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AVALIAÇÃO DOS EFEITOS COMPORTAMENTAIS E NEUROQUÍMICOS INDUZIDOS POR DIFERENTES DOSES DE RESERPINA EM CAMUNDONGOS

DISSERTAÇÃO DE MESTRADO

Catiuscia Molz de Freitas

Santa Maria, RS, Brasil

2014

AVALIAÇÃO DOS EFEITOS COMPORTAMENTAIS E NEUROQUÍMICOS INDUZIDOS POR DIFERENTES DOSES DE RESERPINA EM CAMUNDONGOS

Catiuscia Molz de Freitas

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Orientadora: Profª. Drª. Roselei Fachinetto

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E-mail: catiusciafreitas@hotmail.com

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elaborada por
Catiuscia Molz de Freitas

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

COMISSÃO EXAMINADORA

Roselei Fachinetto, Dr[a]. (UFSM)
(Presidente/Orientador)

Mauro Schneider Oliveira, Dr. (UFSM)

Nilda Berenice de Vargas Barbosa, Dr[a]. (UFSM)

Santa Maria, 02 de outubro de 2014.

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Agradeço a Deus, que nas suas mais diferentes formas de manifestação, está sempre presente guiando meus passos.

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“Os sonhos não determinam o lugar onde vocês vão chegar, mas produzem a força necessária para tirá-los do lugar em que vocês estão... Nessa matemática você só aprende a multiplicar quando aprende a dividir, só consegue ganhar quando aprende a perder, só consegue receber quando aprende a se doar.”

Augusto Cury

RESUMO

Dissertação de Mestrado
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AVALIAÇÃO DOS EFEITOS COMPORTAMENTAIS E NEUROQUÍMICOS INDUZIDOS POR DIFERENTES DOSES DE RESERPINA EM CAMUNDONGOS
AUTOR: CATIUSCIA MOLZ DE FREITAS
ORIENTADORA: ROSELEI FACHINETTO
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A administração de reserpina é considerada um modelo animal para o estudo da discinesia tardia por alguns autores e um modelo para estudo do parkinsonismo por outros. No entanto, apesar de inúmeros trabalhos utilizarem este modelo para avaliar o potencial de substâncias para o tratamento desses distúrbios do movimento, pouco se sabe sobre os mecanismos envolvidos no desenvolvimento das alterações comportamentais presentes no modelo da reserpina. Assim, o presente estudo investigou se as alterações motoras induzidas pela reserpina estão relacionados com alterações em proteínas do sistema dopaminérgico como a tirosina hidroxilase (TH), o transportador de dopamina (TDA) e monoaminoxidase (MAO). Para isso, a reserpina foi administrada subcutaneamente em camundongos nas doses de 0,1, 0,5 ou 1 mg/kg ou veículo (0,2% ácido acético em NaCl 0,9%) durante 4 dias consecutivos. O número de movimentos de mascar no vazio (MMVs), atividade locomotora e exploratória foram avaliados 48 horas (6º dia) e 20 dias (24º dia) após a retirada do tratamento, a fim de avaliar a indução bem como a manutenção das alterações motoras causadas pela reserpina. Foram também analisados a imunoreatividade da TH e do TDA por *Western Blot* e a atividade da MAO-A e MAO-B em estriado e região contendo a *substantia nigra* no 6º e 24º dia. O tratamento com 1 mg/kg de reserpina causou um aumento dos MMVs e hipolocomoção nos animais e este efeito se manteve por pelo menos 20 dias após a retirada da reserpina. Essas alterações foram acompanhadas por uma redução na imunoreatividade do TDA no estriado e redução da TH na *substantia nigra* avaliadas no 6º dia. No 24º dia foi observada uma diminuição na imunoreatividade da TH e do TDA tanto no estriado quanto na *substantia nigra*. A dose de 0,5 mg/kg de reserpina causou alterações comportamentais no 6º dia, mas estas alterações não se mantiveram após a retirada do tratamento e também não foram encontradas alterações neuroquímicas nesta dose. Não foram encontradas diferenças estatisticamente significativas quando os parâmetros comportamentais e neuroquímicos foram avaliados na dose de 0,1 mg/kg de reserpina. Assim, esses resultados sugerem que a reserpina causa alterações em proteínas do sistema dopaminérgico que conduzem às alterações motoras. Desta forma, possíveis intervenções farmacológicas nessas proteínas poderiam aliviar os sintomas motores tanto na doença de Parkinson quanto na discinesia tardia.

Palavras-chave: Reserpina. Tirosina hidroxilase. Transportador de dopamina. Discinesia tardia. Doença de Parkinson.

ABSTRACT

Dissertation of Master's Degree
Post-Graduate Course in Biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria, RS, Brazil

EVALUATION OF BEHAVIORAL AND NEUROCHEMICAL EFFECTS INDUCED BY DIFFERENT DOSES OF RESERPINE IN MICE

AUTHOR: CATIUSCIA MOLZ DE FREITAS
ADVISOR: ROSELEI FACHINETTO
Place and Date of the Defense: Santa Maria, October 2sd, 2014.

Reserpine administration is considered an animal model for the study of tardive dyskinesia by some authors and a model for the study of parkinsonism by others. However, despite several studies used this model to assess the potential of substances to treat these movement disorders, little is known about the mechanisms involved in the development of behavioral changes in reserpine model. Thus, the present study investigated whether behavioral alterations induced by reserpine are related to alterations in dopaminergic system proteins as tyrosine hydroxylase (TH), dopamine transporter (DAT) and monoaminoxidase (MAO). For this, reserpine was administered subcutaneously in mice at doses of 0.1, 0.5 or 1 mg / kg or vehicle (0.2% acetic acid in NaCl 0.9%) for 4 consecutive days. The number of vacuous chewing movements (VCMs), exploratory and locomotor activity were assessed 48 hours (6th day) and 20 days (24th day) after withdrawal of treatment in order to evaluate the induction and maintenance of motor disorders caused by reserpine. It was also analyzed the TH and DAT immunoreactivity by Western blot and the MAO-A and MAO-B activity in striatum and region containing *substantia nigra* on days 6 and 24. Treatment with 1 mg/kg reserpine caused an increase in VCMs and hypolocomotion in animals, and this effect remained for at least 20 days after withdrawal of reserpine. These alterations were accompanied by a reduction in striatal DAT immunoreactivity and a reduction of TH immunoreactivity in the *substantia nigra* evaluated on day 6. On the 24th day was observed a decrease in the DAT and TH immunoreactivity in both striatum and *substantia nigra*. The dose of 0.5 mg/kg reserpine caused behavioral alterations on day 6, but these changes were not maintained after withdrawal of treatment and also neurochemical changes not were found at this dose. We did not find any statistical differences when behavioral and neurochemical parameters were evaluated at a dose of 0.1 mg/kg reserpine. Thereby, these results suggest that reserpine causes changes in dopaminergic system proteins which lead to motor alterations. Thus, possible pharmacological interventions in these proteins could ameliorate motor symptoms in both, Parkinson's disease and tardive dyskinesia.

Keywords: Reserpine. Tyrosine hydroxylase. Dopamine transporter. Tardive dyskinesia. Parkinson's disease.

LISTA DE TABELAS

Manuscrito:

LISTA DE FIGURAS

Manuscrito:

LISTA DE ABREVIATURAS

3-MT	– 3-metoxitiramina
6-OHDA	– 6-hidroxidopamina
ATP-Mg	– trifosfato de adenosina-magnésio
COMT	– catecol-O-metiltransferase
DA	– dopamina
DO	– discinesia orofacial
DOPA	– diidroxifenilalanina
DOPAC	– ácido 3,4-diidroxifenilacético
DP	– doença de Parkinson
DT	– discinesia tardia
HVA	– ácido homovanílico
MAO	– enzima monoaminoxidase
MMVs	– movimentos de mascar no vazio
SNpc	– *substantia nigra pars compacta*
TDA	– Transportador de dopamina
TH	– tirosina hidroxilase
TVMA-2	– Transportador vesicular de monoaminas 2

SUMÁRIO

APRESENTAÇÃO

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

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de manuscrito que será submetido para publicação na revista *Psychopharmacology*, o qual se encontra no item **RESULTADOS**. As seções Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se no próprio manuscrito e representam a íntegra deste estudo.

O item **CONCLUSÕES** encontrado no final desta dissertação, apresenta comentários gerais sobre o manuscrito contido neste trabalho.

As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente às citações que aparecem no item **INTRODUÇÃO** desta dissertação.

1 INTRODUÇÃO

As doenças neurodegenerativas são caracterizadas pela perda seletiva e progressiva de neurônios, as quais levam a déficits cognitivos, comportamentais e físicos que podem causar morbidade e mortalidade nos pacientes (BEAL et al., 2005). São conhecidos seus sintomas clínicos e patológicos, tais como distúrbios do movimento (doença de Parkinson, doença de Huntington) e demências (doença de Alzheimer, esquizofrenia), efeitos que se correlacionam com o tipo de neurônios afetados (dopaminérgicos, GABAérgicos ou neurônios motores) e pela origem (hereditária ou não) (HIRTH, 2010). No entanto, o desconhecimento dos mecanismos que levam a estas patologias acarreta dificuldades tanto em seu tratamento como na busca de fármacos com alvo específico.

1.1 Doença de Parkinson

A doença de Parkinson (DP) foi descrita pela primeira vez, em 1817, pelo médico inglês James Parkinson em sua monografia intitulada *"Essay on the Shaking Palsy"* ("Ensaio da paralisia agitante"). Neste trabalho James Parkinson definiu a enfermidade, determinou os sintomas, descreveu o diagnóstico diferencial e fez considerações a respeito da etiologia e do tratamento desta doença (DAUER e PRZEDBORSKI, 2003). Mais tarde, em 1875, o neurologista francês Jean Martin Charcot sugeriu o nome doença de Parkinson, reconhecendo o mérito do médico inglês que pioneiramente descreveu a doença. Além disso, Charcot identificou disfunções cognitivas presentes na doença, acabando com a ideia de que a doença se tratava apenas de um distúrbio motor (MENESES e TEIVE, 1996).

