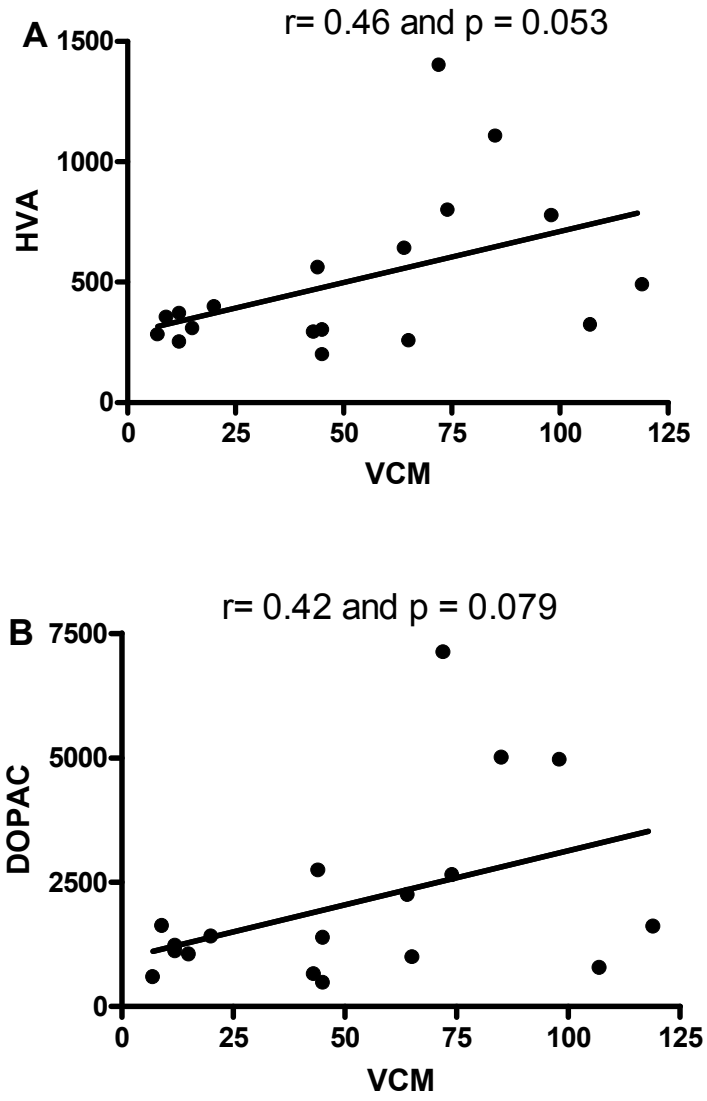

r = 0.53 and p = 0.072
DA
VCM
12500
10000
7500
5000
2500
0
0
25
50
75
100
125

Figure 3:


A
r= 0.46 and p = 0.053
HVA
1500
1000
500
0
0
25
50
75
100
125
VCM
B
r= 0.42 and p = 0.079
DOPAC
7500
5000
2500
0
0
25
50
75
100
125
VCM

4. DISCUSSÃO

A DT consiste no efeito colateral mais grave associado ao uso prolongado de neurolépticos, que acomete um alto percentual de pacientes que fazem uso crônico destes fármacos. Particularmente, a DT é problemática devida, além de sua alta prevalência, à sua natureza incapacitante e falta de um tratamento que seja efetivo. Apesar da grande quantidade de trabalhos propondo mecanismos para o desenvolvimento da DT, sua patofisiologia ainda consiste numa incógnita para estudiosos da área.

Sabe-se que o TDA consiste na principal forma de reduzir os níveis extracelulares de DA, e consequentemente regular a magnitude e a duração do sinal dopaminérgico (Beckman e Quick, 1998; Kahlig e Galli, 2003). De particular importância, recentemente demonstramos que animais experimentais apresentando um alto número de MMV apresentaram uma redução na captação de DA no estriado após tratamento crônico com haloperidol (Fachinetto e cols., 2007a; 2007b). Desta forma, a reduzida captação de DA poderia aumentar os níveis desta monoamina na fenda sináptica alterando sua metabolização e sua taxa de renovação. De acordo com isto, a literatura tem sugerido que a administração crônica de neurolépticos poderia aumentar a renovação de DA devido ao bloqueio dos auto-receptores dopaminérgicos (Casey, 1985; Lohr, 1991; Andreasen e Jorgensen, 2000). Contudo, não existem dados demonstrando a participação das monoaminas e seus metabólitos na DO induzida por flufenazina em ratos.

O primeiro objetivo de nosso estudo foi investigar a ação de um composto antioxidante, disseleneto de difenila num modelo crônico de MMV induzido por tratamento prolongado com flufenazina em ratos.

Tem sido demonstrado que o nível de Se no cérebro consiste num importante fator na etiologia de várias doenças neurodegenerativas (Chang 1983; Zafar e cols., 2003). Além disso, trabalhos prévios de nosso grupo têm demonstrado que os compostos orgânicos de Se possuem um papel protetor em modelos de neurotoxicidade (para revisão ver Nogueira e cols., 2004). Por exemplo, previamente demonstramos que o composto orgânico de Se, disseleneto de difenila, atenuou parâmetros agudos de DO produzidos por haloperidol ou reserpina em ratos (Burger cols., 2004; 2006). Contudo, existem algumas críticas com relação aos modelos agudos de DO. Em modelos agudos, a retirada do tratamento com

neurolépticos diminui e/ou inibe as alterações comportamentais enquanto que em modelos crônicos de DO e na DT em humanos, a retirada do tratamento crônico exacerba a síndrome. Desta forma, modelos agudos de DO têm sido classificados como Parkinsonismo ou síndrome extrapiramidal (SEP), onde a reversão é possível através da administração de fármacos anticolinérgicos (Para revisão ver, Jenner e Marsden, 1983; Egan e cols., 1996; Andreassen e Jorgensen 2000). Além disto, investigamos os efeitos do disseleneto de difenila no modelo crônico de DO bem como seu possível mecanismo de ação. O disseleneto de difenila reduziu a prevalência dos MMV, mas não o número de MMV em ratos tratados com flufenazina.

O mecanismo preciso que contribui para o desenvolvimento da DT continua não elucidado, mas parece envolver múltiplos sistemas neurotransmissores e tipos de receptor (Para revisão ver, Andreassen e Jorgensen, 2000; Gunne e cols., 1984; Lee e cols., 1997; Lohr e cols., 2003). Inicialmente, a supersensibilidade dos receptores de DA D_2 foi proposta como a principal base para explicar a DT, hipótese esta suportada por trabalhos demonstrando um aumento na densidade de receptores D_2 no estriado de ratos após administração aguda (2 a 3 semanas) de neurolépticos, incluindo a flufenazina (Burt e cols., 1977; Boyson e cols., 1988). Contudo, estudos mais recentes têm encontrado níveis elevados de parâmetros relacionados à DO sem uma alteração nos receptores estriatais D_2 após tratamento crônico (18 semanas) de ratos com flufenazina (Van Kampen e Stoessl, 2000). Além disso, existe uma pobre correlação temporal e espacial entre o desenvolvimento da DT e a supersensibilidade dos receptores para DA (Ver para revisão, Gunne e cols., 1984; Gerlach e Casey, 1988). Contudo, outros desequilíbrios na neurotransmissão dopaminérgica poderiam ser importantes para o desenvolvimento da DT. Por exemplo, um tratamento com antisense contra o receptor D_{1A} foi capaz de reduzir os MMV induzidos por flufenazina em ratos (Van Kampen e Stoessl, 2000).

A DA é um neurotransmissor envolvido em várias condições patológicas (See 1993; Miczek e cols., 2002; Mehler-Wex e cols., 2007; van Erp e Miczek, 2007). Apesar da principal forma de regulação da neurotransmissão dopaminérgica ser sua recaptação da fenda sináptica por TDA (Iversen, 1971; Horn, 1990), a modulação da captação de DA não tem sido sistematicamente estudada em modelos animais de DT. Neste trabalho, encontramos que o tratamento crônico de ratos com flufenazina reduz a captação de DA em

fatias estriatais de ratos. Contudo, a redução na captação de DA parece não estar envolvida no desenvolvimento de MMV produzidos por tratamento agudo com flufenazina em ratos. Estes resultados inferem que o tratamento crônico com flufenazina poderia causar uma alteração na disponibilidade de DA na fenda sináptica de neurônios dopaminérgicos extrapiramidais inibindo a captação de DA, a qual pode ser um dos mecanismos de indução de DT por neurolépticos típicos.

Vários fatores podem explicar a redução na captação de DA no estriado de ratos apresentando MMV, incluindo a alteração funcional no TDA e neurodegeneração de células que captam DA. Curiosamente, o tratamento crônico dos ratos com flufenazina (8 meses) causa uma redução significativa na densidade de neurônios da parte central do estriado (Jeste e cols., 1992). Além disso, tem sido demonstrado que alguns neurolépticos podem interagir diretamente com e inibir o TDA (Lee e cols., 1997). Um estudo demonstrou que altas concentrações de flufenazina podem reduzir a captação de DA em sinaptossomas estriatais de ratos, *in vitro* (Westfall e cols., 1976). Somando-se a estes mecanismos propostos, dados da literatura têm demonstrado que o estresse oxidativo diminui a atividade dos TDA (Huang e cols., 2003; Hashimoto e cols., 2004). Considerando as propriedades antioxidantes do disseleneto de difenila, investigamos o papel do estresse oxidativo nos MMV induzidos por flufenazina em ratos.

O estresse oxidativo tem sido proposto como um importante mecanismo na patogênese da DT. É sugerido que o aumento nos níveis da DA causado pelos neurolépticos estimula a produção de substâncias reativas de oxigênio como produtos do metabolismo da DA por via enzimática ou não enzimática (por exemplo, peróxido de hidrogênio e quinonas de DA) (Andreassen e Jorgensen, 2000). Contudo, existem muitos resultados contraditórios nos estudos que investigam o estresse oxidativo na DT. De fato, o tratamento prolongado da DT com Vitamina E tem apresentado pouco sucesso (Lohr e cols., 1987; Egan e cols., 1992; Adler e cols., 1999). Além disso, a vitamina E produziu resultados contraditórios nos parâmetros de DO causados por tratamento crônico com flufenazina em ratos (Sachdev e cols., 1999; Lohr e cols., 2000). Demonstramos que o tratamento com flufenazina ou disseleneto de difenila não alterou os níveis de peroxidação lipídica quando comparados com os animais do grupo controle. Este resultado é contrário a um estudo anterior que demonstrou um aumento na peroxidação lipídica no líquor ou sangue de pacientes tratados

medicamentos fenotiazínicos (Pall e cols., 1987). Contudo, outros estudos clínicos não encontraram nenhuma diferença na peroxidação lipídica no líquor ou no sangue de pacientes discinéticos quando comparados a controles saudáveis ou esquizofrênicos (Brown e cols., 1999; Tsai e cols., 1998).

Com relação à produção de espécies reativas de oxigênio, não detectamos nenhuma diferença significativa na oxidação da DCFH-DA no estriado e *substantia nigra* de ratos tratados somente com flufenazina. Dados da literatura demonstram que o tratamento agudo (3 a 4 semanas) com haloperidol causou um aumento nos MMV e parâmetros de estresse oxidativo em ratos (Naidu e cols., 2003b; Burger e cols., 2005a). A discrepância entre estes dados e os aqui apresentados pode ser explicada pelas diferentes capacidades dos neurolépticos em causar estresse oxidativo. De fato, o haloperidol parece possuir um efeito pró-oxidante, aumentando a peroxidação lipídica *in vitro* (Jeding e cols., 1995). Este efeito pró-oxidante pode indiretamente ser mediado por seus metabólitos tóxicos, os quais causam estresse oxidativo diretamente via disfunção mitocondrial (Avent e cols., 1996; Wright e cols., 1998). Experimentos preliminares realizados por nosso grupo têm demonstrado que a flufenazina *per se* não produz efeito anti ou pró-oxidante *in vitro* (dados não mostrados). Além disso, outro ponto importante a considerar é a relação entre a duração do estresse oxidativo e parâmetros de DO. De fato, Shivakumar e Ravindratnath (1993) têm demonstrado que o tratamento com haloperidol induziu estresse oxidativo somente até um mês após a administração com haloperidol, um evento que não é observado posteriormente até três meses de tratamento em camundongos. Além disso, nós detectamos parâmetros aumentados de DO, mas não de estresse oxidativo em várias regiões cerebrais, 7 meses após administração de haloperidol em ratos recebendo uma dieta normal (Fachinetto e cols., 2005). De maneira interessante, nós não encontramos alterações na peroxidação lipídica ou níveis de espécies reativas de oxigênio no estriado de ratos após uma semana e após 21 dias de tratamento com flufenazina (dados não mostrados). Tem sido relatado que a efetividade dos antioxidantes é reduzida nos casos de DT já instalada (Egan e cols., 1992; Dabiri e cols., 1994) sugerindo seu uso clínico para prevenção, mas não para o tratamento da discinesia já estabelecida. Então, o estresse oxidativo parece estar envolvido no desenvolvimento de discinesia induzida por haloperidol, mas possivelmente não na manutenção da DT. Contudo, apesar de alguns dados da literatura apontarem para uma

disfunção inicial do sistema nigroestriatal decorrente de uma produção aumentada de espécies reativas de oxidativo em ratos tratados com neurolépticos (Lohr e cols., 1991), nós fomos incapazes de observar em nosso modelo. Desta forma, com base em nossos dados, podemos inferir que a redução na atividade do TDA não é devida a um aumento na produção de espécies reativas de oxigênio.

A redução da captação de DA observada após tratamento crônico com haloperidol ou flufenazina (Fachinetto e cols., 2007a ; 2007b) somada às evidências encontradas na literatura de que existe um aumento de síntese de DA, poderia levar a alterações também no metabolismo da dopamina. Então, o segundo objetivo deste trabalho foi investigar o papel da dopamina e de outras monoaminas bem como seus metabólitos na DO aguda e crônica induzida por flufenazina em ratos.

Os resultados deste estudo demonstram que os níveis de monoaminas e sua renovação não foram significativamente alterados pelo tratamento agudo ou crônico com flufenazina. Contudo, nós detectamos algumas tendências ($p<0.10$ a $p>0.05$) a diferenças entre grupos tratados com flufenazina e seus controles, mas que não alcançaram níveis estatisticamente significativos ($p<0.05$). Como o número de animais em alguns grupos é pequeno (3 animais no grupo tratado agudamente com flufenazina) é também há variação no número de animais por grupos (por exemplo, 7 animais no grupo controle e 11 animais no grupo tratado cronicamente com flufenazina), devemos corrigir estas falhas para confirmar o exato papel das monoaminas e seus metabólitos na discinesia. Contudo, nossos resultados preliminares permitem-nos especular a respeito de nossa hipótese.

Em um estudo prévio de nosso grupo, não detectamos uma redução na captação de DA no estriado de ratos tratados com flufenazina (Fachinetto e cols., 2007b). Concordando com nossa idéia de que o metabolismo da DA poderia ser alterado, não detectamos nenhuma alteração significativa nos níveis estriatais de DA, seus metabólitos ou na sua renovação após tratamento agudo com flufenazina, no presente estudo. Por outro lado, encontramos uma tendência a correlacionar negativamente os níveis de DA com o número de MMV em ratos tratados agudamente com flufenazina, indicando que níveis reduzidos de DA no estriado poderiam aumentar a intensidade da DO. De acordo, tem sido descrito que uma redução na DA leva a um desequilíbrio neuronal colinérgico que poderia ser responsável pelo desenvolvimento da SEP, incluindo o parkinsonismo (Neale e cols., 1984;

Rupniak e cols., 1986; para revisão, ver Salamone e cols., 1998). Este dado está de acordo com a idéia de que o tratamento agudo com neurolépticos produz uma síndrome semelhante ao parkinsonimo (para revisão, ver Salamone e cols., 1998).

De forma diferente do tratamento agudo, a administração crônica com flufenazina é capaz de reduzir a captação de DA no estriado de ratos que desenvolveram alta intensidade MMV. Estes resultados inferem que o tratamento crônico com flufenazina poderia aumentar os níveis de DA na fenda sináptica de neurônios dopaminérgicos extrapiramidais, o que poderia ser um possível mecanismo para a DT induzida por neurolépticos. De fato, tem sido demonstrado que o tratamento crônico com flufenazina aumenta os níveis de DA no *nucleus accumbens* e mesencéfalo (Jackson-Lewis e cols., 1991). Além disso, tem sido demonstrado que o tratamento crônico com haloperidol aumenta os níveis de DA e seus metabólitos no estriado (See, 1993). No presente estudo, não fomos capazes de demonstrar um aumento nos níveis de DA no estriado de ratos tratados cronicamente, mas encontramos uma tendência a aumento nos metabólitos de DA, HVA e DOPAC, em ratos apresentando altos níveis de MMV e para uma positiva correlação entre os metabólitos da DA e a intensidade dos MMV. Desta forma, nossos resultados sugerem um aumento no metabolismo e taxa de renovação da DA após tratamento crônico com neurolépticos. Este aumento nos metabólitos da DA observado em modelos animais de DO é também observado em líquor de pacientes apresentando DT (Hsiao e cols., 1993). Além disso, alteração na função do TDA, possivelmente regula enzimas envolvidas na sua síntese e degradação que poderiam estar relacionadas à DT.