A DP é considerada a segunda doença neurodegenerativa mais prevalente, afetando cerca de 1% a 2% da população com mais de sessenta e cinco anos (ALVES et al., 2008), sendo que este percentual aumenta para 3% a 5% em pessoas com mais de oitenta e cinco anos (FAHN, 2003). Este distúrbio motor caracteriza-se, principalmente, pela perda progressiva e seletiva dos neurônios dopaminérgicos da *substantia nigra pars compacta* (SNpc) (COOKSON, 2005; DAWSON e DAWSON, 2003; MOORE et al., 2005), a qual passa a exibir macroscopicamente uma despigmentação na porção ventrolateral

(JELLINGER, 1988; STANDAERT e ROBERSON, 2012). A despigmentação da SNpc é consequência da degeneração dos neurônios dopaminérgicos que contém a neuromelanina (GERLACH e RIEDERER, 1996), resultando em uma diminuição dos níveis de dopamina (DA) no estriado (JELLINGER, 1988; LINDNER et al., 1999).

Desta forma, a via dopaminérgica inibitória que parte da substância negra em direção ao estriado é afetada, levando a falta de DA no estriado que causa um aumento da atividade inibitória pálido-talâmica, dificultando a excitação cortical motora o que conduz aos sintomas motores observados na DP (Figura 1) (STANDAERT e ROBERSON, 2012).

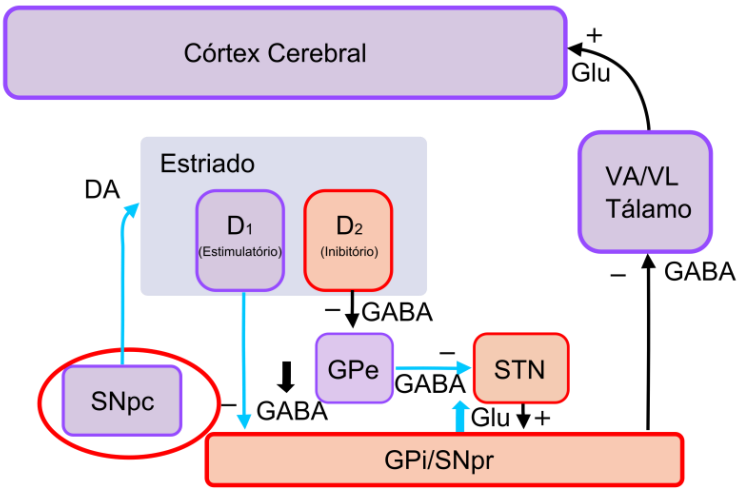

Figura 1 – Os núcleos da base na DP (adaptado de GOODMAN e GILMAN, 2010). (DA: dopamina; D1 e D2: receptores dopaminérgicos; GABA: ácido gama aminobutírico; Glu: glutamato; GPe: globo pálido lateral; GPi: globo pálido medial; STN: núcleo subtalâmico; SNpc: *substantia nigra pars compacta*; SNpr: *substantia nigra pars reticulada*; VA: núcleo ventral anterior; VL: núcleo ventrolateral).

Os sintomas mais comuns da DP são bradiscinesia, tremor de repouso, rigidez e anormalidades posturais (GERLACH e RIEDERER, 1996; KLOCKGETHER, 2004; LINDNER et al., 1999; OBESO et al., 2000; POSTLE et al., 1997). Também podem ser observadas deficiências cognitivas nos pacientes com DP (AARSLAND et al., 2004; MAHIEUX et al., 1998; VERBAAN et al., 2007) como dificuldade de aprendizado (SCHMITT-ELIASSEN et. al., 2007) e déficits de atenção (BRONNICK et al., 2006).

A bradicinesia está relacionada à dificuldade em iniciar o movimento, a pobreza e a lentidão de movimentos observada nos pacientes com DP. Esse é considerado o sintoma que mais incapacita o paciente, pois os movimentos voluntários e automáticos estão reduzidos e as

atividades diárias exigem muito esforço. Além disso, pode-se observar que os passos tornam-se lentos, e o equilíbrio fica comprometido (LIMONGI, 1995).

A rigidez muscular consiste no aumento da resistência que os músculos oferecem quando uma parte do corpo é deslocada passivamente. Isto resulta em uma fragmentação dos movimentos que, ao invés de serem executados de maneira contínua, tornam-se entrecortados (MENESES e TEIVE, 1996). O tremor observado nos pacientes é relativamente lento e ocorre principalmente quando o membro está em repouso. Quando o paciente movimenta um membro, ou durante o sono esse tremor cessa (MENESES e TEIVE, 1996).

A anormalidade postural se caracteriza principalmente pela instabilidade, devido à perda do reflexo postural. As quedas podem se tornar frequentes e, em estágios mais avançados da doença, o paciente pode apresentar dificuldades em permanecer de pé (MENESES e TEIVE, 1996).

Estudos epidemiológicos revelam que menos de 10% dos casos de DP tem etiologia familiar, sendo que a maioria dos casos é esporádica, sem ligação genética aparente (THOMAS e BEAL, 2007). Além disso, vale ressaltar que os sintomas motores na DP somente se manifestam quando a morte dos neurônios dopaminérgicos atinge aproximadamente 50 a 60% na SNpc e 70 a 80% no estriado (AGID, 1991; SCHERMAN et al., 1989).

Atualmente não existe terapia eficaz na prevenção, na cura ou capaz de parar o desenvolvimento da DP. As abordagens terapêuticas atuais são apenas paliativas e visam diminuir os sintomas causados pela doença. A L-dopa (L-3,4-dihidroxifenilalanina) tem sido usada por mais de 40 anos e é o fármaco mais efetivo para atenuar os sintomas motores da DP. Seu mecanismo de ação consiste em sua conversão a dopamina pela atividade da enzima L-aminoácido aromático descarboxilase (ALACHKAR et al., 2010).

Nos estágios iniciais da DP, a terapia com L-dopa é altamente efetiva. No entanto, o tratamento prolongado com este fármaco leva ao agravamento das doenças motoras e ao surgimento de discinesia (NAGATSU e SAWADA, 2009), comprometendo, desta forma, a eficácia clínica da L-dopa (AHLSKOG e MUENTER, 2001). É importante salientar que aproximadamente 50% dos pacientes tratados com L-dopa desenvolvem discinesia cerca de cinco anos após o início do tratamento e os pacientes tratados por mais de 10 anos com L-dopa têm um risco aproximado de 90% de desenvolver a discinesia (AHLSKOG e MUENTER, 2001). Os mecanismos que envolvem tais distúrbios motores não são completamente entendidos o que dificulta também a busca por novas terapias farmacológicas.

No entanto, sabe-se que a DP tem sua gênese relacionada ao sistema dopaminérgico, mais especificamente a via nigroestriatal (Figura 2).

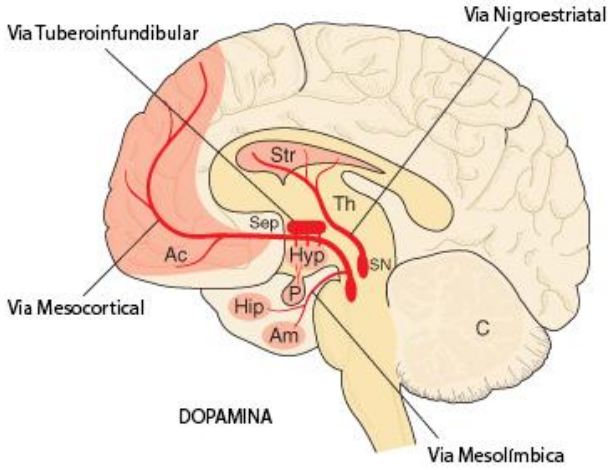

Figura 2 – As vias dopaminérgicas (adaptado de RANG et al., 2012). (Ac: núcleo acumbens; Am: núcleo amigdaloide; C: cerebelo Hip: hipocampo; Hyp: hipotálamo; P: hipófise Sep: septo; SN: *substantia nigra*; Str: estriado; Th: tálamo).

1.2 Antipsicóticos e a discinesia tardia

A esquizofrenia é uma doença psiquiátrica crônica e debilitante que afeta milhões de pessoas no mundo inteiro, e é definida como alterações nas funções mentais e distúrbios comportamentais. Os sintomas da esquizofrenia são classificados conforme suas características, em sintomas positivos os quais incluem delírio, alucinação e desorganização do pensamento, e negativos, que se referem a perda de motivação e oscilação emocional (LEWIS e LIEBERMAN, 2000).

O tratamento mais eficaz para essa psicose inclui a utilização de antipsicóticos. Os primeiros fármacos descritos para o tratamento da esquizofrenia pertencem a classe dos antipsicóticos clássicos ou típicos e incluem fármacos como a clorpromazina, haloperidol e flufenazina, os quais possuem como principal ação farmacológica o bloqueio dos receptores dopaminérgicos D_2 na via mesolímbica (CREESE et al., 1976; DARLING, 1959). Entretanto estes fármacos, além de bloquearem receptores dopaminérgicos nas regiões relacionadas à etiologia da esquizofrenia, induzem efeitos extrapiramidais como a discinesia tardia (DT) por

bloquearem tais receptores também na via nigroestriatal, o que compromete sua eficácia clínica (Figura 2) (ANDREASSEN e JORGENSEN, 2000; ELLENBROEK, 1993).

O desenvolvimento de antipsicóticos de segunda geração ou também chamados de antipsicóticos atípicos (risperidona, olanzapina, etc.) baseou-se na clozapina (KUROKI et al., 2008). A clozapina foi o primeiro antipsicótico que provou ser eficaz no tratamento da esquizofrenia refratária (KANE et al., 1988). Entretanto, estes antipsicóticos causam uma série de efeitos colaterais, entre eles diabetes *mellitus* tipo 2 e agranulocitose (HENDERSON, 2002). Os antipsicóticos atípicos possuem custo muito elevado se comparados aos típicos e, além disso, há inconsistência de dados mostrando sua eficácia superior em relação aos antipsicóticos típicos (LEUCHT et al., 2009). Desta forma os antipsicóticos de primeira geração continuam sendo largamente empregados no tratamento sintomático das psicoses embora apresentem como efeito adverso a DT.

A DT é o principal efeito colateral observado em pacientes que fazem uso crônico de antipsicóticos. Caracteriza-se por movimentos involuntários da região orofacial e incluem movimentos de mastigação, protrusão da língua, estalido dos lábios, movimentos de franzir a face e piscar os olhos e, às vezes, da musculatura dos membros e tronco (KANE, 1995). Este distúrbio do movimento tem uma taxa média de prevalência entre 24% e 30% nos pacientes, sendo que este índice pode variar de 0,5% a 70% (KULKARNI e NAIDU, 2003; LLORCA et al., 2002).