Em conjunto, nossos dados sugerem um mecanismo envolvendo a redução no transporte de DA relacionado à manutenção da DO em ratos. Além disso, podemos sugerir que o aumento de metabolismo de DA poderia estar levando, de alguma forma, a uma inibição no transporte da mesma. No entanto, fica claro que há a necessidade de investigarmos também o papel de enzimas responsáveis pelo metabolismo das monoaminas como, por exemplo, a MAO, neste processo.

5. CONCLUSÕES FINAIS

De acordo com os resultados apresentados nesta tese podemos concluir que:

- Ao tratamento com flufenazina é capaz de induzir uma alta prevalência de DO em ratos, sendo que agudamente esta prevalência fica em torno de 50% e cronicamente em torno de 85%;

- o antioxidante disseleneto de difenila diminuiu a prevalência, mas não alterou a intensidade da DO naqueles animais que apresentaram a DO;

- a redução da captação de dopamina parece estar envolvida na manutenção da DO, mas não no seu desenvolvimento;

- não foram encontradas alterações significativas quando quantificados os níveis de monoaminas e seus metabólitos no tratamento agudo e crônico com flufenazina;

- não encontramos parâmetros de estresse oxidativo alterados no tratamento agudo e crônico com flufenazina em ratos.

6. PERSPECTIVAS

Com base nos resultados obtidos no presente trabalho, faz-se necessário:

- avaliar a atividade da enzima MAO no estriado de ratos tratados aguda e cronicamente com flufenazina;

- investigar possíveis alterações na expressão protéica dos transportadores de dopamina;

- determinar o nível de viabilidade celular em estriado de animais após tratamento prolongado com neurolépticos;

- aumentar e equilibrar o número de animais nos grupo de estudo para esclarecer o papel dos níveis de monoaminas e seus metabólitos na DO induzida por flufenazina em ratos.

DEMAIS TRABALHADOS REALIZADOS DURANTE O PERÍODO DO DOUTORADO:

MEOTTI, F. C. ; FACHINETTO, R. ; MAFFI, L. ; MISSAU, F. C. ; PIZZOLATTI, M. G. ; ROCHA, J. B. T. ; SANTOS, A. R. S. Antinociceptive action of myricitrin: Involvement of the K^+ and Ca^{2+} channels. European Journal of Pharmacology, 567:198-205, 2007.

COLPO, G.; TREVISOL, F.; TEIXEIRA, A.M.; FACHINETTO, R.; PEREIRA, R P; ATHAYDE, M.L.; ROCHA, J. B. T.; BURGER, M. E. Ilex paraguariensis has Antioxidant Potentialand Attenuates Haloperidol-induced Orofacial Dyskinesia and Memory Dysfunction in Rats. Neurotoxicity Research, 12: 1-10, 2007.

TEIXEIRA, A.M.; TREVISOL, F.; COLPO, G.; GARCIA, S.C.; CHARÃO, M.; PEREIRA, R P.; FACHINETTO, R.; ROCHA, J B T; BURGER, M. E. Influence of chronic exercise on reserpine-induced oxidative stress in rats: Behavioral and antioxidant evaluations. Pharmacology, Biochemistry and Behavior, 88: 465-472, 2007.

DALLA CORTE, C. L.; FACHINETTO, R.; COLLE, D.; PEREIRA, R. P.; AVILA, D. S.; VILLARINHO, J. G.; WAGNER, C.; PEREIRA, M.E.; NOGUEIRA, C. W.; SOARES, F.A.A.; ROCHA, J.B.T. Potentially adverse interactions between haloperidol and valerian. Food and Chemical Toxicology, IN PRESS, 2008.

7. REFERÊNCIAS BIBLIOGRÁFICAS

ABÍLIO, V.C., SILVA, R.H., CARVALHO, R.C., GRASSL, C., CALZAVARA, M.B., REGISTRO, S., D'ALMEIDA, V., RIBEIRO, R.A., FRUSSA-FILHO, R. Important role of striatal catalase in aging- and reserpine-induced oral dyskinesia. Neuropharmacology, 47, 263-272, 2004.

ADLER, L.A., ROTROSEN, J., EDSON, R., LAVORI, P., LOHR, J., HITZEMANN, R., RAISCH, D., CALIGURI, M., TRACY, K. Vitamin E treatment for tardive dyskinesia. Arch Gen Psychiatry. 56: 836-841,1999.

AMARA, S.G., KUHAR, M.J. Neurotransmitter transporters: recent progress. Annu Rev Neurosci 16:73–93, 1993.

ANDREASSEN, O.A., AAMO, T.O., JORGENSEN, H.A. Inhibition by memantine of the development of persistent oral dyskinesias induced by long-term haloperidol treatment of rats. Br J Pharmacol., 119, 751-757, 1996.

ANDREASSEN, O.A., FERRANTE, R.J., BEAL, M.F., JORGENSEN, H.A. Oral dyskinesias and striatal lesions in rats after long-term co-treatment with haloperidol and 3-nitropropionic acid. Neuroscience, 87, 639-648, 1998.

ANDREASSEN, O.A., JORGENSEN, H.A. Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats. Implications for tardive dyskinesia? Prog Neurobiol., 61, 525-541, 2000.

ARMSTRONG, A. M., CHESTNUTT, J. E., GORMLEY, M. J., YOUNG FREE, I. S. The effect of dietary treatment on lipid peroxidation and antioxidant status in newly diagnosed noninsulin dependent diabetes. Radic. Biol. Med. 21: 719-726, 1996.

AVENT, K.M., USUKI, E., EYLES, D.W., KEEVE, R., VAN DER SCHYF, C.J., CASTAGNOLI, NJR., POND, S.M. Haloperidol and its tetrahydropyridine derivative (HPTP) are metabolized to potentially neurotoxic pyridinium species in the baboon. Life Sci. 59: 1473-1482, 1996.

BECKMAN, M.L., QUICK, M.W. Neurotransmitter transporters: regulators of function and functional regulation. J Membr Biol 164:1–10, 1998.

BORGES, L.P., BORGES, V.C., MORO, A.V., NOGUEIRA, C.W., ROCHA, J.B., ZENI, G. Protective effect of diphenyl diselenide on acute liver damage induced by 2-nitropropane in rats. Toxicology 210: 1-8, 2005.

BOYSON, S.J., MCGONIGLE, P., LUTHIN, G.R., WOLFE, B.B., MOLINOFF, P.B. Effects of chronic administration of neuroleptic and anticholinergic agents on densities of D2 dopamine and muscarinic cholinergic receptors in rat striatum. J Pharmacol Exp Ther. 244:987-993,1988.

BRANDÃO, R., LARA, F.S., PAGLIOSA, L.B., SOARES, F.A., ROCHA, J.B., NOGUEIRA, C.W., FARINA, M. Hemolytic effects of sodium selenite and mercuric chloride in human blood. Drug Chem Toxicol. 28: 397-407, 2005.

BRENNEISEN, P., STEINBRENNER, H., SIES, H. Selenium, oxidative stress, and health aspects. Mol Aspects Med. 26: 256-267, 2005.

BROMET, E.J., FENNIG, S. Epidemiology and natural history of schizophrenia. Biol Psychiatry. 46, 871–881, 1999.

BROWN, S., BIRTWISTLE, J., ROE, L., THOMPSON, C. The unhealthy lifestyle of people with schizophrenia. Psychol Med., 29, 697-700, 1999.

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

BURGER, M.E., FACHINETTO, R., ZENI, G., ROCHA, J.B. Ebselen attenuates haloperidol-induced orofacial dyskinesia and oxidative stress in rat brain. Pharmacol Biochem Behav. 81**,** 608-615, 2005a.

BURGER, M.E., FACHINETTO, R., ALVES, A., CALEGARI, L., ROCHA, J.B.T. Acute reserpine and subchronic haloperidol treatments change synaptosomal brain glutamate uptake and elicit orofacial dyskinesia in rats. Brain Res. 1031, 202-210, 2005b.

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

BURT, D., CREESE, I., SNYDER, S.H. Antischizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. Science**,** 196, 326-327, 1977.

CADET, J.L., LOHR, J.B., JESTE, D.V. Tardive dyskinesia and Schizophrenic burnout: the possible involvement of cytotoxic free radicals. In: Henn FA, DeLisi LE (Eds). Handbook of schizophrenia: The neurochemistry and pharmacology of schizophrenia. *Elsevier, Amsterdam* p.425-438, 1987.

CALVENT, P.R.V., ARAUJO, C.C.S., BERGAMO, M., ABILIO, V.C., ALMEIDA, V.D., RIBEIRO, R.D.A., FRUSSA-FILHO, R. The mitochondrial toxin 3-nitropropionic acid aggravates reserpine-induced oral dyskinesia in rats. Prog Neuropsychopharmacol & Biol Psychiatry., 26, 401-405, 2002.

CASEY, D.E. Tardive dyskinesia: reversible and irreversible. Psychopharmacology, 2, 88-97, 1985.

CASTILHO, R.F., WARD, W., NICHOLLS, D.G. Oxidative stress, mitochondrial function, and acute glutamate excitotoxicity in cultured cerebellar granule cells. J Neurochem., 72, 1394-1401, 1999.

CAVALLERO, R. e SMERALDI, E. Antipsychotic-induced tardive dyskinesia: recognition, prevention and management. CNS Drugs, 4, 278-293, 1995.

COYLE, J.T., PUTTFARCKEN, P. Oxidative stress, glutamate, and neurodegenerative disorders. Science, 262, 689-695, 1993.

CHANG, L.W. Protective effects of selenium against methylmercury neurotoxicity: a morphological and biochemical study. Exp Pathol. 23: 143-156, 1983.

CHOI, J., JANG, E., PARK, C., KANG, J. Enhanced suscetibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in high-fat diet-induced obesity. Free Radic Biol Med., 38, 806-816, 2005.

COMMANDEUR, J.N.M., ROOSEBOOM, M., VERMEULEN, N.P.E. Chemistry and biological activity of novel selenium-containing compounds. Biological reactive intermediates VI, Adv. Exp. Med. Biol. 500:105, 2001.

COOPER, J.R., BLOOM, F.E., ROTH, R.H. The biochemical basis of Neuropharmacology. Oxford Univ. Press Inc., New York, 2003.

COMBS, G. F., GRAY, P. G. Chemopreventive Agents: Selenium. Pharmacol. Ther. 79 179-192, 1998.

CLARK, L. C., COMBS, G. F., TURNBULL, B. W., SLATE, E., CHALKER, D. K., CHOW, J., DAVIS, L. S., GLOVER, R. A., GRAHAM, G. F., GROSS, E. H.,

KRONGRAD, A., LESHAR, J. L., PARK, H. K., SANDERS, B. B., SMITH, C. L., TAYLOR, J. R. Effects of selenium for cancer prevention in patients with carcinoma of the skin. Journal of the American Medical Association. 276, 1957-1985, 1996.

CRANE, G.E. Tardive dyskinesia in patients treated with major neuroleptics: a review of the literature. Am J Psychiatry., 124, 40-48, 1968.

CRANE, G.E. Persistent dyskinesia. Br J Psychiatry., 122, 395-405, 1973.

CREESE, I., BURT, D., SNYDER, S.H. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science, 192, 481-483, 1976.

DABIRI, L.M., PASTA, D., DARBY, J.K., MOSBACHER, D. Effectiveness of Vitamin E for treatment of long-term tardive dyskinesia. Am J Psychiatry., 151, 925-926, 1994.

DARLING, H.F. Fluphenazine: a preliminary study. Dis Nerv Syst., 20:167-70, 1959.

EGAN, M.F., HYDE, T.M., ALBERS, G.W., ELKASHEF, A., ALEXANDER, R.C., REEVE, A., BLUM, A., SAENZ, R.E., WYATT, R.J. Treatment of tardive dyskinesia with Vitamin E. Am J Psychiatry., 149, 773-777, 1992.

EGAN, M.F., HURD, Y., HYDE, T.M., WEINBERGER, D.R., WYATT, R.J., KLEINMAN, J.E. Alterations in mRNA levels of D2 receptors and neuropeptides in striatonigral and striatopallidal neurons of rats with neuroleptic-induced dyskinesias. Synapse, 18, 178-189, 1994.

EGAN, M.F., HURD, Y., FERGUSON, J., BACHUS, S.E., HAMID, E.H., HYDE, T.M. Pharmacological and neurochemical differences between acute and tardive vacuous chewing movements induced by haloperidol. Psychopharmacology, 127, 337-345, 1996.

EL-BAYOUMY, K. Effects of organoselenium compounds on induction of mouse forestomach tumors by benzo(a)pyrene. Cancer Research. 45, 3631-3635, 1985.

FACHINETTO, R., BURGER, M.B., WAGNER, C., WONDRACEK, D.C., BRITO, V.B., NOGUEIRA, C.W., FERREIRA, J., ROCHA, J.B.T. High fat diet increases the incidence of orofacial dyskinesia and oxidative stress in specific brain regions of rats. Pharmacol Biochem Behav. 81: 585-592, 2005.

FACHINETTO, R., VILLARINHO, J.G., WAGNER, C., PEREIRA, R.P., AVILA, D.S., BURGER, M.E., CALIXTO, J.B., ROCHA, J.B., FERREIRA, J. Valeriana officinalis does not alter the orofacial dyskinesia induced by haloperidol in rats: role of dopamine transporter. Prog Neuropsychopharmacol Biol Psychiatry. 31:1478-1486, 2007a.

FACHINETTO, R., VILLARINHO, J.G., WAGNER, C., PEREIRA, R.P., PUNTEL, R.L., PAIXÃO, M.W., BRAGA, A.L., CALIXTO, J.B., ROCHA, J.B., FERREIRA, J. Diphenyl diselenide decreases the prevalence of vacuous chewing movements induced by fluphenazine in rats. Psychopharmacology (Berl). 194:423-432, 2007b.

FARIA, R.R., ABILIO, V.C., GRASSL, C., CHINEN, C.C., NEGRAO, L.T., DE CASTRO, J.P., FUKUSHIRO, D.F., RODRIGUES, M.S., GOMES, P.H., REGISTRO, S., DE CARVALHO, R.D.E.C., D'ALMEIDA, V., SILVA, R.H., RIBEIRO, R.D.E.A, FRUSSA-FILHO, R. Beneficial effects of vitamin C and vitamin E on reserpine-induced oral dyskinesia in rats: critical role of striatal catalase activity. Neuropharmacology, 48, 993-1001, 2005.

FOLMER, V., SOARES, J.C.M., GABRIEL, D., ROCHA, J.B.T. High-fat diet causes δ-aminolevulinate dehydratase inhibition and lipid peroxidation in mice (*Mus musculus*). J Nutrition., 133, 2165-2170, 2003.

GARDOS, G., COLE, J.O. The prognosis of tardive dyskinesia. Am J Psychiatry., 151, 177-179, 1983.

GERLACH, J., CASEY, D. Tardive Dyskinesia. Acta Psychiatr Scand. 77: 369-378, 1988.

GIROS, B., CARON, M.G. Molecular characterization of the dopamine transporter. Trends Pharmachol Sci., 14:43-49, 1993.

Goodman e Gilman, 2006

GUNNE, L.M., GROWDON, J., GLAESER, B. Oral dyskinesia in rats following brain lesions and neuroleptic drug administration. Psychopharmacology, 77, 134-139, 1982.

GUNNE, L., HÄGGSTRÖM, J., SJÖQUIST, B. Association with persistent neuroleptic-induced dyskinesia of regional changes in brain GABA synthesis. Nature, 309, 347-349, 1984.

HASHIMOTO, K., TSUKADA, H., NISHIYAMA, S., FUKUMOTO, D., KAKIUCHI, T., SHIMIZU, E., IYO, M. Protective effects of N-acetyl-L-cysteine on the reduction of dopamine transporters in the striatum of monkeys treated with methamphetamine. Neuropsychopharmacology, 29, 2018-2023, 2004.

HEALY, D. The Creation of Psychopharmacology. Harvard Univ. Press, Cambridge, MA, pp. 9– 128, 2002.

HENDERSON, D.C. Diabetes mellitus and other metabolic disturbances induced by atypical and antipsychotic agents. Curr diab Rep., 2, 135-140, 2002.

HIPPIUS, H. The history of clozapine. Psychopharmacology, 99, S3– S5, 1989.

HORN, A.S. Dopamine uptake: a review of progress in the last decade. Prog Neurobiol 34: 387-400, 1990.

HSIAO, J.K., COLISON, J., BARTKO, J.J., DORAN, A.R., KONICKI, P.E., POTTER, W.Z., PICKAR, D. Monoamine neurotransmitter interactions in drug-free and neuroleptic-treated schizophrenics. Arch Gen Psychiatry 50:606-614, 1993.

HUANG, C.L., HUANG, N.K., SHYUE, S.K., CHERN, Y. Hydrogen peroxide induces loss of dopamine transporter activity: a calcium-dependent oxidative mechanism. J Neurochem., 86, 1247-1259, 2003.

IVERSEN, L.L. Role of transmitter uptake mechanisms in synaptic neurotransmission. Br J Pharmacol 41: 571-591, 1971.

JACKSON-LEWIS, V., PRZEDBORSKI, S., KOSTIC, V., SUBER, F., FAHN, S., CADET, J.L. Partial attenuation of chronic fluphenazine-induced changes in regional monoamine metabolism by D-alpha-tocopherol in rat brain. Brain Res Bull. 26: 251-258, 1991.

JEDING, I., EVANS, P.J., AKANMU, D., DEXTER, D., SPENCER, J.D., ARUOMA, O.I., JENNER, P., HALLIWELL, B. Characterization of the potential antioxidant and pro-oxidant actions of some neuroleptic drugs. Biochem Pharmacol. 49: 359-365, 1995.

JENNER, P., MARSDEN, C.D. Neuroleptics and tardive dyskinesia. In neuroleptics: Neurochemical, Behavioral and, Clinical perspectives, ed. By J.T. Coyle and S.J. Enna, pp. 223-253, Raven Press, New York, 1983.

JESTE, D.V., POTKIN, S.G., SINHÁ, S., FEDER, S., WYATT, R.J. Tardive dyskinesia-reversible and persistent. Arch Gen Psychiatry., 36, 585-590, 1979.

JESTE, D.V., LOHR, J.B., MANLEY, M. Study of neuropathologic changes in the striatum following 4, 8 and 12 months of treatment with fluphenazine in rats. Psychopharmacology 106: 154-160, 1992.

JORGENSEN, H.A., ANDREASSEN, O.A., HOLE, K. The relationship between motor effects in rats following acute and chronic haloperidol treatment. Psychopharmacology, 116, 89-92, 1994.

KAHLIG, K.M., GALLI, A. Regulation of dopamine transporter function and plasma membrane expression by dopamine, amphetamine, and cocaine. Eur J Pharmacol., 479:153-158, 2003.

KANE, J.M., SMITH, J.M. Tardive dyskinesia: prevalence and risk factors, 1959 to 1979. Arch Gen Psychiatry., 39, 473-481, 1982.

KANE, J., HONIGFELD, G., SINGER, J., MELTZER, H. Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. Arch Gen Psychiatry., 45, 789– 796, 1988.

KANE, J.M. Tardive dyskinesia: epidemiology and clinical presentation. In: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: The Fourth Generation of Progress, vol. 39. Raven Press, Ney York, pp. 1485-1495, 1995.

KANEDA, H., SHIRAKAWA, O., DALE, J., GOODMAN, L., BACHUS, S.E., TAMMINGA, C.A. Co-administration of progabide inhibits haloperidol-induced oral dyskinesias in rats. Eur J Pharmacol., 121, 43-49, 1992.

KLAWANS, H.L., RUBOVITS, R. An experimental model of tardive dyskinesia. J Neural Transm., 33, 235- 246, 1972.

KLOTZ, L.O., KRONCKE, K.D., BUCHCZYK, D.P., SIES, H. Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. J Nut. 133: 1448, 2003.

KONRADI, C., HECKRS, S. Molecular aspects of glutamate dysregulation: implications for schizophrenia and its treatment. Pharmacol Ther., 97, 153-179, 2003.

LAPORTA, M., ARCHAMBAULT, D., ROSS-CHOUINARD, A., CHOUINARD, G. Articulatory impairment associated with tardive dyskinesia. J Nerv Ment Dis., 178, 660-662, 1990.

LEE, S., OH, D., JUNG, S., KIM, Y., CHO, H., KOH, K., LEE, Y. Neuroleptic drugs alter the dopamine transporter-mediated uptake and release of dopamine: a possible mechanism for drug-induced tardive dyskinesia. Pharmacol Res., 35, 447-450, 1997.

LEWIS, D.A., LIEBERMAN, J.A. Catching Up on Schizophrenia: Review Natural History and Neurobiology. Neuron, 28, 325–334, 2000.

LOHR, J.B., JESTE, D.V. Neuroleptic-induced movement disorders: tardive dyskinesia and other tardive symptoms. In: Cavenar Jr, J.O. editor. Psychiatry. Philadelphia (PA): Lippincott., 1-17, 1988.

LOHR, J.B., KUCZENSKI, R., BRACHA, H.S., MOIR, M., JESTE, D.V. Increased indices of free radical activity in the cerebrospinal fluid of patients with tardive dyskinesia. Biol Psychiatry., 28, 535-539, 1990.

LOHR, J.B. Oxygen free radicals and neuropsychiatric illness. Arch Gen Psychiatry., 48, 1097-1106, 1991.

LOHR, J.B., KUCZENSKI, R., NICULESCU, A.B. Oxidative mechanisms and tardive dyskinesia. CNS Drugs, 17, 47-62, 2003.

MAHADIK, P.S., EVANS, D., LAL, H. Oxidative stress and role of antioxidant and ω-3 essential fatty acid supplementation in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry., 25, 463-493, 2001.

MEHLER-WEX, C., RIEDERER, P., GERLACH, M. Dopaminergic dysbalance in distinct basal ganglia neurocircuits: implications for the pathophysiology of Parkinson's disease, schizophrenia and attention deficit hyperactivity disorder. Neurotox Res.10:167-179, 2006.

MICZEK, K.A., FISH, E.W., DE BOLD, J.F., DE ALMEIDA, R.M. Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and gamma-aminobutyric acid systems. Psychopharmacology 163:434-458, 2002.

NAIDU, P.S., SINGH, A., KULKARNI, S.K. Quercetin, a bioflavonoid, attenuates haloperidol-induced orofacial dyskinesia. Neuropharmacology, 44, 1100-1106, 2003a.

NAIDU, P.S., SINGH, A., KAUR, P., SANDHIR, R., KULKARNI, S.K. Possible mechanism of action in melatonin attenuation of haloperidol-induced orofacial dyskinesia. Pharmacol Biochem Behav., 74, 641-648, 2003b.

NEALE, R., GERHARDT, S., LIEBMAN, J.M. Effects of dopamine agonists, catecholamine depletors, and cholinergic and GABAergic drugs on acute dyskinesias in squirrel monkeys. Psychopharmacology., 82:20-26, 1984.

NIEMEGEERS, C.J.E. Pharmacology and mechanism of action of neuroleptics haloperidol and haloperidol decanoate. Workshop haloperidol decanoate. Departament of Pharmacology, Janssen Pharmaceutics Belgium, p. 1-13, 1983.

NOGUEIRA, C.W., MEOTTI, F.C., CURTE, E., PILISSÃO, C., ZENI, G., ROCHA, J.B.T. Investigations into the potencial neurotoxicity induced by diselenides in mice and rats. Toxicology 183: 29–37, 2003.

NOGUEIRA, C.W., ZENI, G., ROCHA, J.B.T. Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem Rev. 104: 6255–6286, 2004.

PALKOVITS, M., BROWNSTEIN, M. Catecholamines in the central nervous system. In: Trendelembreg, U., Weiner, N. (Eds.), Catecholamines II. Springer, Berlin, pp. 1-26, 1989.

PALL, H.S., WILLIAMS, A.C., BLAKE, D.R., LUNEE, J. Evidence of enhanced lipid peroxidation in the cerebrospinal fluid of patients taking phenothiazines. Lancet 2, 596-599, 1987.

PILLAI, A., PARIKH, V., TERRY, A.V.Jr., MAHADIK, S.P. Long-term antipsychotic treatments and crossover studies in rats: Differential effects of typical and atypical agents on the expression of antioxidants enzymes and membrane lipid peroxidation in rat brain. J Psychiatr Res., 41: 372-386, 2007.

POST, A., RUCKER, M., OHL, F., UHR, M., HOLSBOER, F., ALMEIDA, O.F.X., MICHAELIDIS, T.M. Mechanisms underlying the protective potential of alpha-tocopherol (vitamin E) against haloperidol-associated neurotoxicity. Neuropsychopharmacology 26, 397-407, 2002.

RUBINSTEIN, M., MUSCHIETTI, J.P., GERSHANIK, O., FLAWIA, M.M., STEFANO, F.J. Adaptative mechanisms of striatal D1 and D2 dopamine receptors in response to a prolonged reserpine treated mice. J Pharmacol Exp Ther., 252, 810-816, 1990.

RUPNIAK, N.M., BRIGGS, R.S., PETERSEN, M.M., MANN, S., REAVILL, C., JENNER, P., MARSDEN, C.D. Differential alterations in striatal acetylcholine function in rats during 12 months' continuous administration of haloperidol, sulpiride, or clozapine. Clin Neuropharmacol. 9:282-292, 1986.

SACHDEV, P., SAHAROV, T., CATHCART, S. The preventative role of antioxidants (selegiline and vitamin E) in a rat model of tardive dyskinesia. Biol Psychiatry., 46, 1672-1681, 1999.

SADAN, O., BAHAT-STROMZA, M., GILGUN-SHERKI, Y., ATLAS, D., MELAMED, E., OFFEN, D. A novel brain-targeted antioxidant (AD4) attenuates haloperidol-induced abnormal movement in rats: implications for tardive dyskinesia. Clin Neuropharmacol. 28, 285-288, 2005.

SALAMONE, J.D., MAYORGA, A.J., TREVITT, J.T., COUSINS, M.S., CONLAN, A., NAWAB, A. Tremulous jaw movements in rats: a model of parkinsonian tremor. Prog Neurobiol, 56:591-611, 1998.

SALDAÑA, M., BONASTRE, M., AGUILAR, E., MARIN, C. Differential ˙ nigral expression of Bcl-2 protein family in chronically haloperidol and clozapine-treated rats: Role in neurotoxicity and stereotyped behavior. Exp Neurol; 203:302-8, 2006.

SALONEN, J. T.; ALFTHAN, G.; PIKKARAINEN, J. K. et al. Association between cardiovascular death and myocardial infarction and serum selenium in a matchet- pair longitudinal study; **Lancet**, 2, 175-179, 1982.

SANTOS, F.W., ZENI, G., ROCHA, J.B., WEIS, S.N., FACHINETTO, J.M., FAVERO, A.M., NOGUEIRA, C.W. Diphenyl diselenide reverses cadmium-induced oxidative damage on mice tissues. Chem Biol Interact. 151: 159-165, 2005.

SCHMUTZ, J., EICHENBERGER, E. Bindra, J.S., Ledmicer, D. (Eds.), Chronicles of Drug Discovery, vol. 1. Wiley, New York, pp. 39– 59, 1982.

SEE, R.E., ELLISON, G. Intermittent and continuous haloperidol regimens produce different types of oral dyskinesias in rats. Psychopharmacology, 100, 404-412, 1990.

SEE, R.E. Assessment of striatal extracellular dopamine and dopamine metabolites by microdialysis in haloperidol-treated rats exhibiting oral dyskinesia. Neuropsychopharmacology 9: 101-109, 1993.

SHIRAKAWA, O., TAMMINGA, C.A. Basal ganglia $GABA_A$ and dopamine D_1 binding site correlates of haloperidol-induced oral dyskinesias in rat. Exp Neurol., 127, 62-69, 1994.

SNYDER, S.H. The dopamine hypothesis of schizophrenia: focus on the dopamine receptor. Am J Psychiatry., 133, 197-202, 1976.

STEINBRENNER, H., ALILI, L., BILGIC, E., SIES, H., BRENNEISEN, P. Involvement of selenoprotein P in protection of human astrocytes from oxidative damage. Free Radic Biol Med. 40: 1513-1523, 2006.

STOTZ-INGENLATH, G. Epistemological aspects of Eugen Bleuler's conception of schizophrenia in 1911. Med Health Care Philos, 3, 153-159, 2000.

TAMMINGA, C.A., DALE, J.M., GOODMAN, L., KANEDA, H., KANEDA, N.,. Neuroleptic-induced vacuous chewing movements as an animal model of tardive dyskinesia: a study in three rat strains. Psychopharmacology, 102, 474-478, 1990.

TAYLOR, I.J. Clinical evaluation of a new phenothiazine tranquilizer, fluphenazine (prolixin). Am J Psychiatry. 116:457-8, 1959.

TORRES, G.E., GAINETDINOV, R.R., CARON, M.G. Plasma membrane monoamine transporters: structure, regulation and function. Nat Rev Neurosci. 4:13-25, 2003.

TSAI, G., GOFF, D.C., CHANG, R.W., FLOOD, J., BAER, L., COYLE, J.T. Markers of glutamatergic neurotransmission and oxidative stress associated with tardive dyskinesia. Am J Psychiatry., 155, 1207-1213, 1998.

VAN ERP, A.M., MICZEK, K.A. Increased accumbal dopamine during daily alcohol consumption and subsequent aggressive behavior in rats. Psychopharmacology 191:679-688, 2007.

VAN KAMPEN, J.M., STOESSL, A.J. Dopamine D_{1A} receptor function in a rodent model of tardive dyskinesia. Neuroscience 101: 629-635, 2000.

WADDINGTON, J.L. Spontaneous orofacial movements induced in rodents by very long-term neuroleptic drug administration: phenomenology, pathophysiology and putative relationship to tardive dyskinesia. Psychopharmacology, 101, 431- 447, 1990.

WESTFALL, T.C., BESSON, M., GIORGUIEFF, M., GLOWINSKI, J. The role of presynaptic receptors in the release and synthesis of ^{3}H-dopamine by slices of rat striatum. Arch Pharmacol. 292: 279-287, 1976.

WILBER, C. G. Toxicology of selenium. A review clinical. Toxicology, 17, 171-230, 1980.

WOERNER, M.G., KANE, J.M., LIEBERMAN, J.A., ALVIR, J., BERGMANN, K.J., BORENSTEIN, M., SCHOOLER, N.R., MURKHERJEE, S., ROTROSEN, J., RUBINSTEIN, M. e cols. The prevalence of tardive dyskinesia. J Clin Psychopharmacol., 11, 34-42, 1991.

WRIGHT, A.M., BEMPONG, J., KIRBY, M.L., BARLOW, R.L., BLOOMQUIST, J.R. Effects of haloperidol metabolites on neurotransmitter uptake and release: possible role in neurotoxicity and tardive dyskinesia. Brain Res. 788: 215-222, 1998.

YASSA, R. & JESTE, D.V. Gender differences in tardive dyskinesia: a critical review of the literature. Schizophr Bull., 18, 701-715, 1992.

YODER, K.K., HUTCHINS, G.D., MORRIS, E.D., BRASHEAR, A., WANG, C., SHEKHAR, A. Dopamine transporter density in schizophrenic subjects with and without tardive dyskinesia. Schizophr Res; 71:371-5, 2004.

ZAFAR, K.S., SIDDIQUI, A., SAYEED, I., AHMAD, M., SALIM, S., ISLAM, F. Dose-dependent protective effect of selenium in rat model of Parkinson's disease: neurobehavioral and neurochemical evidences. J Neurochem. 84: 438-446, 2003.

ANEXOS
(Artigos que fizeram parte da Dissertação de Mestrado)

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High fat diet increases the incidence of orofacial dyskinesia and oxidative stress in specific brain regions of rats

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Abstract

Haloperidol-induced orofacial dyskinesia (OD) is a putative animal model of tardive dyskinesia (TD) whose pathophysiology has been related to free radical generation and oxidative stress. Schizophrenic patients have been reported to eat a diet higher in fat than the general population and dietary fat intake can lead to an increase in oxidative stress in animal models. The objective of this study was to determine whether association of ingestion of a high fat diet with prolonged haloperidol treatment could lead to OD and oxidative stress in the rat brain. Haloperidol decanoate administration (38 mg/kg, IM, which is equivalent to 1 mg/kg/day) monthly for a period of 6 months to rats fed previously with a high fat and normo fat diets (6 months) caused a increase in vacuous chewing (VCM) and duration of facial twitching (FT). Haloperidol caused a reduction in body weight gain and the loss of body weight occurred after 4 months of treatment with haloperidol. The effects on body weight were more accentuated in HF diet group. HF diet ingestion was associated with an increase in TBARS levels in cerebellum and cerebral cortex (regardless of haloperidol treatment). A significant diet × haloperidol treatment interaction in striatum, subcortical parts and the region containing the substantia nigra was observed for TBARS. In fact, haloperidol caused an increase in TBARS levels of these regions only in rats fed with the HF. These results indicate that a high fat diet caused a transitory increase in haloperidol-induced OD in rats and this in part can be related to the haloperidol-induced oxidative stress in brain structures involved with OD.