A DT apresenta um impacto relevante na qualidade de vida dos pacientes que fazem uso destes medicamentos (KULKARNI e NAIDU, 2003). Um dos aspectos mais graves da DT consiste na persistência da síndrome por meses ou até anos após a retirada do tratamento, ou até mesmo sua irreversibilidade (CASEY, 1985; GLAZER et al., 1990).

Diversos estudos vêm propondo hipóteses para o desenvolvimento da DT, apesar das inconsistências a supersensibilidade dopaminérgica é a hipótese clássica. Segundo está hipótese o bloqueio crônico de receptores dopaminérgicos resulta em um aumento compensatório do número e sensibilidade dos receptores e em consequência um estado hiperdopaminérgico e manifestações clínicas, como a DT (ANDREASSEN e JORGENSEN, 2000; KLAWANS e RUBOVITS, 1972; RUBINSTEIN et al., 1990).

Desta forma, tanto para a discinesia tardia induzida por antipsicóticos quanto para a DP e a discinesia decorrente de fármacos utilizados para o seu tratamento os mecanismos patofisiológicos envolvidos no desenvolvimento destes distúrbios do movimento ainda não estão completamente esclarecidos. Além disso, é importante ressaltar que o sistema

dopaminérgico, mais especificamente a via nigroestriatal tende a ser um ponto em comum à DP e à DT.

Assim, torna-se relevante elucidar o papel de proteínas do sistema dopaminérgico relacionadas com as alterações motoras causadas pelo tratamento com reserpina e se estas alterações são persistentes ao longo do tempo após a retirada do fármaco. Uma vez que o sistema dopaminérgico é um ponto em comum à DP e à DT e que muitos estudos têm buscado tratamentos mais eficazes para estas patologias utilizando o modelo da reserpina, a nossa hipótese é que as alterações motoras induzidas pela reserpina em camundongos estão relacionadas a alterações em proteínas do sistema dopaminérgico como a tirosina hidroxilase e o transportador de dopamina.

1.3 Sistema dopaminérgico

O sistema dopaminérgico possui como neurotransmissor a dopamina, cujo precursor da síntese é o aminoácido tirosina (GOODMAN e GILMAN, 2010). Sua hidroxilação, formando DOPA, é mediada via enzima tirosina hidroxilase (TH), sendo esta a etapa limitante na biossíntese das catecolaminas. A etapa posterior dessa biossíntese é determinada pela DOPA descarboxilase, que catalisa a reação de remoção do grupo carboxila da L-DOPA, tendo como produto final a dopamina (DA) (SILVA, 2007).

A dopamina é então transportada até vesículas de estocagem especializadas, via transportador vesicular de monoaminas 2 (TVMA-2) (GOODMAN e GILMAN, 2010). O armazenamento da dopamina se dá por meio de pequenas vesículas presentes em elevada quantidade nos terminais nervosos. Estas, por sua vez, apresentam duas funções uma vez que mantém o nível de dopamina na terminação nervosa disponível para liberação e agem na mediação desta liberação. Sendo assim, quando um potencial de ação atinge o terminal nervoso, gera a abertura dos canais de cálcio, com seu consequente influxo no terminal nervoso. Devido a elevação do cálcio intracelular ocorre fusão das vesículas com a membrana neuronal, liberando assim o seu conteúdo (SILVA, 2007) (Figura 3).

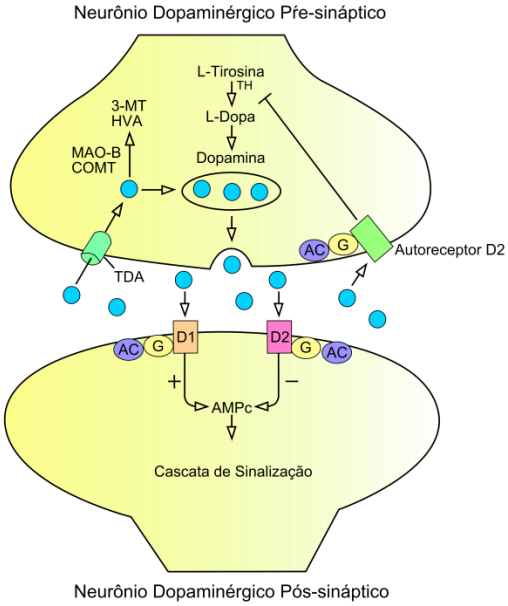

Figura 3 – Sistema Dopaminérgico (adaptado de BLACKSTONE, 2009). (AC: adenilato ciclase; AMPc: adenosina monofosfato cíclico; COMT: catecol O-metiltransferase; D1e D2: classe de receptores de dopamina; G proteína G; HVA: ácido homovanílico; MAO-B: monoaminoxidase B; TDA: transportador de dopamina; TH: tirosina hidroxilase; 3-MT: 3-metoxitiramina).

A dopamina presente no espaço sináptico pode agir em receptores dopaminérgicos exercendo suas ações celulares. Esta catecolamina estando no espaço sináptico pode ser transportada para o interior dos neurônios pré-sinápticos pelo transportador de dopamina (TDA), levando a redução dos níveis de dopamina extracelular (AMARA e KUHAR, 1993). Além disso, a ligação da dopamina em receptores pré-sinápticos D2 inibe sua síntese diminuindo, desta forma, seu armazenamento e liberação (ALBIN et al., 1989; RANG et al., 2012) (Figura 3).

Após suas ações a dopamina pode ser novamente armazenada em vesículas sinápticas ou metabolizada. O metabolismo ocorre principalmente através da atividade da enzima acoplada a membrana mitocondrial monoaminoxidase (MAO) e da catecol-O-metiltransferase (COMT) formando como principais metabólitos o ácido homovanílico (HVA), o ácido 3,4-diidroxifenilacético (DOPAC) e a 3-metoxitiramina (3-MT) (GOODMAN e GILMAN, 2010). Tendo em vista que tanto na DP como na DT o sistema dopaminérgico parece ser um ponto em comum, alguns estudos têm proposto modelos experimentais para o estudo de distúrbios do movimento com ênfase no sistema dopaminérgico.

1.4 Modelos animais de distúrbio motor

Os modelos animais tem sido importantes ferramentas para estudar os mecanismos de diversas patologias e para ajudar a entender os princípios terapêuticos do tratamento dos distúrbios funcionais das doenças humanas (GERLACH e RIEDERER, 1996).

Muitos modelos animais tem sido utilizados experimentalmente no estudo da fisiopatologia dos distúrbios do movimento (BURGER et al., 2004; 2005b; CASTRO et al., 2006; FACHINETTO et al., 2007a; 2007b; NEISEWANDER et al., 1991; 1994; SALAMONE e BASKIN, 1996), dentre eles destacam-se os modelos agudos induzidos por antipsicóticos e o modelos induzidos por reserpina.

1.4.1 Modelos animais de distúrbio motor induzido por antipsicóticos

Nos modelos animais, a discinesia tardia é chamada de discinesia orofacial (DO). Dados da literatura demonstram que o tratamento com antipsicóticos típicos como haloperidol e flufenazina induzem movimentos orais em animais (BUSANELLO et al., 2012; FACHINETTO et al., 2007a; 2007b; PEROZA et al., 2013). O parâmetro mais utilizado para avaliar o desenvolvimento da DO nos animais é o número de movimentos de mascar no vazio (MMVs), o qual é caracterizado por aberturas da boca no plano vertical, com ou sem protrusão de língua (ANDREASSEN e JORGENSEN, 2000).

Assim como a DT em humanos, a DO em animais aparece após semanas de tratamento com os antipsicóticos e o aumento dos MMVs pode persistir após a retirada do fármaco na maioria dos animais (ANDREASSEN et al., 1996, 1998; EGAN et al., 1995). Também, em animais é visto um aumento da DO com o aumento da idade tanto induzida por antipsicóticos quanto espontânea (ANDREASSEN et al., 1996, 1998; JORGENSEN et al., 1994).

Também em modelos animais de DO utilizando antipsicóticos típicos tem sido demonstrado a participação do estresse oxidativo no aparecimento dos movimentos involuntários (BURGER et al., 2005a; NAIDU et al., 2003; POST et al., 2002; SADAN et al., 2005). Além disso, recentemente demonstramos que animais experimentais que desenvolvem DO, em resposta ao tratamento crônico com haloperidol e flufenazina, apresentam níveis

reduzidos de recaptação de DA (FACHINETTO et al., 2007a; 2007b). Ainda, Andreassen e colaboradores (2003) mostraram uma relação entre o desenvolvimento de MMVs e perda de neurônios nigrais em animais.

1.4.2 Modelos animais de distúrbio motor induzido por reserpina

A reserpina é um alcaloide isolado das raízes da planta pertencente ao gênero *Rauwolfia* (DOYLE et al., 1955) cujo mecanismo de ação consiste na inibição do TVMA-2 interferindo com o estoque de aminas biogênicas nas vesículas (METZGER et al., 2002) (Figura 4). Dessa forma, as terminações nervosas perdem a sua capacidade de concentrar e armazenar as monoaminas, como por exemplo, a dopamina. As catecolaminas extravasam no citoplasma, onde são metabolizadas pela MAO intraneural, de modo que pouco ou nenhum neurotransmissor é liberado das terminações nervosas com a despolarização (GOODMAN e GILMAN, 2010). Clinicamente a reserpina é utilizada como anti-hipertensivo, mas seu uso está obsoleto (AL-BLOUSHI et al., 2009).

As alterações motoras induzidas pela reserpina podem ser observadas em animais por aumento na frequência de MMVs e de protrusões de língua, do tempo de tremor facial e de catalepsia (ABÍLIO et al., 2004; BURGER et al., 2004; BUSANELLO et al., 2011; FARIA et al., 2005; NEISEWANDER et al., 1994; PEREIRA et al., 2011). Além disso, a reserpina pode causar hipolocomoção e rigidez muscular, dependendo da dose (DOYLE et al., 1955; FERNANDES et al., 2012; TADAIESKY et al., 2006). Dados da literatura têm demonstrado que animais com MMVs apresentam alteração em parâmetros de estresse oxidativo no estriado (ABÍLIO et al., 2003; BILSKA e DUBIEL, 2007; BURGER et al., 2003; FARIA et al., 2005; FERNANDES et al., 2012; NAIDU et al., 2004).