Keywords: Haloperidol; Orofacial dyskinesia; Tardive dyskinesia; Free radical; Neuroleptics; Neurotoxicity; High fat diet

1. Introduction

Schizophrenia is the major mental disorder that has a lifetime risk of 1% and affects at young age in many cultures around the world (Mahadik et al., 2001). Haloperidol, a typical member of the conventional neuroleptics, is thought to exert its motor side effects through striatal dopamine D_2-receptors (Creese et al., 1976) and sigma-receptors (Walker et al., 1990; Vilner et al., 1995). The neuroleptic efficacy of haloperidol in psychotic patients is somewhat compromised by the drug's liability to cause acute and chronic extrapyramidal side effects, including TD (Andreasen and Jorgensen, 2000). The mean prevalence of TD is 20–25% in subjects receiving classical neuroleptic treatment, but the rate increases strongly with age, and prevalence above 50% has been reported in patients older than 50 years (Kane and Smith, 1982; Woerner et al., 1991; Yassa and Jeste, 1992). The most serious aspect of TD is that it may persist for months or years after drug withdrawal, and in some patients it is irreversible (Crane, 1973; Jeste et al., 1979; Casey, 1985). Some neurochemical hypothesis has been proposed for the development of TD during the last decades. They include dopaminergic hypersensitivity, disturbed balance between dopaminergic and cholinergic systems, dysfunction of striatonigral GABAergic neurons and excitotoxicity (Andreasen and Jorgensen, 2000; Ebadi and Srinivasan, 1995). However, the molecular mechanisms

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responsible for the neuropathophysiology of TD are still not completely understood.

One hypothesis that has gained experimental support in literature is that free radicals may play an important role in the physiopathology of such disorders (Cadet et al., 1986, 1987). In line with this, literature of data indicates that neuroleptic administration can increase the turnover of dopamine and the production of reactive substances as products of dopamine metabolism (Andreasen and Jorgensen, 2000; Casey, 1995; Lohr, 1991; Polydoro et al., 2004). Furthermore, blockage of striatal dopamine receptors can produce an increase in extracellular glutamate (Burger et al., 2005; See and Lynch, 1996), which in turn can increase the production of free radical species (Castilho et al., 1999; Coyle and Puttfarcken, 1993; Tsai et al., 1998).

In line with this hypothesis, several authors have demonstrated the reversion of OD with the administration of antioxidants substances, including FK-506 (Singh et al., 2003), melatonin (Naidu et al., 2003a,b), quercetin (Naidu et al., 2003a,b), ebselen (Burger et al., 2003) and diphenyl–diselenide (Burger et al., 2004). Recently, Abílio et al. (2004) reported that striatal catalase has an important role in the protection of spontaneously hypertensive rats (SHR) against the reserpine-induced OD. Most importantly, patients with TD have elevated markers of oxidative stress in CSF and plasma when compared to controls subjects (Lohr et al., 1990; Tsai and Ikonomidou, 1995; Brown et al., 1998). Additionally some authors have demonstrated that high doses of vitamin E are able to prevent TD in patients under chronic with neuroleptic treatment (Egan et al., 1992; Adler et al., 1993).

Dietary fat intake has been shown to be important in the development of human obesity (Warwick and Schiffman, 1992) and there are also experimental studies showing that high fat diet can be associated with increased oxidative stress in rodents (Storlien et al., 1986, 2000; Folmer et al., 2003) and more recently literature data have indicate that high fat diet may increase the vulnerability of dopaminergic neurons to MPTP (Choi et al., 2005).

Of particular importance, schizophrenic patients have been reported to eat a diet higher in fat than the general population (Brown et al., 1999) and Gardos and Cole (1986) suggested that schizophrenia may confer resistance to the development of tardive dyskinesia. However, there are no data in the literature indicating that excessive fat intake can change the incidence of tardive dyskinesia in schizophrenics. High level of fat intake is considered to be an important factor in the development of insulin resistance and obesity. Schizophrenic individuals appear to have at increased risk for certain obesity-related conditions such as type II diabetes and cardiovascular disease (Mukherjee et al., 1996) in comparison with general population. Metabolic dysfunctions have been associated with antipsychotic treatment including increased levels of circulating leptin and these changes can be an important link in the development of overweight and the insulin resistance syndrome in subjects receiving antipsychotic drugs (Hagg et al., 2001; Haupt et al., 2005; Henderson, 2002; Kraus et al., 1999; Melkersson et al., 2000; Morimoto et al., 1999; Simpson et al., 2001).

In line with this, over production of reactive oxygen species (ROS) and antioxidant depletion have been associated with the diabetes manifestation (Hunt et al., 1988; Wolff and Dean, 1987), OD in animal models (Naidu et al., 2003a,b; Burger et al., 2003) and TD in humans (Andreasen and Jorgensen, 2000; Lohr et al., 2003). These considerations raise the possibility that a relation among neuroleptic treatment and diet can exist. Furthermore, it is plausible to suppose that some exacerbation of their pro-oxidant activity could occur by simultaneous exposure to them.

The aim of this study consisted in investigate the effects of the normo fat (NF) and high fat (HF) diets on the development of OD haloperidol-induced and TBARS in brain regions as measure of oxidative stress.

2. Materials and methods

2.1. Drugs

Haloperidol decanoate (Janssen Pharmaceutical); ketamine (Dopalen/ Division VetBrands/ Sespo-Brasil). Haloperidol was injected intramuscularly (I.M.) and Ketamine was injected intraperitoneally (i.p.).

2.2. Animals and diets

Male Wistar rats (2 months old), weighing between 270 and 320 g, from our own breeding colony (Animal Householding, UFSM, Brasil) were kept in wire cages with free access to the diets and water, in a room with controlled temperature (22±3 °C) and in 12-h light/dark cycle with lights on at 7:00 am. The animals were maintained and used in accordance 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 São Paulo, Brasil.

The rats were randomly divided into two groups, with 16 animals each, and a fed either a NF or a HF diet. The composition of the diets is shown in Table 1. Food was placed daily before the beginning of the dark cycle. Food offering was adjusted in such a way that leftovers were less than 10%. Diets were prepared weekly and stored at 4 °C. Rats received the diets for 13 months and were monthly weighed.

2.3. Induction of orofacial dyskinesia

Chronic OD haloperidol-induced occurred after 6 months of the treatment with diets, when the rats were divided in two subgroups. The NF control group ($n=7$) received NF diet and vegetable oil solution intramuscularly (I.M.). The NF haloperidol group ($n=7$) received NF diet and haloper-

Table 1

Composition of the diets	High fat diet (HF)	Normo fat diet (NF)
	(g/kg)	
Protein	75.0	76.0
Carbohydrate	483.0	765.0
Fiber	35.00	37.00
Fat acid saturated	125.00	8.00
Fat acid unsaturated	230.0	62.0
Salt mixture[1]	48.00	48.00
Caloric content (cal/g)	5.35	3.91

[1]The salt mixture has the following composition (g/kg): KCl, 96.3; $MgSO_4$, 56.7; $ZnCl_2$, 0.4; $CuCO_4$, 0.7; $MnSO_4$, 1.2; bonemeal, 449; salt ligth, 152. The values were retired from Andriguetto (1986).

idol decanoate (I.M.), the HF control group ($n=7$) received HF diet and vegetal oil (I.M.) and the HF haloperidol ($n=9$) group received HF diet and haloperidol decanoate (I.M.). The haloperidol groups received the depot neuroleptic drug, haloperidol decanoate (Janssen Pharmaceutical) at a dose of 38 mg/kg, the equivalent of 1 mg/kg/day of unconjugated haloperidol. Injections were given intramuscularly each 4 weeks during one period of 7 months.

2.4. Behavioral testing

One behavioral analyze was realized in the beginning of treatment with the diets (0 month) and compared with 6 months of diet intake. Haloperidol treatment was started after 6 months of experimental diets intake. We examined the effect of haloperidol decanoate after 28 days (1 month) of the first injection, because it is well established in the literature that haloperidol-treated animals develop orofacial dyskinesia (Andreassen et al., 1998, 2003; Egan et al., 1999; Hamid et al., 1998). The others evaluations were performed 2, 3, 4, 5, 6, 7 months of haloperidol treatment. To quantify the occurrence of orofacial dyskinesia rats were placed individually in cages ($20\times20\times19$ cm) and hand operated counters were employed to vacuous chewing (VCMs) frequency and stopwatches were employed to score the duration of twitching of the facial musculature (FT).

VCMs are referred as a single mouth opening in the vertical plane not directed towards physical material. If VCMs or FT occurred during a period of grooming they were not taken into account. The behavioral parameters of orofacial dyskinesia were measured continuously for 6 min after a period of 2 min adaptation. During the observation sessions, mirrors were placed under the floor and behind the backwall of the experimental cage to permit observation when the animal was faced away from the observer. The behavioral tests were always conducted by four observers blind.

2.5. Experimental procedure

After 30 days of the last administration of drugs and 24 h of last section of behavioral quantification, all the rats were injected with the anesthetic ketamine (1 mg/kg) (Dopalen/ Division VetBrands/ Sespo-Brasil). After the rats were killed for decapitation, the brains were immediately excised and the cerebellum, cerebral cortex, striatum, region containing the substantia nigra and subcortical parts of the brain were separated, weighed and homogenized in 10 volumes (w/v) of 10 mM Tris–HCl, pH 7.5. The homogenates were centrifuged for 10 min at 1800 $\times g$ and the supernatant was used to TBARS determination as described early on (Ohkawa et al., 1979; Rossato et al., 2002).

2.6. Statistical analysis

Data were analyzed by a two- and three-way ANOVA, followed by Duncan's post hoc tests when appropriated. F values are presented in the text only the p value associated with it was <0.05. Significance was considered when $p<0.05$.

3. Results

Diet had no effect on body weight gain (Fig. 1). Two-way ANOVA (2 diets $\times$ 7 weight determinations) revealed no

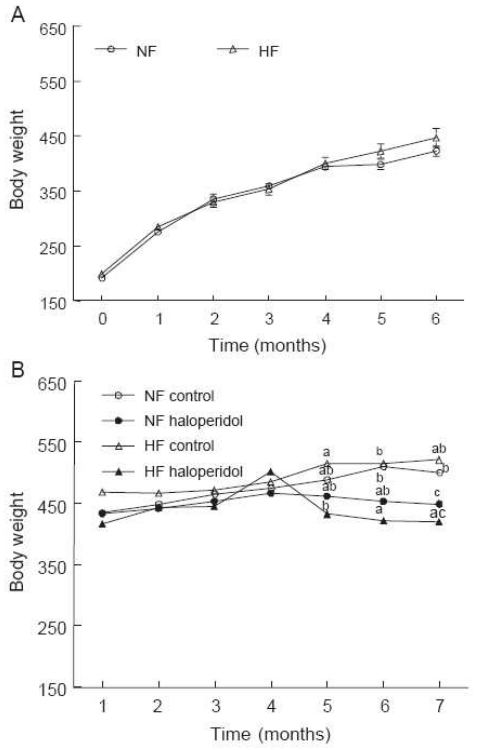

Fig. 1. A) Body weight in rats fed normo fat (NF) and high fat (HF) diets for 6 months. B) Body weight in rats receiving diets and haloperidol treatment. The symbols indicates a significant difference (detected by Duncan's multiple range test) between the groups into same month ($p<0.05$).

significant effects. Haloperidol treatment caused a significant reduction in body weight of rats in both dietary groups. However, rats from HF diet started to loss body weight before of the animals from NF diet. This was evidenced by a significant interaction between body weight and haloperidol treatment with $F(6, 150)=8.50$ and $p<0.001$.

Ingestion of the HF for 6 months caused an increase in the facial twitching frequency, when compared to rats fed with the diet containing normal fat (NF) content (Fig. 2; $p<0.01$). Main effect of haloperidol ($F(1,28)=25$).

Haloperidol caused a marked increase on VCM. However, the effect of haloperidol varied depending on the dietary treatment. In fact, rats on HF diet treated with haloperidol displayed an increase in VCM from the 3rd to the 5th month of treatment, when compared to animals from NF diet. This was evidenced by a significant third order interaction (diet × haloperidol × months) with $F(6,138)=6.33$ and $p<0.001$.

Haloperidol caused a marked increase on FT. However, the effect of haloperidol varied depending on the dietary treatment. In fact, rats on HF diet treated with haloperidol displayed an increase in FT from the 4th to the 6th month of treatment, when compared to animals from NF diet. This was evidenced by a significant interaction between haloperidol × months with $F(1,26)=74.56$ and $p<0.001$ (Fig. 3).

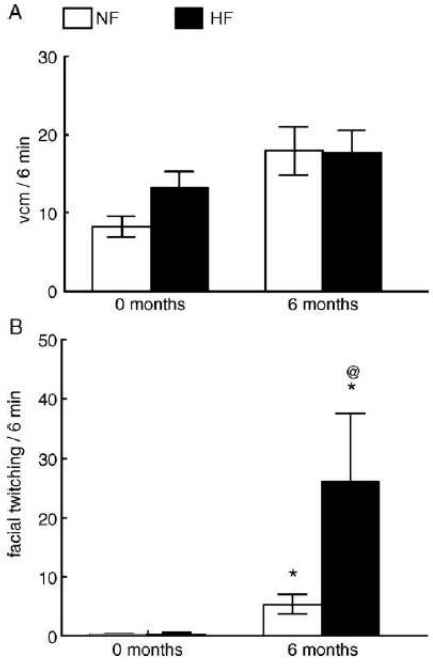

Fig. 2. Effects of the diet on orofacial dyskinesia. Vacuous chewing movements (A) and facial twitching (B) frequency in 6 min in the beginning of treatment (0 months) and after 6 months of treatment with the NF and HF diet. The values are indicated as mean ± S.E.M. @Indicates a significant difference between the groups ($p<0.05$) and *indicates a significant difference into the same diet in relation to the beginning of treatment by ANOVA.

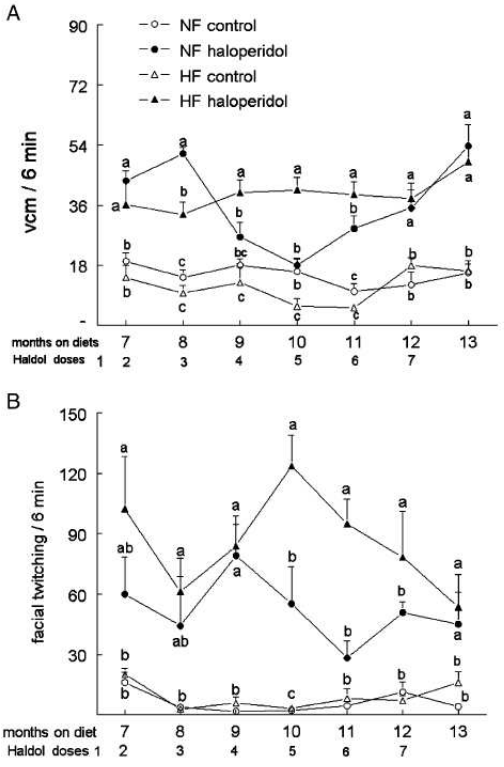

Fig. 3. Effects of haloperidol (1 mg/kg/day) or vehicle (vegetable oil) long-term administration on vacuous chewing movements (A) and facial twitching (B) frequency concomitant of treatment with NF and HF diet. Values are express as means. Symbols indicates a significant difference between means of groups ($p<0.05$) in the same behavioral session. Two-way ANOVA following by Duncan's tests.

Two-way ANOVA of cortical TBARS levels revealed a significant main effect of diet ($F(1,26)=7.73$ and $p<0.01$), indicating that HF increased oxidative stress in this region. In fact, the levels of TBARS in HF group (575.8 ± 40.2) was significantly higher than that of NF group (442.3 ± 13.2). However, post hoc comparisons by Duncan's multiple range test indicated a significant difference only between rats treated with haloperidol. Two-way ANOVA of striatal TBARS levels revealed a significant interaction of diet and haloperidol ($F(1,26)=6.6$ and $p<0.05$), indicating that HF associated with haloperidol administration increased oxidative stress in this region (Fig. 4).