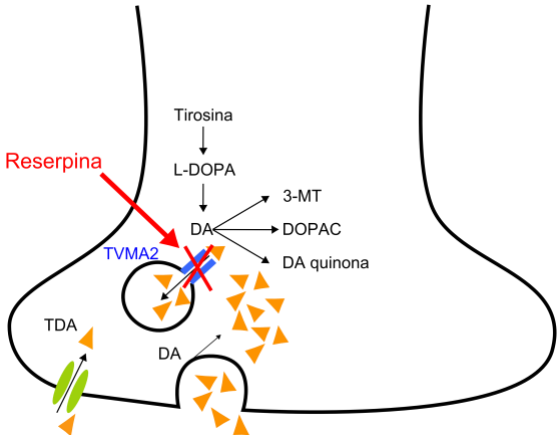

Figura 4 – Mecanismo de ação da reserpina (bloqueio do TVMA-2) (adaptado de QI et al., 2008). (3-MT: 3-metoxitiramina; DA: dopamina; DA quinona: dopamina quinona; DOPAC: ácido 3,4-diidroxifenilacético; L-dopa: L-3,4-dihidroxifenilalanina; TDA: transportador de dopamina; TVMA2: transportador vesicular de monoaminas).

Acredita-se que a reserpina cause danos motores principalmente via interferência no metabolismo da dopamina, e subsequente acumulação de produtos neurotóxicos provenientes do metabolismo oxidativo deste neurotransmissor em estruturas cerebrais que participam do controle dos movimentos, como o estriado (ALUF et al., 2011; FERNANDES et. al., 2012). Esta ação da reserpina mimetiza, pelo menos em parte, o *turnover* aumentado da dopamina em terminais dopaminérgicos que tem sido verificados no curso da DP (ABÍLIO et al., 2004; BILSKA e DUBIEL, 2007; NAIDU et al., 2004) e também nas fases iniciais do tratamento com antipsicóticos que leva a DO.

Estudos com modelos de reserpina em coelhos ajudaram a elucidar o papel da dopamina na patogênese da DP (BERTLER, 1961). Esses achados levaram a descoberta de medicamentos para o tratamento dessa doença como, por exemplo, a L-dopa (ANTONY et al., 2011; CARLSSON et al., 1957; 1958; COTZIAS et al., 1967; HORNYKIEWICZ, 2002; YAHR et al., 1969).

Muitos autores têm demonstrado que animais tratados com reserpina desenvolvem DO, caracterizada pelo aumento dos movimentos de mascar no vazio, de protrusões de língua e do tempo de tremor facial, o qual é considerado por vários autores como um modelo de DT (BURGER et al., 2004; BUSANELLO et al., 2011; FARIA et al., 2005; NEISEWANDER et al., 1991; 1994; RECKZIEGEL et al., 2013), no entanto outros autores sugerem que esse alcaloide é considerado um modelo farmacológico de parkinsonismo por interferir com o estoque das catecolaminas, resultando na depleção de monoaminas nos nervos terminais o que conduz a hipolocomoção e rigidez muscular (COLPAERT, 1987; DAWSON et al., 2000;

MENZAGHI et al., 1997; SALAMONE e BASKIN, 1996). Desta forma, os modelos de DT têm sido contrastados contra o modelo da DP (DUTRA et al., 2002).

A TH é um marcador da síntese de dopamina e sua expressão é um indicador específico da produção de dopamina (LIMA et al., 2012). Um estudo recente demonstrou que uma redução nos níveis de TH induzida por repetidas administrações de reserpina pode promover déficits cognitivos em animais (SANTOS et al., 2013). Outro marcador é o TDA, o qual tem um papel importante em distúrbios que alteram a plasticidade neuronal dopaminérgica, desde que este transportador é a principal via para a captação da dopamina extracelular e para regulação da magnitude e duração da sinalização dopaminérgica (BECKMAN e QUICK, 1998; KAHLIG e GALLI, 2003). De fato, estudos demonstram que pacientes com DT ou com DP apresentam níveis reduzidos do TDA (HARRINGTON et al., 1996; YODER et al., 2004). Além disso, alterações nesses marcadores ocorrem em roedores com parkinsonismo induzido por 6-OHDA (LUNDBLAD et al., 2005; TADAIESKY et al., 2008; TRONCI et al., 2012) e em animais com discinesia orofacial induzida por haloperidol (FACHINETTO et al., 2007b). No entanto não há estudos avaliando esses parâmetros no modelo experimental da reserpina utilizando diferentes doses.

Considerando estes aspectos, hipotetiza-se que os MMVs induzidos pela reserpina em camundongos estão relacionados a alterações em proteína do sistema dopaminérgico como a tirosina hidroxilase, o transportador de dopamina e a monoaminoxidase. Uma vez que muitos estudos têm buscado tratamentos mais eficazes tanto para a DP quanto para a DT utilizando o modelo da reserpina, torna-se necessário a caracterização dos efeitos da reserpina e se estes são persistentes ao longo do tempo após a retirada do fármaco. Além disso, torna-se relevante elucidar o papel de proteínas do sistema dopaminérgico relacionadas com as alterações causadas pelo tratamento com reserpina, uma vez que o sistema dopaminérgico parece ser um ponto em comum à DP e à DT.

2 OBJETIVOS

2.1 Objetivo geral

O objetivo geral deste estudo consistiu em investigar os efeitos comportamentais induzidos por diferentes doses de reserpina em camundongos e sua relação com alterações em parâmetros relacionados ao sistema dopaminérgico.

2.2 Objetivos específicos

Em camundongos tratados com diferentes doses de reserpina avaliados 2 e 20 dias após a última injeção de reserpina:

- Verificar parâmetros locomotores e os movimentos de mascar no vazio;
- Investigar a atividade da enzima monoaminoxidase no estriado e região contendo a *substantia nigra*;
- Determinar possíveis alterações na tirosina hidroxilase e no transportador de dopamina no estriado e região contendo a *substantia nigra*.

3 RESULTADOS

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de um manuscrito, o qual se encontra aqui organizado. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se no próprio manuscrito. O **manuscrito** está disposto na forma em que será submetido para publicação na revista ***Psychopharmacology*.**

3.1 Manuscrito

EFEITOS COMPORTAMENTAIS INDUZIDOS POR DIFERENTES DOSES DE RESERPINA EM CAMUNDONGOS: RELAÇÃO COM O TRANSPORTADOR DE DOPAMINA E A TIROSINA HIDROXILASE

Manuscrito

BEHAVIORAL EFFECTS INDUCED BY DIFFERENT DOSES OF RESERPINE IN MICE: RELATIONSHIP WITH DOPAMINE TRANSPORTER AND TYROSINE HYDROXYLASE

Catiuscia Molz de Freitas; Alcindo Busanello; Luis Ricardo Peroza; Larissa Finger Schaffer; Caroline Queiroz Leal; Bárbara Nunes Krum; João Batista Teixeira da Rocha; Roselei Fachinetto.

BEHAVIORAL EFFECTS INDUCED BY DIFFERENT DOSES OF RESERPINE IN MICE: RELATIONSHIP WITH DOPAMINE TRANSPORTER AND TYROSINE HYDROXYLASE

[1]Catiuscia Molz de Freitas; [2]Alcindo Busanello; [1]Luis Ricardo Peroza; [2]Larissa Finger Schaffer; [3]Caroline Queiroz Leal; [3]Bárbara Nunes Krum; [1]João Batista Teixeira da Rocha; [1,2*]Roselei Fachinetto.

[1]*Programa de Pós-Graduação em Bioquímica Toxicológica, Universidade Federal de Santa Maria, RS, Brazil.*
[2]*Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria, RS, Brazil.*
[3]*Curso de Farmácia, Universidade Federal de Santa Maria, RS, Brazil.*

Corresponding author:
Drª. Roselei Fachinetto
Centro de Ciências da Saúde
Departamento de Fisiologia e Farmacologia
97105-900, Santa Maria, RS, Brazil
Tel: x21-55-3220 8096
Fax: x21-55-3220 8241
e-mail: roseleirf@gmail.com

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ABSTRACT

Rationale Reserpine, a monoamine-depleting agent which blocks the vesicular monoamine transporter, has been used experimentally as an animal model to study several neurological disorders, such as tardive dyskinesia and Parkinson's disease.

Objective The purpose of this study was to examine if motor deficits induced by reserpine in mice are related to alterations in dopaminergic system proteins as tyrosine hydroxylase (TH), dopamine transporter (DAT) and monoaminoxidase (MAO).

Methods Mice received either vehicle or reserpine (0.1, 0.5 or 1 mg/kg s.c.) for four consecutive days. Two or twenty days after reserpine withdrawal, behavioral and neurochemical changes were evaluated.

Results Reserpine at dose of 0.5 and 1 mg/kg increased the number of vacuous chewing movements (VCMs) and induced hypolocomotion, being this effect maintained for at least 20 days after its withdrawal. Additionally, these alterations were accompanied by reduction in DAT striatal immunoreactivity and in TH immunoreactivity in *substantia nigra* evaluated on day 6. Twenty days after the last administration of reserpine the group that received 1 mg/kg reserpine showed decreased of striatal TH and DAT immunoreactivity, reduced DAT and TH immunoreactivity in *substantia nigra*.

Conclusions These findings suggest that pharmacological blockage of vesicular monoamine transporter 2 (VMAT2) by reserpine caused neurochemical alterations in dopaminergic system proteins which seem be related to motor damage.

Keywords: Parkinson's disease. Tardive dyskinesia. Dopamine. Tyrosine hydroxylase. Dopamine transporter.

Introduction

Neurological disorders, as Parkinson's disease (PD) and tardive dyskinesia (TD), are associated with abnormal movements which are characterized by impairment of motor function mainly due alterations in nigro-striatal dopaminergic system (Lotharius and Brundin 2002; Andreassen et al. 2003). It is important to emphasize their high prevalence in the worldwide population which makes them clinically relevant (Dorsey et al. 2007; Aquino and Lang 2014). However, despite numerous studies about pathological conditions involving abnormal movements, their etiology remains incompletely understood.

Animal models have been used to study the pathophysiology of movement disorders (Abílio et al. 2003; Castro et al. 2006; Fachinetto et al. 2007a, 2007b; Salamone et al. 2008). One useful model for the study of the extrapyramidal symptoms related to parkinsonism and TD in experimental animals is by using reserpine (Neisewander et al. 1991, 1994; Salamone and Baskin 1996; Dutra et al. 2002; Busanello et al. 2011; Reckziegel et al. 2013; Reis et al. 2013).