Post hoc comparisons by Duncan's multiple range test indicated a significant difference in the group HF treated haloperidol than others groups. Two-way ANOVA of subcortical TBARS levels revealed a significant interaction of diet and haloperidol ($F(1,26)=8.94$ and $p<0.01$), indicating that HF ingestion associated with haloperidol administration increased oxidative stress in this region of the brain. Two-way ANOVA of TBARS levels of the

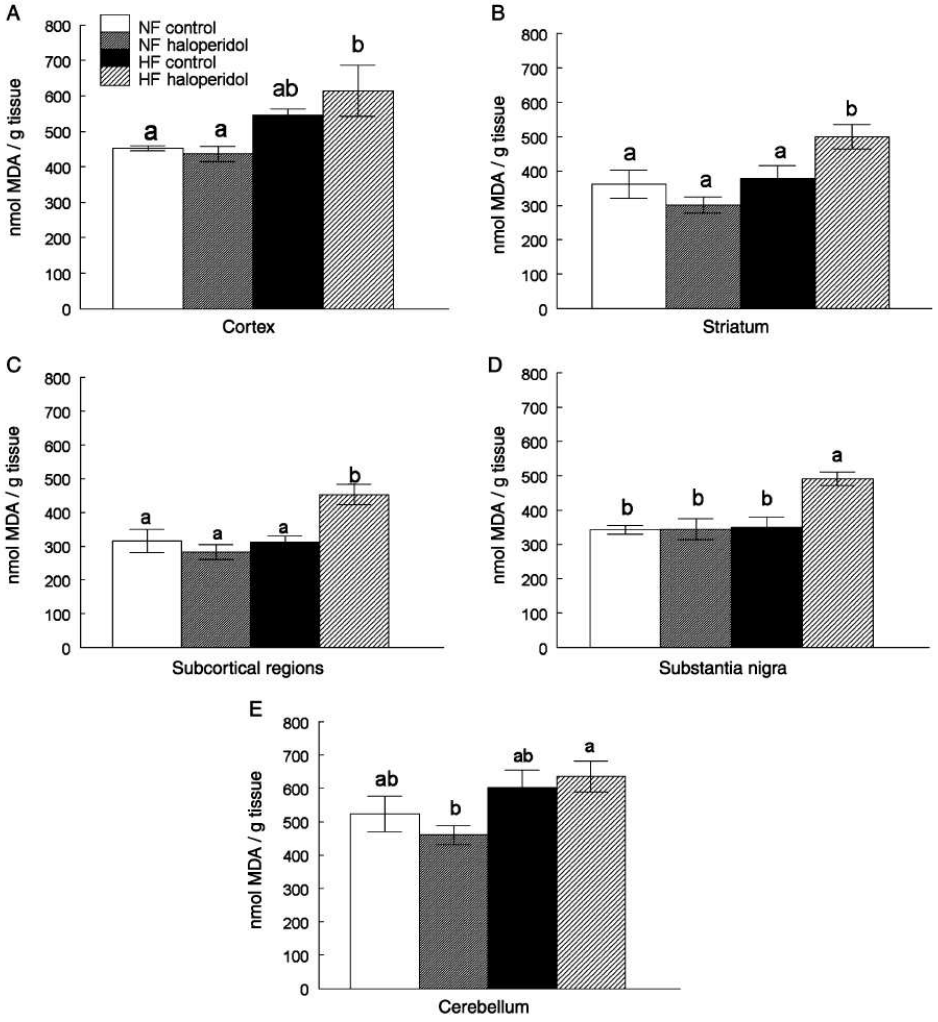

Fig. 4. Thiobarbituric acid-reactive species (TBARS) concentrations in cerebral cortex (A), striatum (B), subcortical parts (C), region of substantia nigra (D) and cerebellum (E) in rats after 13 months in a NF or HF diet and after treatment with haloperidol for 7 months. Values are means±S.E.M. Symbols represent significant differences between the groups ($p<0.05$). Two-way ANOVA followed by Duncan's multiple range tests.

region containing the substantia nigra revealed a significant interaction of diet and haloperidol ($F(1,26)=9.72$ and $p<0.01$), indicating that HF increased oxidative stress in this region. Two-way ANOVA of TBARS levels of cerebellum revealed a significant main effect of diet ($F(1,26)=3.27$ and $p<0.01$), indicating that HF increased oxidative stress in this region of brain. In fact, TBARS levels in cerebellum of rats fed the HF (608.4±33.6) were significantly higher than that detected in cerebellum of rats raised on NF (493.3±31.7). However, post hoc comparisons by Duncan's multiple range test indicated a significant difference only between rats treated with haloperidol.

4. Discussion

The results of the present study indicate that the high fat diet caused an increase in the incidence of OD in rats. However, this effect was transitory and disappeared one month latter. Additionally, we observed that a concomitant ingestion of the high fat diet with haloperidol administration

resulted in an increase in OD in rats. This effect was also transitory and disappeared when the treatment with haloperidol continued. For the case of VCM, the disappearance resulted from an increase in the OD of rats maintained in the normal diet. For the case of facial twitching, the disappearance was a consequence of a more complex change in OD in both groups. One factor that could contribute to modify the level of FT is the reduction in food intake after prolonged haloperidol administration. In fact, the haloperidol effects are complicated by the fact that the treatments caused a relative loss in body weight which was most exaggerated in the haloperidol plus high fat diet group. The uncontrolled effect of bodyweight loss could in part explain some of the variations listed above. This hypothesis, although tentative, is in accordance with expanding literature data indicating that food restriction reduces the production of oxidative stress in mammals (Armeni et al., 2003) and can also release neurotrophic factors (Mattson, 2000). These results indicate that the two behavioral measures did not necessarily reflect the same pathophysiological effect of neuroleptics and indicate that they can be independently modulated by exogenous factors, including aging and food ingestion. In line with this, in a previous study we observed that ebselen (Burger et al., 2003), an antioxidant agent, affected in different ways these behavioral measures in rats exposed to reserpine.

Literature data indicates that exposure to haloperidol causes an increase in cerebral oxidative stress (Andreasen and Jorgensen, 2000; Casey, 1995; Lohr, 1991; Clow et al., 1980; Slivka and Cohen, 1985; Tse et al., 1976; Abílio et al., 2002, 2003) that may be causally linked to an increase in orofacial dyskinesia after neuroleptic treatment. The results of the present investigation indicated that long-term consumption of the high fat diet caused an increase in oxidative stress in cerebral cortex and cerebellum, as indicated by a significant effect of diet regardless of the haloperidol treatment. Of particular importance for OD, haloperidol caused an increase in TBARS production in the high fat diet group specifically in the regions of the brain that are thought to be involved in the genesis of tardive dyskinesia (Lohr et al., 2003; Tsai and Ikonomidou, 1995), i.e., striatum and the region containing the substantia nigra. However, the increase in TBARS production in these regions cannot exclusively account for the increase in the OD, because there were no significant differences in the OD parameters between the two dietary groups treated with haloperidol in the end of the observation period.

Taken together the results of the present investigation indicate that high fat diets ingestion for a long period can have some transitory behavioral effects on rats. Furthermore, here we demonstrated for the first time that simultaneous ingestion of high fat diet and chronic haloperidol administration caused transitory exacerbation of orofacial dyskinesia in rats, however the animal model cannot to reflect the same effects in humans. Although literature data indicate that neuroleptic-induced orofacial dyskinesia is associated with oxidative stress, here we are unable to establish such correlation, because haloperidol increased the brain oxidative stress only in rats maintained on a high fat diet and the incidence of OD was similar between the high fat and normal fat diets groups. One explanation to these finds may reside on an anticipation of oxidative stress in high fat diet fed rats treated with haloperidol that is previous to development of OD in rats. This is agreement with Andreassen et al. (1998) and Calvent et al. (2002) where the nitropropionic acid administration can potentiate the orofacial dyskinesia of rats. The disappearance of differences between NF and HF diet groups may be a consequence of a complex interaction with other factors that affect OD in rodents, particularly the age of the animals (Kane and Smith, 1982; Woerner et al., 1991; Yassa and Jeste, 1992) or others compensatory mechanisms such as neurotransmission plasticity that could follow the neuronal consequences of oxidative stress and could also be influenced by aging.

5. Conclusion

In conclusion, the results of the present investigation demonstrated that high fat diet ingestion can enhance the OD produced by a typical neuroleptic used for the treatment of schizophrenia. It is known that schizophrenic patients eat more fat than the general population (Brown et al., 1999); however, there are no data in the literature indicating that the incidence of TD is more frequent in those schizophrenic patients eating diets with high content of fat.

Although it is still premature to extrapolate the relevance of our findings to man, we realize that epidemiological studies should be carried out to determine a possible increase in the incidence of TD in patients fed with more fatty diets. However, we must emphasize that the animal model used here did not replicate the situation found in humans. In fact, the chronic use of haloperidol and others neuroleptics are frequently associated with obesity in schizophrenic patients and in our rat model haloperidol cause loss of weight.

In spite of this, haloperidol-treated rats showed OD, indicating that in rats obesity is not a mandatory factor for the development of OD.

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References

Abílio VC, Vera JAR, Ferreira LSM, Duarte CRM, Carvalho RC, Grassl CC, et al. Effects of melatonin on orofacial movements in rats. Psychopharmacology 2002;161:340–7.

Abílio VC, Araújo CC, Bergamo M, Calvente PR, D'Almeida V, Ribeiro R, et al. Vitamin E attenuates reserpine-induced oral dyskinesia and striatal oxidized glutathione/reduced glutathione ratio (GSSG/GSH) enhancement in rats. Prog Neuro-Psychopharmacol Biol Psychiatry 2003;2: 7109–14.

Abílio VC, Silva RH, Carvalho RC, Grassl C, Calzavara MB, Registro S, et al. Important role of striatal catalase in aging- and reserpine-induced oral dyskinesia. Neuropharmacology 2004;47:263–72.

Adler LA, Peselow E, Rotrosen J, Duncam E, Lee M, Rosenthal BA, et al. Vitamine E treatment of tardive dyskinesia. Am J Psychiatry 1993;150: 1405–7.

Andreasen OA, Jorgensen HA. Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats Implications for tardive dyskinesia? Prog Neurobiol 2000;61:525–41.

Andreassen OA, Ferrante RJ, Beal MF, Jorgensen HA. Oral dyskinesias and striatal lesions in rats after long-term co-treatment with haloperidol and 3-nitropropionic acid. Neuroscience 1998;87:639–48.

Andreassen OA, Ferrante RJ, Aamo TO, Beal MF, Jorgensen HA. Oral dyskinesias and histopathological alterations in substantia nigra after long-term haloperidol treatment of old rats. Neuroscience 2003;122: 717–25.

Andriguetto JM. Normas e padrões de nutrição e alimentação. Curitiba, Brazil: Nutrição editora e publicitária LTDA; 1986. p. 1–3.

Armeni T, Principato G, Quiles JL, Pieri C, Bompadre S, Battino M. Mitochondrial dysfunctions during aging: vitamin E deficiency or caloric restriction—two different ways of modulating stress. J Bioenerg Biomembranes 2003;35:181–91.

Brown K, Reid A, White T, Hukin S, Jonhstone C, Glen A. Vitamin E, lipids, and lipid peroxidation products in tardive dyskinesia. Biol Psychiatry 1998;43:863–7.

Brown S, Birtwistle J, Roe L, Thompson C. The unhealthy lifestyle of people with schizophrenia. Psychol Med 1999;29:697–700.

Burger ME, Alves A, Callegari L, Athayde FR, Nogueira CW, Zeni G, et al. Ebselen attenuates reserpine-induced orofacial dyskinesia and oxidative stress in rat striatum. Prog Neuro-Psychopharmacol Biol Psychiatry 2003;27:135–40.

Burger ME, Fachinetto R, Calegari L, Paixão MW, Braga AL, Rocha JBT. Effects on age on orofacial dyskinesia reserpine-induced and possible protection of diphenyl–diselenide. Brain Res Bull 2004;64:339–45.

Burger ME, Fachinetto R, Alves A, Calegari L, Rocha JBT. Acute reserpine and subchronic haloperidol treatments change synaptosomal brain glutamate uptake and elicit orofacial dyskinesia in rats. Brain Res 2005;1031:202–10.

Cadet JL, Lohr JB, Jeste DV. Free radical and tardive dyskinesia. Trends Neurosci 1986;9:107–8.

Cadet JL, Lohr JB, Jeste DV. Tardive dyskinesia and schizophrenic burnout: the possible involvement of cytotoxic free radicals. In: Henn FA, DeLisi LE, editors. Handbook of schizophrenia: the neurochemistry and pharmacology of schizophrenia. Amsterdam: Elsevier; 1987. p. 425–38.

Calvent PRV, Araujo CCS, Bergamo M, Abilio VC, Almeida VD, Ribeiro RDA, et al. The mitochondrial toxin 3-nitropropionic acid aggravates reserpine-induced oral dyskinesia in rats. Prog Neuro-Psychopharmacol Biol Psychiatry 2002;26:401–5.

Casey DE. Tardive dyskinesia: reversible and irreversible. Psychopharmacology 1985;2:88–97.

Casey DE. Tardive dyskinesia—pathophysiology. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York: Raven Press, Ltd.; 1995. p. 1497–502.

Castilho RF, Ward W, Nicholls DG. Oxidative stress, mitochondrial function, and acute glutamate excitotoxicity in cultured cerebellar granule cells. J Neurochem 1999;72:1394–401.

Choi J, Jang E, Park C, Kang J. Enhanced susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in high-fat diet-induced obesity. Free Radic Biol Med 2005;38:806–16.

Clow A, Theodorou A, Jenner P, Marsden CD. Cerebral dopamine function in rats following withdrawal from one year of continuous neuroleptic administration. Eur J Pharmacol 1980;63:135–44.

Coyle JT, Puttfarcken P. Oxidative stress, glutamate and neurodegenerative disorders. Science 1993;262:689–95.

Crane GE. Persistent dyskinesia. Br J Psychiatry 1973;122:395–405.

Creese I, Burt D, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 1976;192:481–3.

Ebadi M, Srinivasan SK. Pathogenesis, prevention and treatment of neuroleptic-induced movement disorders. Pharmacol Rev 1995;47:575–604.

Egan MF, Hyde TM, Albers GW, Elkashef A, Alexander RC, Reeve A, et al. Treatment of tardive dyskinesia with vitamin E. Am J Psychiatry 1992;149:773–7.

Egan MF, Hurd Y, Ferguson J, Bachus SE, Hamid EH, Hyde TM. Pharmacological and neurochemical differences between acute and tardive vacuous chewing movements induced by haloperidol. Psychopharmacology 1999;127:337–45.

Folmer V, Soares JCM, Gabriel D, Rocha JBT. High-fat diet causes δ-aminolevulinate dehydratase inhibition and lipid peroxidation in mice (*Mus musculus*). J Nutr 2003;133:2165–70.

Gardos G, Cole JO. Neuroleptics and tardive dyskinesia in non schizophrenic patients. In: Casey DE, Cole G, editors. Tardive dyskinesia and neuroleptics: from dogma to reason. Washington, DC: American Psychiatric Press; 1986. p. 56–74.

Hagg S, Soderberg S, Ahren B, Olsson T, Mjorndal T. Leptin concentrations are increased in subjects treated with clozapine or conventional antipsychotics. J Clin Psychiatry 2001;62:843–8.

Hamid EH, Hyde TM, Baca SM, Egan MF. Failure to down regulate NMDA receptors in the striatum and nucleus accumbens associated with neuroleptic-induced dyskinesia. Brain Res 1998;796:291–5.

Haupt DW, Luber A, Maeda J, Melson AK, Schweiger JA, Newcomer JW. Plasma leptin and adiposity during antipsychotic treatment of schizophrenia. Neuropsychopharmacology 2005;30:184–91.

Henderson DC. Diabetes mellitus and other metabolic disturbances induced by atypical antipsychotic agents. Curr Diab Rep 2002;2:135–40.

Hunt JV, Dean RT, Wolff SP. Hydroxyl radical production and autoxidative glycosylation, glucose oxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and aging. Biochem J 1988;256:205–12.

Jeste DV, Potkin SG, Sinhá S, Feder S, Wyatt RJ. Tardive dyskinesia—reversible and persistent. Arch Gen Psychiatry 1979;36:585–90.

Kane JM, Smith JM. Tardive dyskinesia: prevalence and risk factors, 1959 to 1979. Arch Gen Psychiatry 1982;39:473–81.

Kraus T, Haack M, Schuld A, Hinze-Selch D, Kühn M, Uhr M, et al. Body weight and leptin plasma levels during treatment with antipsychotic drugs. Am J Psychiatry 1999;156:312–4.

Lohr JB. Oxygen free radicals and neuropsychiatric illness. Arch Gen Psychiatry 1991;48:1097–106.

Lohr JB, Kuczenski R, Bracha HS, Moir M, Jeste DV. Increased indices of free radical activity in the cerebrospinal fluid of patients with tardive dyskinesia. Biol Psychiatry 1990;28:535–9.

Lohr JB, Kuczenski R, Niculescu AB. Oxidative mechanisms and tardive dyskinesia. CNS Drugs 2003;17:47–62.

Mahadik PS, Evans D, Lal H. Oxidative stress and role of antioxidant and ω-3 essential fatty acid supplementation in schizophrenia. Prog Neurol Psychiatry 2001;25:463–93.

Mattson MP. Neuroprotective signaling and the aging brain: take away my food and let me run. Brain Res 2000;886:47–53.

Melkersson KI, Hulting AL, Brismar KE. Elevated levels of insulin, leptin, and blood lipids in olanzapine-treated patients with schizophrenia or related psychoses. J Clin Psychiatry 2000;61:742–9.

Morimoto T, Yamamoto Y, Mobarakeh JI, Yanai K, Watanabe T, Yamatadoni A. Involvement of the histaminergic system in leptin-induced suppression of food intake. Physiol Behav 1999;67:679–83.

Mukherjee S, Decina P, Bocola V, Saraceni F, Scapicchio PL. Diabetes mellitus in schizophrenic patients. Compr Psychiatry 1996;37:68–73.

Naidu PS, Singh A, Kaur P, Sandhir R, Kulkarni SK. Possible mechanism of action in melatonin attenuation of haloperidol-induced orofacial dyskinesia. Pharmacol Biochem Behav 2003;74:641–8.