Reserpine is a monoamine-depleting agent which blocks the vesicular monoamine transporter (Metzger et al. 2002). This blockage interferes with the storage of monoamines in intracellular vesicles, causing monoamine depletion in nerve terminals (Henry et al. 1998). Therefore, there is an increase in extracellular monoamines levels, as the dopamine, leading to an increase in the metabolism of these substances by monoaminoxidase (MAO), resulting in the production of neurotoxic products. (Lotharius and Brundin 2002; Caudle et al. 2008).

Reserpine is considered a good animal model for orofacial dyskinesia (OD) by some authors and a Parkinson's disease model by others. Animals treated with this monoamine-depleting agent develop vacuous chewing movements (VCMs) which are characteristic in OD (Neisewander et al. 1991, 1994; Burger et al. 2004; Faria et al. 2005; Busanello et al. 2011; Reckziegel et al. 2013) and were related to a decrease in dopamine uptake in rats (Fachinetto et al. 2007a; 2007b). However, other authors have suggested that reserpine may provide a pharmacological model of Parkinsonism (Colpaert 1987; Salamone and Baskin 1996; Menzaghi et al. 1997; Dawson et al. 2000) because it leads to the induction of hypolocomotion, catalepsy and muscular rigidity (Gerlach and Riederer 1996; Dutra et al. 2002).

Then, we hypothesized that VCMs induced by reserpine in mice are related to alterations in dopaminergic system proteins as tyrosine hydroxylase (TH), dopamine transporter (DAT) and MAO. The aim of the present study was to evaluate the effects of different doses of reserpine on behavioral parameters in mice. We also investigated if the alterations were accompanied by neuronal changes by measuring TH and DAT immunoreactivity and MAO activity in striatum and region containing the *substantia nigra*. In addition, we investigated the possible persistence of behavioral alterations after the withdrawal of reserpine treatment.

Materials and methods

Animals

Albino Swiss mice weighing 25-35g were kept in cages of 4-5 animals each, with controlled temperature (22±2 °C) and under a 12 h light/dark cycle (lights on at 7:00 a.m.). Food and water were provided *ad libitum*. All experiments were performed in accordance to the guidelines of the National Council of Control of Animal Experimentation (CONCEA). This protocol was approved by the Ethics Commission on Animal Use of the Federal University of Santa Maria under process number 078/2013.

Drugs

Reserpine was obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Reserpine was dissolved in 0.2% glacial acetic acid (Sigma-Aldrich, St. Louis, MO, USA) and 0.9% NaCl (Sigma-Aldrich, St. Louis, MO, USA). Vehicle consisted of 0.2% glacial acetic acid and 0.9% NaCl. Pargyline, clorgyline and kynuramine was obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA).

Experimental design

Mice were randomly divided into four groups: (I) control group; (II) reserpine 0.1 mg/kg group; (III) reserpine 0.5 mg/kg group; (IV) reserpine 1 mg/kg group. Animals received subcutaneous injections of vehicle or different doses of reserpine (Carvalho et al. 2006) once a day, for 4 consecutive days. The behavioral parameters were measured on days 6 (48 h after the 4^{th} injection) and 24 (20 days after the 4^{th} injection) (Fig. 1).

Behavioral testing

Quantification of vacuous chewing movements (VCMs)

VCMs was evaluated before the treatment (basal evaluation) and on days 6 and 24 of experimental protocol as depicted in Fig. 1. To quantify the occurrence of VCMs, mice were placed individually in cages (20x20x19 cm) containing one mirror under the floor to permit

the observation of de VCMs, when the animals were away from the observer. The VCMs were measured continuously during 6 min after a period of 6 min of adaptation (Busanello et al. 2011). VCMs are defined as single mouth openings in the vertical plane not directed towards physical object. If VCMs occurred during a period of grooming, they were not taken into account. Experimenters were always blind to treatments.

Open Field test

To evaluate possible changes in spontaneous locomotor and exploratory activity caused by treatment with reserpine, mice were placed in the center of an open field arena, divided into nine parts (Broadhurst 1960; Busanello et al. 2011). The number of lines crossed and the frequency of rearing were measured for 5 min.

Tissue preparation and biochemical assays

After the last behavioral test, on day 6 and day 24, mice were killed by cervical dislocation and the brains were rapidly dissected and put on ice. The striatum and the region containing the *substantia nigra* were separated and immediately frozen on powdered dry ice and thereafter stored at -80ºC.

Determination of MAO activity

Monoamine oxidase (MAO) activity was determined by measuring the kynuramine oxidation to 4-hydroxiquinoline (Villarinho et al. 2012; Reis et al. 2014). The striatum and region containing the *substantia nigra* were homogenized in assay buffer (16.8 mM Na_2HPO_4, 10.6 mM KH_2PO_4, 3.6 mM KCl, pH 7.4). Brain homogenates, containing 0.25 mg of protein, were pre-incubated at 37ºC with 250 nM pargyline (selective MAO-B inhibitor) and 250 nM clorgyline (selective MAO-A inhibitor) for 5 min, for MAO-A and MAO-B activity estimation. The reaction was started by the addition of 60 mM kynuramine in the reaction mixture and then incubated at 37ºC for 30 min. The reaction was stopped with 10% trichloroacetic acid (TCA). The samples were centrifuged at 3.000*g* for 8 min and the supernatant was used to estimate the MAO activity. It was added 1 ml of 1N NaOH with an equal volume of supernatant. The product of reaction was measured spectrofluorimetrically at

315 nm for excitation and 380 nm for emission. Results were expressed as nmol of 4-HQ/min/mg of protein (Villarinho et al. 2012).

Western Blotting analyze

The striatum was homogenized in 400µL of lysis buffer (4% SDS, 2 mM EDTA, 50 mM Tris, 0.5 mM Na_2VO_4, 2µg/mL aprotinin, 0.1 mM benzamidine, 0.1 mM PMSF) and the region containing the *substantia nigra* was homogenized in 800µL of the same buffer, boiled for 6 minutes and then centrifuged at 8.000 rpm at 4ºC for 10 minutes. The supernatant was used to determine protein concentration by Lowry method. Then, it was added to the samples 10% glycerol and 8% 2-mercaptoethanol. The proteins (30µg for the striatum and 60µg for the region containing the *substantia nigra*) were resolved by 10% SDS-PAGE and transferred onto nitrocellulose membrane (Millipore, USA). The proteins on the membrane were stained with a ponceau solution (0.5% ponceau plus 5% glacial acetic acid in water), as a loading control (Romero-Calvo et al. 2010). After staining, the membranes were dried, scanned, and quantified. Membranes were then processed using the SNAP ID system (Millipore, USA), blocked with 1% bovine serum albumin, incubated with an anti-DAT (1:1000; Millipore; AB2231) or anti-TH (1:1000; Millipore; AB152). After, the membranes were incubated with alkaline phosphatase-coupled secondary antibody (1:3000; Millipore). The reaction was determined by a colorimetric assay using nitroblue tetrazolium (NBT)/5-bromo-4-chloro-3-indolyl phosphate (BCIP) as substrate (Trevisan et al. 2013). The membranes were dried, scanned and quantified. Finally, all values were normalized using ponceau quantification.

Statistical analysis

The behavioral parameters were analyzed by ANOVA with repeated measures followed by Duncan's multiple range tests. Data from MAO activity and western blot were analyzed by one-way ANOVA followed by the Duncan's multiple range tests when appropriate. All values are expressed as mean ± S.E.M. Differences were considered statistically significant with $p < 0.05$.

Results

Effect of different doses of reserpine on VCMs in mice

Statistical analysis revealed a significant interaction between reserpine treatment and time ($F_{(6,24)} = 3.565$; $p = 0.011$) on the number of VCMs. *Post-hoc* analysis demonstrated that treatment with 0.5 or 1 mg/kg of reserpine increased the number of VCMs when compared with the control group on day 6 ($p < 0.01$ and $p < 0.001$ respectively). The effect of reserpine treatment on VCMs remained at least 20 days after the last injection of reserpine only at a dose of 1 mg/kg ($p < 0.05$) (Fig. 2).

Effect of different doses of reserpine on spontaneous locomotor activity in mice

Reserpine caused a marked and dose-dependent decrease on locomotor activity, represented by the number of crossings in the open field test. The treatment with 0.5 or 1 mg/kg reserpine decreased the locomotor activity 48 h after the last injection ($F_{(3,42)} = 15.93$, $p < 0.001$ and $p < 0.001$ respectively) (Fig. 3A). The reduction on locomotor activity observed in mice treated with 1mg/kg of reserpine remained until 24th day ($F_{(3,15)} = 4.20$, $p < 0.05$) (Fig. 3A). Any significant effect was observed in the group treated with reserpine at a dose of 0.1 mg/kg neither on day 6 nor day 24 compared with control group.

Similarly, mice treated with 0.5 or 1 mg/kg of reserpine presented a reduction in the exploratory activity on day 6 ($F_{(3,42)} = 10.69$, $p < 0.05$ and $p < 0.001$, respectively) (Fig. 3B). The exploratory activity remained decreased until the end of experimental period in group treated with 1 mg/kg reserpine ($F_{(3,15)} = 3,75$, $p < 0.05$), represented by the number of rearing in the open field test. Any significant effect was observed in the group treated with reserpine at a dose of 0.1 mg/kg neither on day 6 nor day 24 compared with control group (Fig. 3B).

Effect of different doses of reserpine on MAO activity in striatum and *substantia nigra* of mice

To evaluate if the treatment with reserpine affects the enzyme responsible for monoamine oxidation, we determine MAO activity. The treatment with 0.1, 0.5 or 1 mg/kg of reserpine during 4 consecutive days did not alter the MAO-A or MAO-B activity in striatum or *substantia nigra* on day 6 (Table 1). However, mice treated with 0.5 or 1 mg/kg of reserpine presented an increase on MAO-B activity compared with mice treated with 0.1 mg/kg ($F_{(3,11)} = 4.37$, $p < 0.05$) in the striatum 20 days after the 4th injection (Table 1). However, no

difference was observed between groups on the MAO-A activity in striatum or *substantia nigra* on 24th day.

Effect of different doses of reserpine in TH immunoreactivity in striatum and *substantia nigra* of mice

We also tested the possible involvement of reserpine at the immunoreactivity of TH in striatum and in region containing the *substantia nigra*. No change in striatal TH immunoreactivity was observed in mice treated with reserpine at 48 h after the last administration of reserpine (Fig. 4A). In contrast a decrease in TH immunoreactivity occurred in striatum of mice that received 1mg/kg of reserpine compared with other groups on 24th day ($F(3,15) = 2.99$, $p < 0.05$) (Fig. 4C). Furthermore, *post hoc* analysis revealed that the treatment with 1mg/kg reserpine during 4 consecutive days decreased TH immunoreactivity in *substantia nigra* when compared to control group on day 6 ($F(3,11) = 4.55$, $p < 0.01$) (Fig. 5A) and when compared with group treated with 0.5 mg/kg of reserpine on day 24 of the experimental period ($p < 0.05$) (Fig. 5C).

Effect of different doses of reserpine on DAT immunoreactivity in striatum and *substantia nigra* of mice

To determine whether the inhibition of VMAT2 by reserpine affects dopaminergic terminal function, we examined the immunoreactivity of DAT in striatum and region containing the *substantia nigra*. Western blot analysis revealed a decrease in DAT immunoreactivity in striatum at a dose of 1 mg/kg 48 h after the last administration of reserpine ($F(3,11) = 2.18$, $p < 0.05$) (Fig. 4B). Moreover, DAT immunoreactivity remained reduced in striatum of mice at least 20 days after the withdrawal of 1 mg/kg of reserpine compared all other groups ($F(3,15) = 1.34$, $p < 0.05$) (Fig. 4D).

A significant reduction in DAT immunoreactivity was also evidenced 20 days after withdrawal of 1 mg/kg reserpine compared with all other groups in region containing the *substantia nigra* ($F(3,15) = 7.55$, $p < 0.05$) (Fig. 5D). Furthermore mice treated for 4 days with 0.1 mg/kg reserpine presented an increase in DAT immunoreactivity which was significantly different from control group on day 24 (20 days after the 4th injection) ($p < 0.05$) (Fig. 5D). By the other hand, no changes in DAT immunoreactivity were observed between groups treated with reserpine on day 6 in region containing the *substantia nigra* (Fig. 5B).

Discussion

In the present study it was investigated if the behavioral (mainly VCMs) parameters induced by reserpine are related to alterations in dopaminergic system proteins. Reserpine at dose of 1 mg/kg induced VCMs characteristic of OD and motor impairment indicative of parkinsonian-like symptoms in mice. In addition, these alterations remained 20 days after withdrawal of the treatment. Of particular importance, reserpine at dose of 1 mg/kg caused alterations in DAT and TH immunoreactivity in both striatum and *substantia nigra.*

Different animal models have been proposed to study oral movement disturbances, because they can be associated with brain disorders observed in Parkinson's disease and tardive dyskinesia (TD). Particularly reserpine has been extensively used in the literature as an animal model of tardive dyskinesia since it induces VCMs (Neisewander et al. 1994; Burger et al. 2004; Faria et al. 2005; Busanello et al. 2011; Reckziegel et al. 2013). Tardive dyskinesia is a severe side effect of long-term treatment with typical antipsychotics characterized by involuntary movements of the orofacial region and, sometimes, musculature of the members and trunk (Kane 1995; Andreassen and Jørgensen 2000). Unlike, some authors believe that model of involuntary oral movements present features similar to tremors-related symptoms found in patients with PD (Steinpreis et al. 1993; Salamone and Baskin 1996; Menzaghi et al. 1997; Dawson et al. 2000). Independent if reserpine model is OD or parkinsonism, the VCMs are the main symptoms observed in the experimental animals and highly correlated to TD (Andreassen and Jørgensen 2000). However the mechanisms which could be involved in VCMs are not clear. Then, we hypothesized that VCMs induced by reserpine in mice are related to alterations in dopaminergic proteins as tyrosine hydroxylase (TH), dopamine transporter (DAT) and MAO.

Our first aim was to evaluate if the damage caused by reserpine could be maintained after its withdrawal and the dose necessary to promote permanent VCMs. Here we demonstrated that 0.5 and 1mg/kg of reserpine increased the number of VCM evaluated 48 h after the last injection and VCM remained increased at least 20 days after withdrawal of the treatment only in the higher dose, suggesting that the depletion of monoamines by reserpine caused a damage that is not easily reversed. Similarly, others studies have demonstrated an increase in number of VCMs with reserpine administration (Neisewander et al. 1994; Dutra et al. 2002; Abílio et al. 2004; Faria et al. 2005; Pereira et al. 2011; Fernandes et al. 2012) as well as the maintenance of VCMs after the last administration of reserpine (Neisewander et al. 1994). Moreover, in our study the withdrawn from the treatment with reserpine resulted in

partial recovery of VCMs in mice treated with 0.5mg/kg of reserpine. VCMs remain unchanged in the animals treated with 0.1 mg/kg of reserpine.

It was also evaluated if the doses that caused sustained VCMs were able to decrease spontaneous locomotor activity. These raise on number of VCMs were accompanied by a decrease on motor activity 48 h after the last injection of reserpine and these alterations remained 20 days after withdrawal of the treatment with 0.5 and 1mg/kg of reserpine. Additionally the dose of 0.1mg/kg of reserpine did not presented motor alterations.

As it is known reserpine causes a blockage of VMAT2 leading to a depletion in monoamines with their consequent oxidative metabolism, we examined if motor alterations promoted by reserpine could be related to alterations in presynaptic markers of dopaminergic system. In this sense, the dopamine transporter, is especially important as a marker of damage to the striatal dopaminergic terminals in PD and also DT (Miller et al. 1997). The dopamine transporter (DAT) is a transmembrane protein that regulates extracellular dopamine levels through reuptake of the released transmitter into pre-synaptic dopaminergic neurons, where dopamine is either degraded or repackaged into vesicles for release (Caudle et al. 2007). Our data shows that mice treated with 0.1 and 0.5 mg/kg of reserpine presented similar striatal DAT immunoreactivity. In contrast there was a reduction in DAT immunoreactivity in striatum 48 h after the 4th injection in animals receiving the highest dose of reserpine and this effect remained at least 20 days after withdrawal of the reserpine treatment. Studies have demonstrated that the generation of dopamine quinones, as well as reactive oxygen species, as a consequence of cytosolic accumulation of dopamine, can interact with DAT, resulting in its alteration of expression and function, which may explain the decrease in DAT observed here (Berman et al. 1996; Whitehead et al. 2001). Moreover, high concentrations of dopamine per se have been demonstrated to decrease dopamine transporter function in vitro (Berman et al. 1996). Previous data of our group reported a decrease in dopamine uptake in experimental animals presenting VCMs induced by antipsychotics (Fachinetto et al., 2007a; b). Additionally, a reduction in DAT immunoreactivity in *substantia nigra* with the highest dose used here is only seen on 24^{th} day as well as evidenced in model of PD where the dopaminergic damage begins in the striatal terminal before reaching the cell body in the *substantia nigra* (Caudle et al. 2007).

Furthermore, the reduction of tyrosine hydroxylase levels in the *substantia nigra* is also a hallmark feature of neuronal loss that occurs in PD (Olanow and Tatton 1999) and OD (Mazurek et al. 1998; Andreassen et al. 2003). TH is the rate-limiting enzyme in catecholamine biosynthesis, and its expression constitutes a specific indicator of dopamine

production (Lima et al. 2012). Interestingly, a significant decrease in TH immunoreactivity was observed 48 h and also 20 days after reserpine withdrawal of mice treated with 1 mg/kg of reserpine in *substantia nigra*. Considering that TH is a reliable marker of dopaminergic neuron terminal integrity and neuronal loss (Caudle et al. 2006), our data suggest that these alterations to the nigrostriatal dopaminergic system were likely due interference in dopamine homeostasis caused by pharmacological blockage of VMAT2 by reserpine. Although no change was observed in the striatal TH immunoreactivity on 6th day, a significant reduction in TH immunoreactivity was observed on 24th day in striatum in mice treated with 1 mg/kg of reserpine. Furthermore in low doses used (0.1 and 0.5 mg/kg) we did not observe changes in TH immunoreactivity evaluated in 48 h and 20 days after the last injection of reserpine.

As mentioned before, reserpine depletes brain levels of catecholamine by reducing vesicular storage (Metzger et al. 2002). Interestingly, a report by Caudle and cols. (2007) demonstrated that transgenic mice expressing only 5% of VMAT2 presented a decreased striatal dopamine and decreased expression of DAT and TH. The VMAT2 deficient animals have increased oxidative damage, dopamine terminals dysfunction and eventual neurodegeneration of the nigrostriatal dopamine system. As can be seen, some alterations present in the VMAT2-deficient animals are similar to those found in the animals treated with reserpine. Additionally, studies in patients with PD post mortem showed reduced DAT and VMAT2 mRNA expression in the *substantia nigra* (Harrington et al. 1996). These findings show that alterations in VMAT2 can be one of the factors related to the development of neurological disorders, which would favor the use of reserpine to study substances with potential therapeutic in animal models.

Monoamine oxidase, in the brain, catalyses the oxidative deamination of several neurotransmitters such as dopamine, noradrenaline, and serotonin (Soto-Otero et al. 2001).Neurological disorders, including Parkinson's disease have been associated with oxidative stress and increasing MAO-B activity in the central nervous system (Good et al. 1996; Volz and Gleiter 1998). So, we also investigated if reserpine is causing some alterations in MAO activity and, consequently, could be associated to the decrease observed DAT e TH immunoreactivity. However in this study we did not observe change in MAO-A or MAO-B activity in the *substantia nigra* and the striatum of mice treated with any dose of reserpine on day 6. Interestingly, on day 24 a significant increase in MAO-B activity was observed in striatum in higher doses of reserpine tested (0.5 and 1 mg/kg). However, we believe that these changes in MAO activity are not directly related motor alterations since the dose of 0.5mg/kg reserpine did not maintain these alterations over time.

In conclusion, our data demonstrate that pharmacological blockage of VMAT2 by higher dose of reserpine (1 mg/kg) caused behavioral alterations and these behavioral signs remained after interruption of treatment. The motor alterations are correlated with dopaminergic terminal dysfunction due a decrease of TH and DAT immunoreactivity. In addition, these neurochemical alterations are cellular indicators of reduced activity the dopaminergic neurons that could precede nigral neuron death. However more studies are necessary to investigate which pathways can be directly related with alterations in DAT e TH immunoreactivity and whether pharmacological interventions in them could ameliorate motor symptoms in PD and TD.