Naidu PS, Singh A, Kaur P, Kulkarni SK. Quercetin, a bioflavonoid, attenuates haloperidol-induced orofacial dyskinesia. Neuropharmacology 2003;44:1100–6.

Ohkawa H, Ohishi H, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.

Polydoro M, Schröder N, Lima MNM, Caldana F, Laranja D, Bromberg E, et al. Haloperidol- and clozapine-induced oxidative stress in the rat brain. Pharmacol Biochem Behav 2004;78:751–6.

Rossato JI, Ketzer LA, Centurião FB, Silva SJN, Lûdtke DS, Zeni G, et al. Antioxidant properties of new chalcogenides against lipid peroxidation in rat brain. Neurochem Res 2002;27:297–303.

See RE, Lynch AM. Duration dependent increase in striatal glutamate following prolonged fluphenazine administration in rats. Eur J Pharmacol 1996;308:279–82.

Simpson MM, Goetz RR, Devlin MJ, Goetz SA, Walsh BT. Weight gain and antipsychotic medication: differences between antipsychotic-free and treatment periods. J Clin Psychiatry 2001;62:694–700.

Singh A, Naidu PS, Kulkarni SK. Possible antioxidant and neuroprotective mechanisms of FK-506 in attenuating haloperidol-induced orofacial dyskinesia. Eur J Pharmacol 2003;477:87–94.

Slivka A, Cohen G. Hydroxyl radical attack on dopamine. J Biol Chem 1985;260:15466–72.

Storlien LH, James DE, Burleigh KM, Crisholm DJ, Kraegen EW. Fat feeding causes widespread in vivo insulin resistance, decrease energy expenditure, and obesity in rats. Am J Physiol 1986;251:E576–83.

Storlien LH, Higgins JA, Thomas TC, Brown MA, Wang HQ, Huang XF, et al. Diet composition and insulin action in animal models. Br J Nutr 2000;83:S85–90.

Tsai L, Ikonomidou C. Toward an understanding of tardive dyskinesia: chronic haloperidol enhances NMDA toxicity in mouse striatum. Soc Neurosci Abstr 1995;1721:1744.

Tsai G, Goff DC, Change RW, Flood J, Bair L, Coyle JT. Markers of glutamatergic neurotransmission and oxidative stress associated with tardive dyskinesia. Am J Psychiatry 1998;155:1207–13.

Tse DCE, McCreery RL, Adams RN. Potential oxidative pathways of brain catecholamines. J Med Chem 1976;19:37–40.

Vilner BJ, Costa BR, Bowen WD. Cytotoxic effects of sigma ligands: sigma receptor-mediated alterations in cellular morphology and viability. J Neurosci 1995;15:117–34.

Walker JM, Bower WD, Walker FO, Matsumoto RR, De Costa B, Rice CK. Sigma receptors: biology and function. Pharmacol Rev 1990;42:355–402.

Warwick ZS, Schiffman SS. Role of dietary fat in calorie intake and weight gain. Neurosci Biobehav Rev 1992;16:585–96.

Woerner MG, Kane JM, Lieberman JA, Alvir J, Bergmann KJ, Borenstein M, et al. The prevalence of tardive dyskinesia. J Clin Psychopharmacol 1991;11:34–42.

Wolff SP, Dean RT. Glucose autoxidation and protein modification—the potential role autoxidative glycosylation in diabetes. Biochem J 1987;245:243–50.

Yassa R, Jeste DV. Gender differences in tardive dyskinesia: a critical review of the literature. Schizophr Bull 1992;18:701–15.

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Valeriana officinalis does not alter the orofacial dyskinesia induced by haloperidol in rats: Role of dopamine transporter

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Abstract

Chronic treatment with classical neuroleptics in humans can produce a serious side effect, known as tardive dyskinesia (TD). Here, we examined the effects of *V. officinalis*, a medicinal herb widely used as calming and sleep-promoting, in an animal model of orofacial dyskinesia (OD) induced by long-term treatment with haloperidol. Adult male rats were treated during 12 weeks with haloperidol decanoate (38 mg/kg, i.m., each 28 days) and with *V. officinalis* (in the drinking water). Vacuous chewing movements (VCMs), locomotor activity and plus maze performance were evaluated. Haloperidol treatment produced VCM in 40% of the treated rats and the concomitant treatment with *V. officinalis* did not alter either prevalence or intensity of VCMs. The treatment with *V. officinalis* increased the percentage of the time spent on open arm and the number of entries into open arm in the plus maze test. Furthermore, the treatment with haloperidol and/or *V. officinalis* decreased the locomotor activity in the open field test. We did not find any difference among the groups when oxidative stress parameters were evaluated. Haloperidol treatment significantly decreased [^{3}H]-dopamine uptake in striatal slices and *V. officinalis* was not able to prevent this effect. Taken together, our data suggest a mechanism involving the reduction of dopamine transport in the maintenance of chronic VCMs in rats. Furthermore, chronic treatment with *V. officinalis* seems not produce any oxidative damage to central nervous system (CNS), but it also seems to be devoid of action to prevent VCM, at least in the dose used in this study.

Keywords: Dopamine uptake; Haloperidol; Medicinal plant; Oxidative stress; Tardive dyskinesia; *V. officinalis*

1. Introduction

Neuroleptic drugs are used in the treatment of severe psychiatric disorders, especially schizophrenia. Haloperidol is a classical or typical neuroleptic that is widely used in the treatment of schizophrenic patients. Its pharmacological action involves the blockage of dopamine D_2 receptors (Creese et al., 1976). However, chronic use of haloperidol can be associated with the development of TD in 20–25% of the patients (Kane and Smith, 1982) and its prevalence increases strongly with age (Kane and Smith, 1982; Woerner et al., 1991; Yassa and Jeste, 1992). TD is characterized by involuntary and abnormal movements of the orofacial region, and sometimes, trunk and

Abbreviations: CNS, central nervous system; CSF, cerebro spinal fluid; DCF, 2',7'-dichlorofluorescein; DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate; DNPH, 2,4-dinitrophenylhydrazine; GABA, γ-aminobutyric acid; Halo, Haloperidol; OD, orofacial dyskinesia; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TD, tardive dyskinesia; Val, *Valeriana officinalis*; VCMs, vacuous chewing movements; *V. officinalis*, *Valeriana officinalis*.

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members musculature that can appear during treatment with neuroleptic drugs or after its withdrawal (Kane, 1995). The main clinical problem is that TD is irreversible in the majority of the cases (Casey, 1985; Crane, 1973; Jeste et al., 1979).

Although some hypotheses have been postulated to explain at molecular and cellular levels this syndrome, the exact mechanisms involved in TD development remain unclear. The classical hypothesis to explain TD development is that typical neuroleptics can develop dopaminergic supersensitivity after its chronic use (Andreassen and Jorgensen, 2000; Burt et al., 1977; Klawans and Rubovits, 1972; Rubinstein et al., 1990). The chronic blockage of dopamine receptors for these drugs can produce a compensatory increase in the number and the sensitivity of dopaminergic receptors, which could culminate in a hyperdopaminergic state and clinical symptoms, such as TD (Cavallero and Smeraldi, 1995; Kane, 1995). Besides receptors, the dopamine transporter (DAT) could be also implicated in TD development since dopamine uptake by DAT is the primary pathway for the clearance of extracellular dopamine and consequently for the regulation of the magnitude and duration of dopaminergic signaling (Beckman and Quick, 1998; Kahlig and Galli, 2003). In fact, it has been demonstrated that TD patients present reduced levels of DAT (Yoder et al., 2004). Others neurochemical hypothesis has been proposed for the development of TD during the last decades. They include disturbed balance between dopaminergic and cholinergic systems, dysfunction of striatonigral GABAergic neurons, excitotoxicity promoted by glutamate and overproduction of free radicals (Andreassen and Jorgensen, 2000; Cadet et al., 1986, 1987; Lohr, 1991).

Valerian root (*Valeriana officinalis L.*, Valerianaceae) has been used for centuries as a calming and sleep-promoting herb (McCabe, 2002; Morazzoni and Bombardelli, 1995) and it is among the most widely used medicinal herbs (Fugh-Berman and Cott, 1999). Although its exact mechanism of action is not well understood, studies indicate that the CNS effect of valerian might occur through interaction with GABA, melatonin, adenosine or serotonin systems in the brain (Abourashed et al., 2004). These data suggests that *V. officinalis* could be useful for TD treatment since several of its pharmacological targets are related with TD development (Araujo et al., 2005; Fibiger and Lloyd, 1984; Morselli et al., 1985; Peixoto et al., 2004; Raghavendra et al., 2001; Rosengarten et al., 2006; Tamminga et al., 1979).

Thus, the aims of the present study were to investigate the possible action of the *V. officinalis* on a chronic model of OD induced by long-term treatment with haloperidol and also to investigate the role of dopamine uptake in maintenance of OD induced by chronic treatment with haloperidol in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 270–320 g and with age from 3 to 3.5 months, from our own breeding colony were kept in cages of 3 or 4 animals each, with continuous access to foods and *V. officinalis* or its vehicle (ethanol 1%) in a room with controlled temperature (22±3 °C) and on a 12-h light/dark cycle with lights on at 7:00 am. The animals were maintained and used in accordance to the guidelines of the Brazilian Association for Laboratory Animal Science (COBEA).

2.2. Drugs

Haloperidol decanoate was a gift from Cristália (São Paulo, Brazil). A standard tincture of *V. officinalis* (10 g of valerian roots per 100 mL of ethanol) was obtained from Bio extracts (São Paulo, Brazil).

2.3. Treatments

The rats were divided into four groups: control group received soy oil (that was the haloperidol vehicle, i.m.) and ethanol 1% (in the drinking water, that was *V. officinalis* vehicle); *V. officinalis* group received soy oil (i.m.) and *V. officinalis* 1% (in the drinking water); haloperidol group received haloperidol decanoate (i.m.) and ethanol 1% (in the drinking water); and haloperidol plus *V. officinalis* group received haloperidol decanoate (i.m.) and *V. officinalis* 1% (in the drinking water). The number of animals in each group that received treatment was 12, 14, 10 and 14 for control, *V. officinalis*, haloperidol and haloperidol plus *V. officinalis* groups, respectively. Haloperidol decanoate (a slow-releasing preparation of haloperidol) or its vehicle were administered intramuscularly (i.m.) every 28 days (38 mg/Kg, i.m.) that is equivalent to 1 mg/kg/day of unconjugated haloperidol. *V. officinalis* was administered in the drinking water in a proportion of 1% (final concentration of 100 mg/mL). The dosage was calculated every week by the amount of water drunk assuming equal drinking among the four animals. Thus, each animal received *V. officinalis* extract in a dosage about 200–250 mg/Kg/day.

V. officinalis and its vehicle were placed daily before the beginning of the dark cycle. It was not observed a reduction in liquid intake among the groups (data not shown).

V. officinalis treatment started 15 days before the administration of haloperidol. The treatment with haloperidol was carried out during 12 weeks concomitantly with *V. officinalis*.

2.4. Behavioral analysis

2.4.1. Quantification of VCMs

Behavior measurement of VCMs was assessed before the treatment with haloperidol or its vehicle (basal evaluation), as previously described. The effect of drugs on behavior was examined every 15 days beginning on the 15th day after the first haloperidol injection (that occurred on same day of the basal behavior) during a period of 12 weeks. To quantify the occurrence of VCMs, rats were placed individually in cages (20×20×19 cm) and hand operated counters were employed to quantify VCMs frequency. VCMs are defined as single mouth openings in the vertical plane not directed towards physical material. If VCMs occurred during a period of grooming they were not taken into account. The behavioral parameters of OD were measured continuously for 6 min after a period of

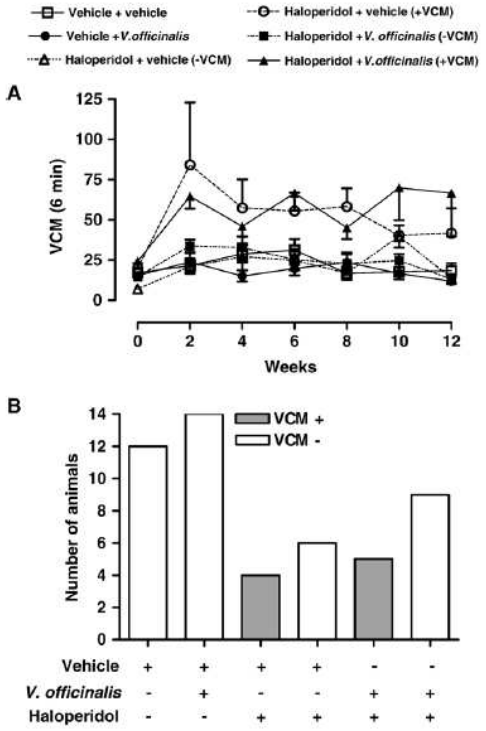

Fig. 1. Effects of *V. officinalis* on haloperidol-induced orofacial dyskinesia. A) Number of vacuous chewing movements (VCM) for 6 min during long-term treatment. Values are presented as means ± S.E.M. (Control, $n=12$; *V. officinalis*, $n=14$; haloperidol (−VCM), $n=6$; haloperidol (+VCM), $n=4$; haloperidol + *V. officinalis* (−VCM), $n=9$; haloperidol + *V. officinalis* (+VCM), $n=5$). B) Prevalence of VCMs in rats under long-term treatment with haloperidol and/or *V. officinalis*.

6 min adaptation. During the observation sessions, mirrors were placed under the floor of the experimental cage to permit observation when the animal was faced away from the observer. Experimenters were always blind to treatments.

It was previously reported that the treatment with neuroleptic drugs does not result in the development of VCMs in all treated rats (Kane and Smith, 1982; Shirakawa and Tamminga, 1994). In the present study, we have also verified similar results about the prevalence of neuroleptic-induced VCMs. In our laboratory, control rats present maximally 40 VCMs during a period of 6 min. Thus, in this study, we analyzed the rats that developed neuroleptic-induced VCM (+VCM, more than 40 VCMs) separately from those that did not develop neuroleptic-induced VCM (−VCMs, less than 40 VCMs), as described previously (Andreassen et al., 2003; Egan et al., 1994; Shirakawa and Tamminga, 1994).

2.4.2. Open field test

To analyze changes in spontaneous locomotor activity caused by treatment with haloperidol and/or *V. officinalis*, the animals were placed individually in the center of an open-field arena (40 × 40 × 30 cm) with black plywood walls and a white floor divided into 9 equal squares, as previously described (Broadhurst, 1960). The number of line crossings was measured over 2 min and taken as an indicator of locomotor activity.

2.4.3. Elevated plus maze

To evaluate the anxiety-like state caused by treatment with haloperidol and/or *V. officinalis*, animals were exposed to an elevated plus maze (Chopin et al., 1985; Pellow et al., 1985). The number of head dippings and the time spent into open or closed arms were recorded over a 2 min session. The percentage of the time spent on open arm and the percentage of the entries into the open arms were calculated, as follows: time spent or number of entries into the open arm/total time or total number of the entries into closed and open arm X 100, respectively.

2.5. Tissue preparations

Rats were killed about 24 h after the last session of behavioral quantification (on the 28th day after the last administration of haloperidol). The brains were immediately excised and put on ice. The cortex, striatum and region containing the substantia nigra were separated, weighed and homogenized in 10 volumes (w/v) of 10 mM Tris–HCl, pH 7.4. A portion of the striatum was dissected for slices used for the [^{3}H] dopamine uptake assay.

2.5.1. [^{3}H] dopamine uptake

[^{3}H] dopamine uptake was carried out as described by Holz and Coyle (1974) with some modifications. To measure [^{3}H] dopamine uptake, the striatum was cut into 400 μm slices, which were washed with a buffered solution (1) consisting of 127 mM NaCl, 1.2 mM Na_2HPO_4, 5.36 mM KCl, 0.44 mM

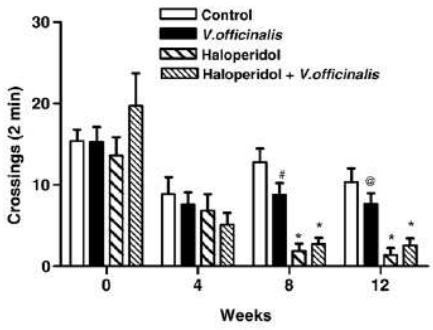

Fig. 2. Effects of *V. officinalis* on open field test in rats. Number of crossings in 2 min. Values of number of crossings are presented as means ± S.E.M. (Control, $n=12$; *V. officinalis*, $n=14$; haloperidol, $n=10$; haloperidol + *V. officinalis*, $n=14$). One way ANOVA followed by Duncan's multiple range tests. * represents significant differences from control group and # represents significant differences from control, haloperidol and haloperidol plus *V. officinalis* groups. @ represents significant differences from haloperidol and haloperidol plus *V. officinalis* groups.