References

Abílio VC, Araujo CC, Bergamo M, Calvente PR, D'Almeida V, Ribeiro Rde A, Frussa-Filho, R (2003) Vitamin E attenuates reserpine-induced oral dyskinesia and striatal oxidized glutathione/reduced glutathione ratio (GSSG/GSH) enhancement in rats. Prog Neuropsychopharmacol Biol Psychiatry 27:109-114.

Abílio VC, Silva RH, Carvalho RC, Grassl C, Calzavara MB, Registro S et al (2004) Important role of striatal catalase in aging- and reserpine-induced oral dyskinesia. Neuropharmacology 47:263–272.

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 JR, 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.

Aquino CCH, Lang AE (2014) Tardive dyskinesia syndromes: current concepts. Parkinsonism Relat Disord 20 S1:S113-S117.

Berman SB, Zigmond MJ, Hastings TG (1996) Modification of dopamine transporter function: effect of reactive oxygen species and dopamine. J. Neurochem. 67:593–600.

Broadhurst PL (1960) Experiments in psychogenetics. In: Experiments in personality **(**Eysenk HJ, ed), pp76. London: Routledge e Kegan Paul Books.

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

Busanello A, Barbosa NB, Peroza LR, Farias LE, Burger ME, Barreto KP, Fachinetto R (2011) Resveratrol protects against a model of vacuous chewing movements induced by reserpine in mice. Behav Pharmacol 22:71-75.

Carvalho RC, Patti CC, Takatsu-Coleman AL, Kameda SR, Souza CF, Garcez-Do-Carmo L, Abílio VC, Frussa-Filho R, Silva RH (2006) Effects of reserpine on the plus-maze discriminative avoidance task: Dissociation between memory and motor impairments. Brain Res 1122:179-183.

Castro JP, Frussa-Filho R, Fukushiro DF, Silva RH, Medrano WA, Ribeiro Rde A, Abílio VC (2006) Effects of baclofen on reserpine-induced vacuous chewing movements in mice. Brain Res Bull 68:436-441.

Caudle WM, Richardson JR, Delea KC, Guillot TS, Wang M, Pennell KD, Miller GW (2006) Polychlorinated biphenyl-induced reduction of dopamine transporter expression as a precursor to Parkinson's disease associated dopamine toxicity. Toxicol Sci 92:490–499.

Caudle WM, Richardson JR, Wang MZ, Taylor TN, Guillot TS, McCormack AL, Colebrooke RE, Di Monte DA, Emson PC, Miller GW (2007) Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. J. Neurosci. 27:8138–8148.

Caudle WM, Colebrooke RE, Emson PC, Miller GW (2008) Altered vesicular dopamine storage in Parkinson's disease: a premature demise. Trends Neurosci 31:303–308.

Colpaert FC (1987) Pharmacological characteristics of tremor, rigidity and hypokinesia induced by reserpine in rat. Neuropharmacology 26:1431–1440.

Dawson L, Chadha A, Megalou M, Duty S (2000) The group II metabotropic glutamate receptor agonist, DCG-IV, alleviates akinesia following intranigral or intraventricular administration in the reserpine treated rat. Br J Pharmacol 129:541–546.

Dorsey ER, Constantinescu R, Thompson JP, Biglan KM, Holloway RG, Kieburtz K, Marshall FJ, Ravina BM, Schifitto G, Siderowf A, Tanner CM (2007) Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology 68:384-386.

Dutra RC, Andreazza AP, Andreatini R, Tufik S, Vital MA (2002) Behavioral effects of MK-801 on reserpine-treated mice. Prog Neuropsychopharmacol Biol Psychiatry 26:487-495.

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.

Faria RR, Abílio VC, Grassl C, Chinen CC, Negrão LT, de Castro JP, Fukushiro DF, Rodrigues MS, Gomes PH, Registro S, de Carvalho Rde C, D'Almeida V, Silva RH, Ribeiro Rde A, 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.

Fernandes VS, Santos JR, Leão AHFF, Medeiros AM, Melo TG, Izídio GS, Cabral A, Ribeiro RA, Abílio VC, Ribeiro AM, Silva RH (2012) Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's disease. Behav Brain Res 231:154-163.

Gerlach M, Riederer P (1996) Animal models of Parkinson's disease: an empirical comparison with the phenomenology of disease in man. J Neural Transm 103:987–1041.

Good PF, Werner P, Hsu A, Olanow CW, Perl DP (1996) Evidence of neuronal oxidative damage in Alzheimer's disease. Am. J. Pathol 149:21-28.

Harrington KA, Augood SJ, Kingsbury AE, Foster OJ, Emson PC (1996) Dopamine transporter (Dat) and synaptic vesicle amine transporter (VMAT2) gene expression in the substantia nigra of control and Parkinson's disease. Brain Res Mol Brain Res 36:157–162.

Henry JP, Sagne C, Botton D, Isambert MF, Gasnier B (1998) Molecular pharmacology of the vesicular monoamine transporter. Adv Pharmacol 42:236–239.

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

Lima MMS, Andersen ML, Reksidler AB, Ferraz AC, Vital MABF, Tufik S (2012) Paradoxical sleep deprivation modulates tyrosine hydroxylase expression in the nigrostriatal pathway and attenuates motor deficits induced by dopaminergic depletion. CNS Neurol Disord Drug Targets 11:359–68.

Lotharius J, Brundin P (2002) Pathogenesis of Parkinson's disease: dopamine, vesicles and α-synuclein. Nat Rev Neurosci 3:932-942.

Mazurek MF, Savedia SM, Bobba RS, Garside S, Rosebush PI (1998) Persistent loss of tyrosine hydroxylase immunoreactivity in the substantia nigra after neuroleptic withdrawal. J Neurol Neurosurg Psychiatry 64:799–801.

Menzaghi F, Whelan KT, Risbrough VB, Rao TS, Lloyd GK (1997) Interactions between a novel cholinergic ion channel agonist, SIB-1765F and L-DOPA in the reserpine model of Parkinson's disease in rats. J Pharmacol Exp Ther 280:393-401.

Metzger RR, Brown JM, Sandoval V, Rau KS, Elwan MA, Miller GW, Hanson GR, Fleckenstein AE (2002) Inhibitory effect of reserpine on dopamine transporter function. Eur J Pharmacol 456:39-43.

Miller GW, Staley JK, Heilman CJ, Perez JT, Mash DC, Rye DB, Levey AI (1997) Immunochemical analysis of dopamine transporter protein in Parkinson's disease. Ann Neurol 41:530–539.

Neisewander JL, Lucki I, McGonigle P (1991) Neurochemical changes associated with the persistence of spontaneous oral dyskinesia in rats following chronic reserpine treatment. Brain Res 558:27-35.

Neisewander JL, Castañeda E, Davis DA (1994) Dose-dependent differences in the development of reserpine-induced oral dyskinesia in rats: support for a model of tardive dyskinesia. Psychopharmacology (Berl) 116:79-84.

Olanow CW, Tatton WG (1999) Etiology and pathogenesis of Parkinson's disease. Annu Rev Neurosci 22:123–144.

Pereira RP, Fachinetto R, Prestes A de D, Wagner C, Sudati JH, Boligon AA, Athayde ML, Morsch VM, Rocha JB (2011) *Valeriana officinalis* ameliorates vacuous chewing movements induced by reserpine in rats. J Neural Transm 118:1547–1557.

Reckziegel P, Peroza LR, Schaffer LF, Ferrari MC, de Freitas CM, Bürger ME, Fachinetto R (2013) Gallic acid decreases vacuous chewing movements induced by reserpine in rats. Pharmacol Biochem Behav 104:132-137.

Reis EM, Ropke J, Busanello A, Reckziegel P, Leal CQ, Wagner C, Boligon AA, Athayde ML, Fachinetto R. Effect of Hypericum perforatum on different models of movement disorders in rats. Behav Pharmacol 23:623-627.

Reis EM, Schreiner Neto FW, Cattani VB, Peroza LR, Busanello A, Leal CQ, Boligon AA, Lehmen TF, Libardoni M, Athayde ML, Fachinetto R (2014) Antidepressant-like effect of *Ilex paraguariensis* in rats. Biomed Res Int. doi: 10.1155/2014/958209

Romero-Calvo I, Ocon B, Martinez-Moya P, Suarez MD, Zarzuelo A, Martinez-Augustin O, de Medina FS (2010) Reversible ponceau staining as a loading control alternative to actin in Western blots. Anal Biochem 401:318–320.

Salamone J, Baskin P (1996) Vacuous jaw movements induced by acute reserpine and low-dose apomorphine: possible model of parkinsonian tremor. Pharmacol Biochem Behav 53:179-183.

Salamone JD, Ishiwari K, Betz AJ, Farrar AM, Mingote SM, Font L, Hockemeyer J, Müller CE, Correa M (2008) Dopamine/adenosine interactions related to locomotion and tremor in animal models: Possible relevance to parkinsonism. Parkinsonism Relat Disord 14 Suppl2: S130-S134.

Soto-Otero R, Méndez-Álvarez E, Hermida-Ameijeiras A, Sánchez-Sellero I, Cruz-Landeira A, Lamas MLR (2001) Inhibition of brain monoamine oxidase activity by the generation of hydroxyl radicals Potential implications in relation to oxidative stress. Life Sciences 69:879-889.

Steinpreis RE, Baskin P, Salamone JD (1993) Vacuous jaw movements induced by sub-chronic administration of haloperidol: interactions with scopolamine. Psychopharmacology 111:99-105.

Trevisan G, Hoffmeister C, Rossato MF, Oliveira SM, Silva MA, Ineu RP, Guerra GP, Materazzi S, Camilla Fusi C, Nassini R, Geppetti P, Ferreira J (2013) Transient receptor potential ankyrin receptor stimulation by hydrogen peroxide is critical to trigger pain during monosodium urate-induced inflammation in rodents. Arthritis Rheum 65:2984-2995.

Villarinho JG, Oliveira SM, Silva CR, Cabreira TN, Ferreira J (2012) Involvement of monoamine oxidase B on models of postoperative and neuropathic pain in mice. Eur J Pharmacol 690:107-114.

Volz HP, Gleiter CH (1998) Monoamine oxidase inhibitors. A perspective on their use in the elderly. Drugs Aging 13:341-355.

Whitehead RE, Ferrer JV, Javitch JA, Justice JB (2001) Reaction of oxidized dopamine with endogenous cysteine residues in the human dopamine transporter. J Neurochem 76:1242–1251.