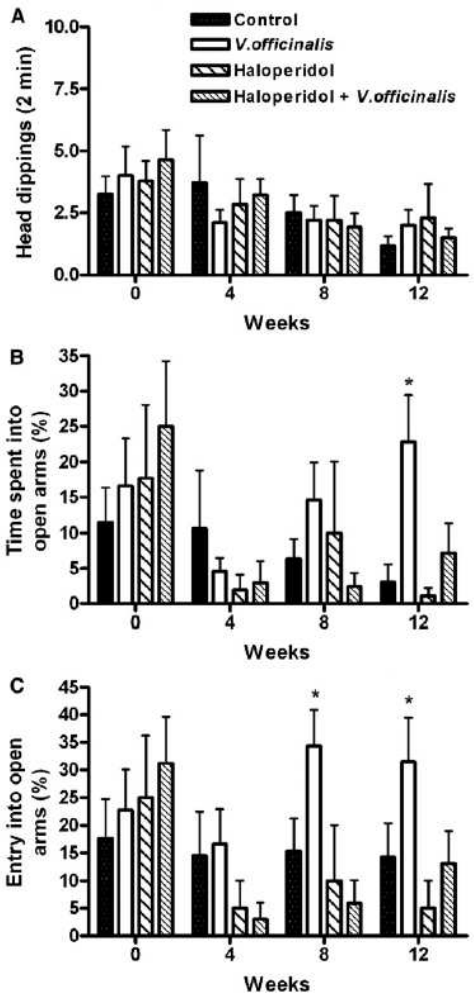

Fig. 3. Effects of *V. officinalis* and haloperidol on plus maze test in rats. A) Number of head dippings for 2 min, B) percentage of the time spent on the open arms for 2 min, C) percentage of entries into the open arms. Values are presented as means±S.E.M. (Control, $n=12$; *V. officinalis*, $n=14$; haloperidol, $n=10$; haloperidol+*V. officinalis*, $n=14$). One way ANOVA followed by Duncan's multiple range tests. * represents significant differences among the groups into the same period of observation.

KH_2PO_4, 0.95 mM $MgCl_2$, 0.70 mM $CaCl_2$, 10 mM glucose, and 1 mM Tris–HCl, pH 7.4. Slices (0.2–0.3 mg protein) were further pre-incubated in 96 well-polycarbonate plates for 15 min at 35 °C with the buffered solution plus selegiline 1 μM. [^{3}H] dopamine was added to the incubation medium and uptake was carried out for 10 min at 35 °C, after which the reaction was stopped by five washes of 30 s each with 1 mL of iced-cold solution 1, containing 1 μM selegiline and 100 μM cocaine. Immediately after washing, 0.25 mL of 0.5 M NaOH and 0.2% sodium dodecyl sulfate (SDS) was added to the slices that were digested by 10 min incubation at 60 °C. Aliquots of the lysates were taken for protein content measurement by the Lowry et al. (1951) method. For determination of the intracellular amount of dopamine, liquid scintillation counting was used. Results were expressed as [^{3}H] dopamine uptake per mg of protein.

2.5.2. Oxidative stress parameters

To evaluate the levels of reactive oxygen species (ROS), the homogenates were centrifuged for 10 min at 1500 ×g. Just after the centrifugation, an aliquot of supernatant was used for 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) oxidation. DCFH-DA-oxidation was determined spectrofluorimetrically, using 7 μM of DCFH-DA. Fluorescence was determined at 488 nm for excitation and 520 nm for emission. A standard curve was carried out using increasing concentrations of 2',7'-dichlorofluorescein (DCF) incubated in parallel (Pérez-Severiano et al., 2004). The results were analyzed as percentage in relation to control group.

To assess lipid peroxidation, we quantified thiobarbituric acid reactive substances (TBARS). The homogenates were centrifuged for 10 min at 1500× g. Just after the centrifugation, an aliquot of 200 μL or of supernatant was incubated for 1 h at 37 °C and then used for lipid peroxidation quantification as earlier described (Ohkawa et al., 1979).

To verify protein carbonyl, cortical and nigral tissue were homogenized in 10 volumes (w/v) of 10 mM Tris–HCl buffer pH 7.4. The protein carbonyl content was determined by the method described by Yan et al. (1995), with some modifications. Briefly, homogenates were diluted 1:8 in 10 mM Tris–HCl buffer pH 7.4 and 1 mL aliquots were mixed with 0.2 mL of 2,4-dinitrophenylhydrazine (10 mM DNPH) or 0.2 mL HCl (2 M). After incubation at room temperature for 1 h in a dark ambient, 0.5 mL of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing 3% SDS), 2 mL of heptane (99.5%) and 2 mL of ethanol (99.8%) were added sequentially, and mixed with vortex agitation for 40 s and centrifuged for 15 min. After that, the protein isolated from the interface was washed two times with 1 mL of ethyl acetate/ethanol 1:1 (v/v) and suspended in 1 mL of denaturing buffer. Each DNPH sample was read at 370 nm against the corresponding HCl sample (blank), and total carbonylation calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1} according to Levine et al. (1990).

To verify superoxide dismutase (SOD) activity, cortex, striatum or substantia nigra were adequately diluted to 40 volumes with Tris–HCl 10 mM (pH 7.5) and the assay was performed according to the method of Misra and Fridovich (1972). Briefly, epinephrine rapidly auto oxidizes at pH 10.2 producing adrenochrome, a pink colored product that can be detected at 480 nm. The addition of samples (10, 25, 50 μL) containing SOD inhibits the auto-oxidation of epinephrine. The rate of inhibition was monitored during 180 s at intervals of 30 s. The amount of enzyme required to produce 50% inhibition at 25 °C was defined as one unit of enzyme activity. The SOD activity was expressed as units/g of protein.

Table 1
Effects of haloperidol and *V. officinalis* treatments on oxidative stress parameters (mean±S.E.M; with control, $n=12$; *V. officinalis*, $n=14$; haloperidol (−VCM), $n=6$; haloperidol (+VCM), $n=4$; haloperidol+*V. officinalis* (−VCM), $n=9$; haloperidol+*V. officinalis* (+VCM), $n=9$) (Val, *V. officinalis* treatment; Halo, Haloperidol treatment; −VCM; +VCM)

Brain regions	Lipid peroxidation	ROS levels	SOD activity	Protein carbonyl
	(nmol of MDA/g tissue)	(% of control)	(U/mg protein)	(nmol carbonyl/mg protein)
Cortex				
Control	95.5±5.7	112.0±16.5	377.5±39.6	26.8±3.0
Val	93.9±6.4	92.8±6.1	434.1±29.9	25.6±2.7
Halo−VCM	89.0±9.9	84.1±9.8	348.7±65.4	30.2±2.3
Halo+VCM	85.5±6.1	87.0±13.4	426.6±97.7	28.0±5.2
Val+Halo−VCM	104.5±8.2	88.0±5.5	382.0±60.8	33.0±3.6
Val+Halo+VCM	99.2±15.9	107.8±11.6	425.1±109.4	34.7±10.8
Striatum				
Control	62.2±6.7	107.5±16.7	722.7±77.1	–
Val	64.9±6.1	95.1±8.4	781.7±48.2	–
Halo−VCM	49.6±2.4	131.1±22.3	721.3±92.4	–
Halo+VCM	50.4±7.2	83.0±21.8	614.8±93.9	–
Val+Halo−VCM	55.9±7.2	103.7±8.9	653.8±92.2	–
Val+Halo+VCM	49.4±4.9	143.8±27.5	787.3±124.0	
Substantia nigra				
Control	88.9±10.5	75.1±9.0	388.8±56.4	29.7±3.4
Val	76.6±3.9	87.1±4.2	459.3±52.1	30.7±3.1
Halo−VCM	65.9±2.9	87.5±9.6	400.3±46.5	34.8±9.6
Halo+VCM	66.8±9.3	97.5±9.9	533.0±39.3	24.4±0.6
Val+Halo−VCM	82.2±9.8	89.1±4.2	433.8±59.4	30.5±3.3
Val+Halo+VCM	80.5±10.0	100.6±11.5	481.1±56.3	28.1±3.1

Protein content was measured by method of Lowry et al. (1951) and bovine serum albumin was used as standard.

2.6. Statistical analysis

Data from behavioral parameter were analyzed by one-way or two-way ANOVA. F values are presented in the text only if p value associated with it was <0.05. Prevalence data were analyzed by the Chi-square test. Data from TBARS, ROS quantification, SOD activity, carbonyl content and [^{3}H] dopamine uptake were analyzed by one-way ANOVA, followed by Duncan's Post Hoc tests when appropriate. A possible relationship between oxidative stress parameters, VCM, and [^{3}H] dopamine uptake were also determined using linear regression analysis using SPSS 10.1 for Windows. Significance was considered when $p<0.05$.

3. Results

3.1. Effects of V. officinalis on VCMs induced by long-term treatment with haloperidol

Haloperidol caused a marked increase on VCMs when compared with its vehicle ($F(5,44)=10.41$, $p<0.001$; Fig. 1A). In fact, a significant interaction between haloperidol and time treatment ($F(30,264)=2.27$ and $p<0.001$) was observed in this case. Treatment with haloperidol induced a VCMs prevalence of 40% compared to its vehicle (Chi-square=4.05; $p<0.05$; Fig. 1B), with 4 out of 10 animals actually having VCMs. The treatment with *V. officinalis* was not able to reduce neither the prevalence nor the intensity of VCMs in those rats that developed VCMs. In fact, the co-treatment of haloperidol with *V. officinalis* developed VCMs in 35.7% of the rats.

3.2. Effects of long-term treatment with V. officinalis and haloperidol on locomotor activity in rats

Haloperidol caused a marked and time-dependent decrease on locomotor activity, represented by the number of crossings in the open field test. In fact, a significant interaction between haloperidol x time treatment ($F(3,138)=12.12$ and $p<0.001$) was observed. *V. officinalis* administered alone also caused a significant decrease in locomotor activity only 8 weeks after haloperidol administration ($F(3,46)=15.43$ and $p<0.001$ (Fig. 2). The effect of concomitant treatment with *V. officinalis* and haloperidol was similar to haloperidol treated group.

3.3. Effects of long-term treatment with V. officinalis and haloperidol on plus maze test in rats

There was a significant effect of the time on head dippings ($F(3,138)=5,72$ and $p<0.05$; Fig. 3A). Long-term treatment with haloperidol did not cause any effect on head dippings in rats. Similarly, *V. officinalis* alone or with haloperidol also did not cause any effect on this parameter.

Long-term treatment with haloperidol did not cause any effect neither in the percentage of the time spent on open arm nor in the percentage of entries into the open arm when compared to

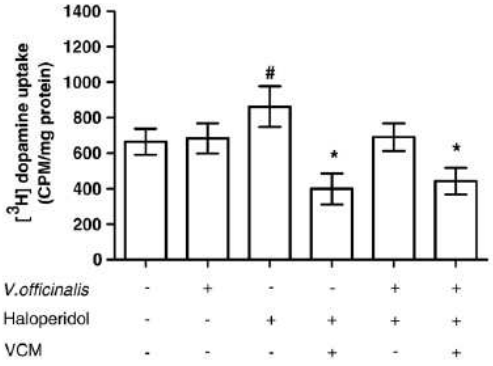

Fig. 4. Effects of long-term treatment with haloperidol and *V. officinalis* on [^{3}H] dopamine uptake (CPM/mg protein) in slices from striatum of rats. Data (mean±SEM, with control, n=12; *V. officinalis*, n=14; haloperidol (−VCM), n=6; haloperidol (+VCM), n=4; haloperidol+*V. officinalis* (−VCM), n=9; haloperidol+*V. officinalis* (+VCM), n=9) were analyzed by one-way ANOVA followed by Duncan's multiple range tests. * represents significant difference from control group and # represents significant differences from animals treated with haloperidol (+VCMs).

control group (Fig. 3B and C). *V. officinalis* alone caused a significant increase in the percentage of the time spent on open arm 12 weeks after haloperidol administration (Fig. 3B). Furthermore, there was a significant difference of *V. officinalis* from other groups in the percentage of the entries into the open arm 8 and 12 weeks after haloperidol administration (Fig. 3C). Also, a significant effect of the time was observed in the percentage of time spent into the open arm ($F(3,138)=3.99$; $p<0.05$) and in the number of entries into the open arm ($F(3,138)=3.35$; $p<0.05$). The treatment with *V. officinalis* did not cause neither effect in the percentage of the time spent into open (Fig. 3B) nor in the percentage of entries into the open arm in rats treated concomitantly with haloperidol (Fig. 3C).

3.4. Effects of haloperidol and V. officinalis on oxidative stress parameters

There was no significant difference among the groups in DCFH-DA-oxidation levels, TBARS, carbonyl content groups and SOD activity in rats under long-term treatment with haloperidol and *V. officinalis* (Table 1).

3.5. Effects of haloperidol and V. officinalis on [^{3}H] dopamine uptake

Haloperidol treatment in association with VCM development decreased [^{3}H] dopamine uptake in striatal slices when compared to control group ($p<0.05$) (Fig. 4). *V. officinalis* co-treatment did not protect against haloperidol-induced [^{3}H] dopamine uptake reduction in those rats that developed VCM (Fig. 4). In rats co-treated with both drugs that did not develop VCM, the level of [^{3}H] dopamine uptake was similar to vehicle levels (Fig. 4). *V. officinalis* administration alone did not alter [^{3}H] dopamine uptake in rats.

4. Discussion

TD is a serious side effect caused by long-term treatment with neuroleptic drugs. Particularly, it is problematic due to its high prevalence and the lack of effective treatment. Our current study shows that *V. officinalis* was not effective in reducing OD prevalence or intensity in rats under chronic treatment with haloperidol. *V. officinalis* showed a significant effect in to maintain rats on the open arm of the elevated plus maze. The chronic treatment with *V. officinalis* and/or haloperidol did not cause any effect on oxidative stress parameters. Furthermore, the reduction in dopamine uptake in striatum seems to have an important role in the development of OD in rats, an effect not altered by chronic treatment with *V. officinalis*.

It has been demonstrated that long-term treatment with neuroleptic drugs is capable of producing OD in rats and TD in humans. However the mechanisms that can be involved are not clear. In the present study, we found that long-term treatment with haloperidol caused a prevalence of OD in 40% of treated rats. Accordingly, a previous study showed that chronic treatment with haloperidol develops significant OD 45–55% in rats with 6 months of treatment and approximately 65–75% after 12 months of treatment (Kaneda et al., 1992).

We have demonstrated that there was a significant reduction in dopamine uptake in the animals presenting OD in relation to the control group and group that did not develop OD. These results imply that chronic treatment with haloperidol could be causing an overflow of dopamine into the synaptic cleft of extrapyramidal dopaminergic neurons, which may be one of the possible mechanisms of typical neuroleptic-induced TD. Furthermore, *V. officinalis* could not prevent the reduction in dopamine uptake nor OD. In accordance with our findings, recent data from literature have demonstrated that haloperidol can decrease the striatal expression of dopamine transporter in rats (Saldaña et al., 2006). Several factors might explain the reduction of dopamine uptake in the striatum of rats presenting OD, including neurodegeneration of cells that uptake dopamine and alteration in dopamine transport function. Moreover, it has been shown that some neuroleptics, including haloperidol, can directly interact with and inhibit the dopamine transporter in vitro (Lee et al., 1997).

Literature data have shown that oxidative stress can decrease the activity of dopamine transporters (Hashimoto et al., 2004; Huang et al., 2003). Thus, we investigated oxidative stress parameters in this model of OD. A hypothesis has postulated that free radicals could have an important role in the development of TD (Lohr et al., 1990, 2003). In humans, there are some studies showing that patients with TD had an increase in oxidative stress parameters in plasma and cerebral spinal fluid (CSF) (Brown et al., 1998; Lohr et al., 1990; Pall et al., 1987). In rats, acute OD has been related to an increase in oxidative stress parameters (Abílio et al., 2004; Andreassen et al., 2003; Burger et al., 2005a, b; Faria et al., 2005; Naidu et al., 2003) and treatment with antioxidant substances seems to be efficacious to reduce OD (Burger et al., 2003, 2004, 2005a; Naidu et al., 2003; Singh et al., 2003). However, oxidative stress could be important in beginning of the events that culminate in OD. In fact, it was detected increase in OD and oxidative stress in several brain regions one month

after haloperidol treatment in rats (Burger et al., 2005a; Naidu et al., 2003). On the other hand, we have detected increased OD, but not oxidative stress in the same brain regions 7 months after haloperidol treatment (Fachinetto et al., 2005). Here, we did not find any alteration in oxidative stress parameters evaluated after 3 months of neuroleptic treatment, suggesting that oxidative stress seems to be involved in the development of acute OD (Abílio et al., 2004; Andreassen et al., 2003; Burger et al., 2005a,b; Faria et al., 2005; Naidu et al., 2003) but not in the maintenance of chronic OD. Accordingly, Shivakumar and Ravindratnath (1993) have shown that the treatment with haloperidol induced oxidative stress up to 1 month after the administration. However, after this period, authors did not find changes in markers of oxidative stress in mice brain up to 3 months of haloperidol treatment. Furthermore, some studies have demonstrated no correlation between oxidative stress and OD or TD (Boomershine et al., 1999; Sachdev et al., 1999; Tsai et al., 1998).

Although the etiology of TD is unclear, reduction in GABA is thought to be important in this syndrome. In fact, it has been described a decrease in the GAD activity and in the levels of GABA in brain regions of monkeys with dyskinetic symptoms induced by neuroleptics (Gunne et al., 1984). The mechanism of action of *V. officinalis* seems to be related with the potentiation of GABAergic transmission via direct and/or indirect agonist effect (Mennini et al., 1993; Ortiz et al., 1999; Santos et al., 1994). Considering these effects, *V. officinalis* could be efficacious against TD. However, *V. officinalis* treatment was not able to alter the prevalence or the intensity of haloperidol-induced OD, at least in the dose used in this experiment.

V. officinalis is clinically used to relieve anxiety and improve symptoms of insomnia (Della Loggia et al., 1981; Kennedy et al., 2006; McCabe, 2002; Morazzoni and Bombardelli, 1995; Oliva et al., 2004; Sakamoto et al., 1992). Thus, we investigated the effects of *V. officinalis* in the locomotor activity and anxiety-like behavior to evaluate if the treatment was capable of producing pharmacological effects. Supporting the effectiveness of treatment used here, *V. officinalis* was able to produce hypolocomotion and anxiolytic-like effect in the treated rats when assessed in open field and plus maze tests 8 weeks after the beginning of the treatment with haloperidol. It has been reported that anxiolytic effects appears acutely in response to drugs (Carobrez and Bertoglio, 2005). However, Vorbach et al. (1996) reported that approximately 2–4 weeks of therapy with valerian is needed to achieve significant improvements in sleep disturbances. In our study, probably because we used a low and nontoxic dose of *V. officinalis*, the anxiolytic-like effect of this herb appeared only 8 weeks after haloperidol administration. Of particular importance to select the dose used in this study, we have considered the fact that the treatment with a dose of 500 mg/kg of *V. officinalis* during 7 has caused oxidative stress in liver of mice (Al-Majed et al., 2006). Thus, as we used a chronic model, our dose was 200–250 mg/kg to avoid signals of toxicity. In fact, an important finding of our study was that the chronic treatment with *V. officinalis* did not cause any alteration on oxidative stress parameters neither in the CNS nor in liver and kidney (F.A.A Soares, unpublished data). More studies must be carried out to elucidate the toxic potential of *V. officinalis* treatment. However, further studies must be carried out to elucidate the exact mechanisms through haloperidol treatment reduces dopamine uptake.

5. Conclusion

Taken together, our data suggest that the oxidative stress seems not to have an important role in maintenance of OD. Moreover, a mechanism involving the reduction of dopamine transport related with the maintenance of chronic OD in rats can be involved. Therefore, the chronic treatment with *V. officinalis* seems not produce any oxidative damage to CNS. However, *V. officinalis* seems not effective in preventing or treating OD in rats.

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References

Abílio VC, Silva RH, Carvalho RC, Grassl C, Calzavara MB, Registro S, et al. Important role of striatal catalase in aging- and reserpine-induced oral dyskinesia. Neuropharmacology 2004;47:263–72.

Abourashed EA, Koetter U, Brattstrom A. In vitro binding experiments with a Valerian, hops and their fixed combination extract (Ze91019) to selected central nervous system receptors. Phytomedicine 2004;11:633–8.

Al-Majed AA, Al-Yahya AA, Al-Bekairi AM, Al-Shabanah OA, Qureshi S. Studies on the cytological and biochemical effects of valerian in somatic and germ cells of Swiss albino mice. Food Chem Toxicol 2006;44:1830–7.

Andreassen OA, Jorgensen HA. Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats. Implications for tardive dyskinesia? Prog Neurobiol 2000;61:525–41.

Andreassen OA, Ferrante RJ, Aamo TO, Beal MF, Jorgensen HA. Oral dyskinesias and histopathological alterations in substantia nigra after long-term haloperidol treatment of old rats. Neuroscience 2003;122: 717–25.

Araujo NP, Abilio VC, Silva RH, Pereira RC, Carvalho RC, Gonzalez C, et al. Effects of topiramate on oral dyskinesia induced by reserpine. Brain Res Bull 2005;64:331–7.

Beckman ML, Quick MW. Neurotransmitter transporters: regulators of function and functional regulation. J Membr Biol 1998;164:1–10.

Boomershine KH, Shelton PS, Boomershine JE. Vitamin E in the treatment of tardive dyskinesia. Ann Pharmacother 1999;33:1195–202.

Broadhurst PL. Experiments in psychogenetics. In: Eysenk HJ, editor. Experiments in personality. London: Routledge & Kegan Paul; 1960. p. 76.

Brown K, Reid A, White T, Henderson T, Hukin S, Johnstone C, et al. Vitamin E, lipids, and lipid peroxidation products in tardive dyskinesia. Biol Psychiatry 1998;43:863–7.

Burger ME, Alves A, Callegari L, Athayde FR, Nogueira CW, Zeni G, et al. Ebselen attenuates reserpine-induced orofacial dyskinesia and oxidative stress in rat striatum. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:135–40.

Burger ME, Fachinetto R, Calegari L, Paixão MW, Braga AL, Rocha JBT. Effects on age on orofacial dyskinesia reserpine-induced and possible protection of diphenyl-diselenide. Brain Res Bull 2004;64:339–45.

Burger ME, Fachinetto R, Zeni G, Rocha JBT. Ebselen attenuates haloperidol-induced orofacial dyskinesia and oxidative stress in rat brain. Pharmacol Biochem Behav 2005a;81:608–15.

Burger ME, Fachineto R, Alves A, Callegari L, Rocha JB. Acute reserpine and subchronic haloperidol treatments change synaptosomal brain glutamate uptake and elicit orofacial dyskinesia in rats. Brain Res 2005b;1031:202–10.

Burt D, Creese I, Snyder SH. Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 1977;196:326–7.

Cadet JL, Lohr JB, Jeste DV. Free radical and tardive dyskinesia. Trends Neurosci 1986;9:107–8.

Cadet JL, Lohr JB, Jeste DV. Tardive dyskinesia and schizophrenic burnout: the possible involvement of cytotoxic free radicals. In: Henn FA, DeLisi LE, editors. Handbook of schizophrenia: the neurochemistry and pharmacology of schizophrenia. Amsterdam: Elsevier; 1987. p. 425–38.

Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci Biobehav Rev 2005;29:1193–205.

Casey DE. Tardive dyskinesia: reversible and irreversible. Psychopharmacology 1985;2:88–97.

Cavallero R, Smeraldi E. Antipsychotic-induced tardive dyskinesia: recognition, prevention and management. CNS Drugs 1995;4:278–93.

Chopin P, Stenger A, Couzinier JP, Briley M. Indirect dopaminergic effects of tofisopam, a 2,3-benzodiazepine, and their inhibition by lithium. J Pharm Pharmacol 1985;37:917–9.

Crane GE. Persistent dyskinesia. Br J Psychiatry 1973;122:395–405.

Creese I, Burt D, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 1976;192: 481–3.

Della Loggia R, Tubaro A, Redaelli C. Evaluation of the activity on the mouse CNS of several plant extracts and a combination of them. Riv Neurol 1981;51:297–310.

Egan MF, Hurd Y, Hyde TM, Weinberger DR, Wyatt RJ, Kleinman JE. Alterations in mRNA levels of D2 receptors and neuropeptides in striatonigral and striatopallidal neurons of rats with neuroleptic-induced dyskinesias. Synapse 1994;18:178–89.

Fachinetto R, Burger MB, Wagner C, Wondracek DC, Brito VB, Nogueira CW, et al. High fat diet increases the incidence of orofacial dyskinesia and oxidative stress in specific brain regions of rats. Pharmacol Biochem Behav 2005;81:585–92.

Faria RR, Abilio VC, Grassl C, Chinen CC, Negrao LT, de Castro JP, et al. Beneficial effects of vitamin C and vitamin E on reserpine-induced oral dyskinesia in rats: critical role of striatal catalase activity. Neuropharmacology 2005;48:993–1001.

Fibiger HC, Lloyd KG. Neurobiological substrates of tardive dyskinesia: the GABA hypothesis. Trends Neurosci 1984;7:462–4.

Fugh-Berman A, Cott JM. Dietary supplements and natural products as psychotherapeutic agents. Psychosom Med 1999;61:712–28.

Gunne L, Häggström J, Sjöquist B. Association with persistent neuroleptic-induced dyskinesia of regional changes in brain GABA synthesis. Nature 1984;309:347–9.

Hashimoto K, Tsukada H, Nishiyama S, Fukumoto D, Kakiuchi T, Shimizu E, et al. Protective effects of *N*-acetyl-L-cysteine on the reduction of dopamine transporters in the striatum of monkeys treated with methamphetamine. Neuropsychopharmacology 2004;29:2018–23.

Holz RW, Coyle JT. The effects of various salts, temperature and the alkaloids veratridine and batrachotoxin on the uptake of ^{3}H-dopamine into synaptosomes from rat striatum. Mol Pharmacol 1974;10:746–58.

Huang CL, Huang NK, Shyue SK, Chern Y. Hydrogen peroxide induces loss of dopamine transporter activity: a calcium-dependent oxidative mechanism. J Neurochem 2003;86:1247–59.

Jeste DV, Potkin SG, Sinhá S, Feder S, Wyatt RJ. Tardive dyskinesia—reversible and persistent. Arch Gen Psychiatry 1979;36:585–90.

Kahlig KM, Galli A. Regulation of dopamine transporter function and plasma membrane expression by dopamine, amphetamine, and cocaine. Eur J Pharmacol 2003;479:153–8.

Kane JM. Tardive dyskinesia: epidemiology and clinical presentation. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress, vol. 39. New York: Raven Press; 1995. p. 1485–95.

Kane JM, Smith JM. Tardive dyskinesia: prevalence and risk factors, 1959 to 1979. Arch Gen Psychiatry 1982;39:473–81.

Kaneda H, Shirakawa O, Dale J, Goodman L, Bachus SE, Tamminga CA. Co-administration of progabide inhibits haloperidol-induced oral dyskinesias in rats. Eur J Pharmacol 1992;121:43–9.

Kennedy DO, Little W, Haskell CF, Scholey AB. Anxiolytic effects of a combination of *Melissa officinalis* and *Valeriana officinalis* during laboratory induced stress. Phytother Res 2006;20:96–102.

Klawans Jr HL, Rubovits R. An experimental model of tardive dyskinesia. J Neural Transm 1972;33:235–46.

Lee S, Oh D, Jung S, Kim Y, Cho H, Koh J, et al. Neuroleptic drugs alter the dopamine transporter-mediated uptake and release of dopamine: a possible mechanism for drug-induced tardive dyskinesia. Pharmacol Res 1997;35: 446–50.

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990;86:464–78.

Lohr JB. Oxygen radicals and neuropsychiatric illness: some speculations. Arch Gen Psychiatry 1991;48:1097–106.

Lohr JB, Kuczenski R, Bracha HS, Moir M, Jeste DV. Increased indices of free radical activity in the cerebrospinal fluid of patients with tardive dyskinesia. Biol Psychiatry 1990;28:535–9.

Lohr JB, Kuczenski R, Niculescu AB. Oxidative mechanisms and tardive dyskinesia. CNS Drugs 2003;17:47–62.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265.

McCabe S. Complimentary herbal and alternative drugs in clinical practice. Perspect Psychiatr Care 2002;38:98–107.

Mennini T, Bernasconi P, Bombardelli E, Morazzoni P. In vitro study on the interaction of extracts and pure compounds from *Valeriana officinalis* roots with GABA, benzodiazepine and barbiturate receptors in the rat brain. Fitoterapia 1993;64:291–300.

Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase. J Biol Chem 1972;247:3170–5.

Morazzoni P, Bombardelli E. *Valeriana offcinalis*: traditional use and recent evaluation of activity. Fitoterapia 1995;66:99–112.

Morselli PL, Fournier V, Bossi L, Musch B. Clinical activity of GABA agonists in neuroleptic- and L-dopa-induced dyskinesia. Psychopharmacology 1985;2:128–36.

Naidu PS, Singh A, Kulkarni SK. Quercetin, a bioflavonoid, attenuates haloperidol-induced orofacial dyskinesia. Neuropharmacology 2003;44: 1100–6.

Ohkawa H, Ohishi H, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.

Oliva I, Gonzalez-Trujano ME, Arrieta J, Enciso-Rodriguez R, Navarrete A. Neuropharmacological profile of hydroalcohol extract of *Valeriana edulis* ssp. procera roots in mice. Phytother Res 2004;18:290–6.

Ortiz JG, Nieves-Natal J, Chavez P. Effects of *Valeriana officinalis* extracts on [^{3}H]flunitrazepam binding, synaptosomal [^{3}H] GABA uptake, and hippocampal [^{3}H]GABA release. Neurochem Res 1999;24:1373–8.

Pall HS, Williams AC, Blake DR, Lunec J. Evidence of enhanced lipid peroxidation in the cerebrospinal fluid of patients taking phenothiazines. Lancet 1987;2:596–9.

Peixoto MF, Araújo NP, Silva RH, Castro JP, Fukushiro DF, Faria RR, et al. Effects of gabaergic drugs on reserpine-induced oral dyskinesia. Behav Brain Res 2004;160:51–9.

Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arms entries in elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.

Pérez-Severiano P, Rodríguez-Pérez M, Pedraza-Chaverrí J, Maldonado PD, Medina-Campos ON, Ortíz-Plata A, et al. S-Allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats. Neurochem Int 2004;45:1175–83.

Raghavendra V, Naidu PS, Kulkarni SK. Reversal of reserpine-induced vacuous chewing movements in rats by melatonin: involvement of peripheral benzodiazepine receptors. Brain Res 2001;904:149–52.

Rosengarten H, Bartoszyk GD, Quartermain D, Lin Y. The effect of chronic administration of sarizotan, 5-HT1A agonist/D3/D4 ligand, on haloperidol-induced repetitive jaw movements in rat model of tardive dyskinesia. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:273–9.

Rubinstein M, Muschietti JP, Gershanik O, Flawia MM, Stefano FJ. Adaptative mechanisms of striatal D1 and D2 dopamine receptors in response to a prolonged reserpine treated mice. J Pharmacol Exp Ther 1990;252:810–6.

Sachdev P, Saharov T, Cathcart S. The preventative role of antioxidants (selegiline and vitamin E) in a rat model of tardive dyskinesia. Biol Psychiatry 1999;46:1672–81.

Sakamoto T, Mitani Y, Nakajima K. Psychotropic effects of Japanese valerian root extract. Chem Pharm Bull 1992;40:758–61.

Saldaña M, Bonastre M, Aguilar E, Marin C. Differential nigral expression of Bcl-2 protein family in chronically haloperidol and clozapine-treated rats: role in neurotoxicity and stereotyped behavior. Exp Neurol 2006;203:302–8.

Santos MS, Ferreira F, Cunha AP, Carvalho AP, Macedo T. An aqueous extract of valerian influences the transport of GABA in synaptosomes. Planta Med 1994;60:278–9.

Shirakawa O, Tamminga CA. Basal ganglia $GABA_A$ and dopamine D_1 binding site correlates of haloperidol-induced oral dyskinesias in rat. Exp Neurol 1994;127:62–9.

Shivakumar BR, Ravindratnath V. Oxidative stress and thiol modification induced by chronic administration of haloperidol. J Pharmacol Exp Ther 1993;265:1137–41.

Singh A, Naidu PS, Kulkarni SK. Possible antioxidant and neuroprotective mechanisms of FK-506 in attenuating haloperidol-induced orofacial dyskinesia. Eur J Pharmacol 2003;477:87–94.

Tamminga CA, Crayton JW, Chase TN. Improvement in tardive dyskinesia after muscimol therapy. Arch Gen Psychiatry 1979;36:595–8.

Tsai G, Goff DC, Chang RW, Flood J, Baer L, Coyle JT. Markers of glutamatergic neurotransmission and oxidative stress associated with tardive dyskinesia. Am J Psychiatry 1998;155:1207–13.

Vorbach EU, Gortelmayer R, Bruning J. Therapie von Insomnien: Wirksamkeit und Vertrag-lichkeit eines Baldrian-Praparates. Psychopharmakotherapie 1996;3:109–15.

Woerner MG, Kane JM, Lieberman JA, Alvir J, Bergmann KJ, Borenstein M, et al. The prevalence of tardive dyskinesia. J Clin Psychopharmacol 1991;11:34–42.

Yan LJ, Traber MG, Packer L. Spectrophotometric method for determination of carbonyls in oxidatively modified apolipoprotein B of human low-density lipoproteins. Anal Biochem 1995;228:349–51.

Yassa R, Jeste DV. Gender differences in tardive dyskinesia: a critical review of the literature. Schizophr Bull 1992;18:701–15.

Yoder KK, Hutchins GD, Morris ED, Brashear A, Wang C, Shekhar A. Dopamine transporter density in schizophrenic subjects with and without tardive dyskinesia. Schizophr Res 2004;71:371–5.