FIGURE LEGENDS

Fig. 1: Experimental design. Numerals within the arrow represent the number of days from day 0. The reserpine treatment started in the first day until fourth day. On day 6 (48hs after the last injection) and day 24 (20 days after withdrawal of treatment) was performed the biochemical assays. Triangle represents VCMs analysis and circle represents open field test.

Fig. 2: Effect of treatment with different doses of reserpine (0.1, 0.5, and 1mg/kg, s.c., for 4 days) in mice on VCMs during 6 min. Data are expressed as means ± SEM of nine to eleven animals per group on day 6 and four to five per group on day 24. **p < 0.01 or ***p < 0.001 compared with control group; ^{a}p < 0.05 or ^{aa}p < 0.01 compared with 0.1 mg/kg reserpine group (ANOVA with repeated measures followed by Duncan's multiple range test).

Fig. 3: Effect of the treatment with different doses of reserpine (0.1, 0.5, and 1mg/kg, s.c. for 4 days) on day 6 or 24 of the experimental period. (A) Number of crossing and (B) Frequency of rearing in the open field test. Data are expressed as means ± SEM of nine to eleven animals per group on day 6 and four to five per group on day 24. *p < 0.05, **p < 0.01 or ***p < 0.001 compared with control group; ^{aa}p < 0.01, ^{aaa}p < 0.001 compared with 0.1 mg/kg reserpine group (ANOVA with repeated measures followed by Duncan's multiple range test).

Fig. 4: Western blot analysis of DAT and TH in striatum of mice treated with different doses of reserpine (0.1, 0.5, and 1mg/kg, s.c. for 4 days) or its vehicle. (A) TH immunoreactivity and (B) DAT immunoreactivity on day 6. (C) TH immunoreactivity and (D) DAT immunoreactivity on day 24 represented by relative optical density (ROD). Data are expressed as means ± SEM of three to four animals per group. *p < 0.05 compared with control group; $^{\#}$p < 0.05 compared with group control, 0.1 mg/kg and 0.5 mg/kg reserpine; $^{@}p$

< 0.05 compared with 0.5 mg/kg reserpine (one-way ANOVA followed by Duncan's multiple range test).

Fig. 5: Western blot analysis of DAT and TH in region containing the *substantia nigra* of mice treated with different doses of reserpine (0.1, 0.5, and 1mg/kg, s.c. for 4 days) or its vehicle. (A) TH immunoreactivity and (B) DAT immunoreactivity on day 6. (C) TH immunoreactivity and (D) DAT immunoreactivity on day 24 analyzed by relative optical density (ROD). Data are expressed as means ± SEM of three to four animals per group. *p < 0.05 or **p < 0.01 compared with control group; $^{\#}$p < 0.05 compared with group control, 0.1 mg/kg and 0.5 mg/kg reserpine; $^{@}p$ < 0.05 compared with 0.5 mg/kg reserpine (one-way ANOVA followed by Duncan's multiple range test).

FIGURE 1:

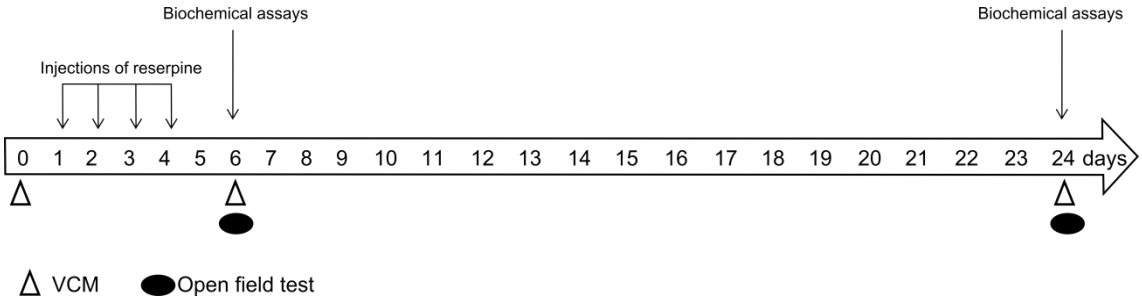

FIGURE 2:

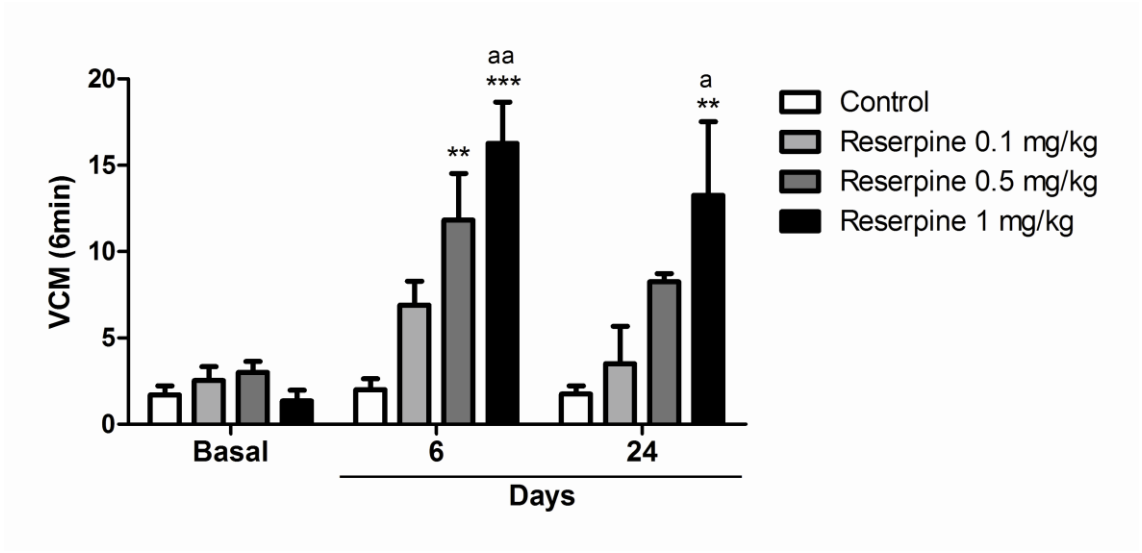

FIGURE 3:

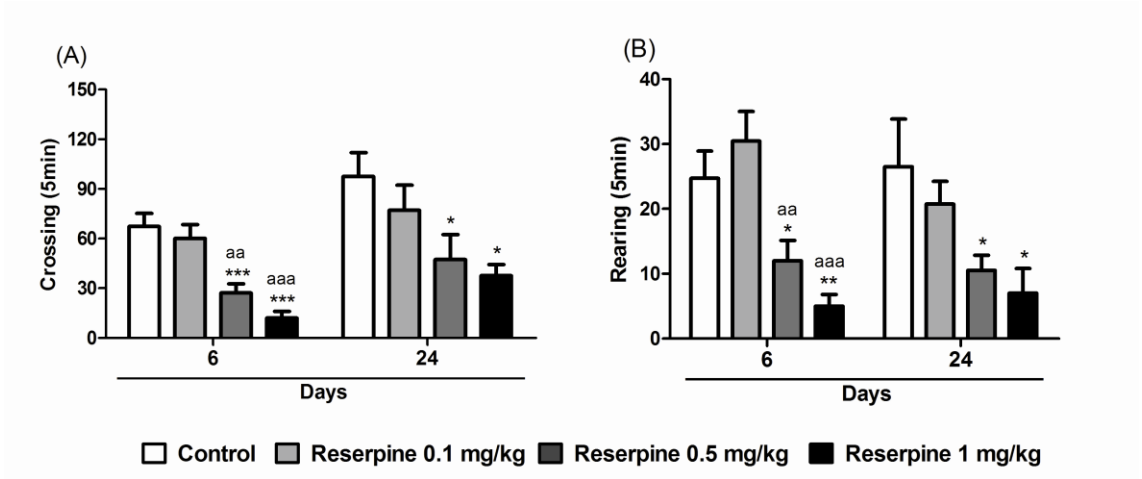

FIGURE 4:

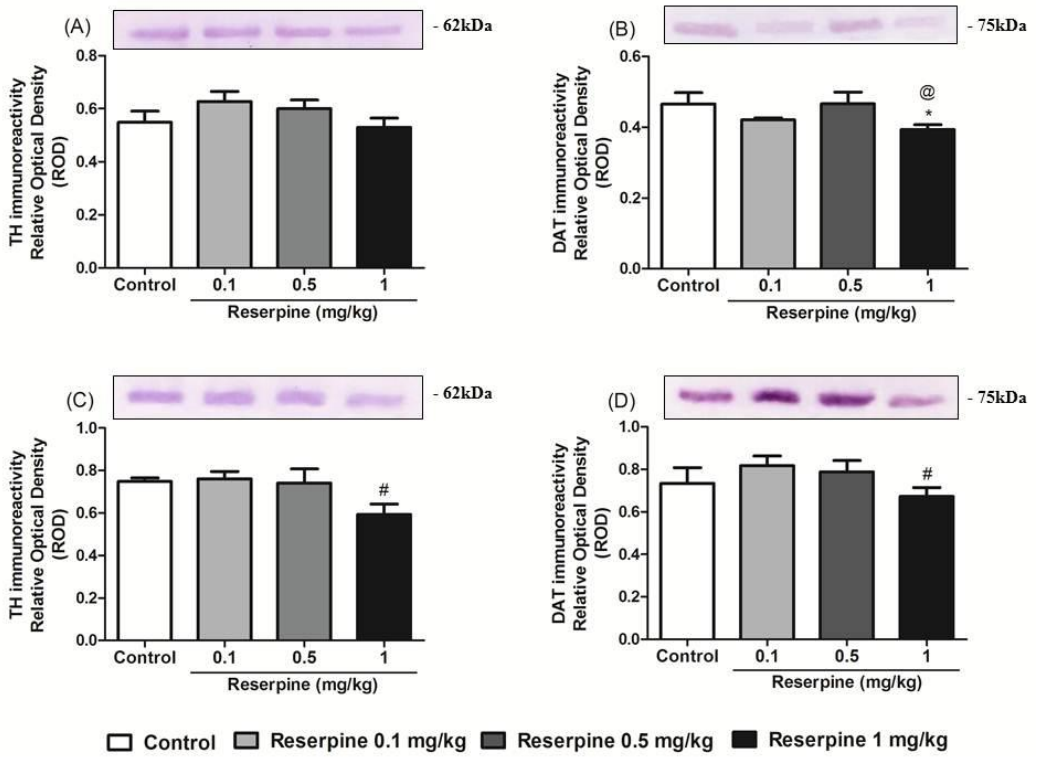

FIGURE 5:

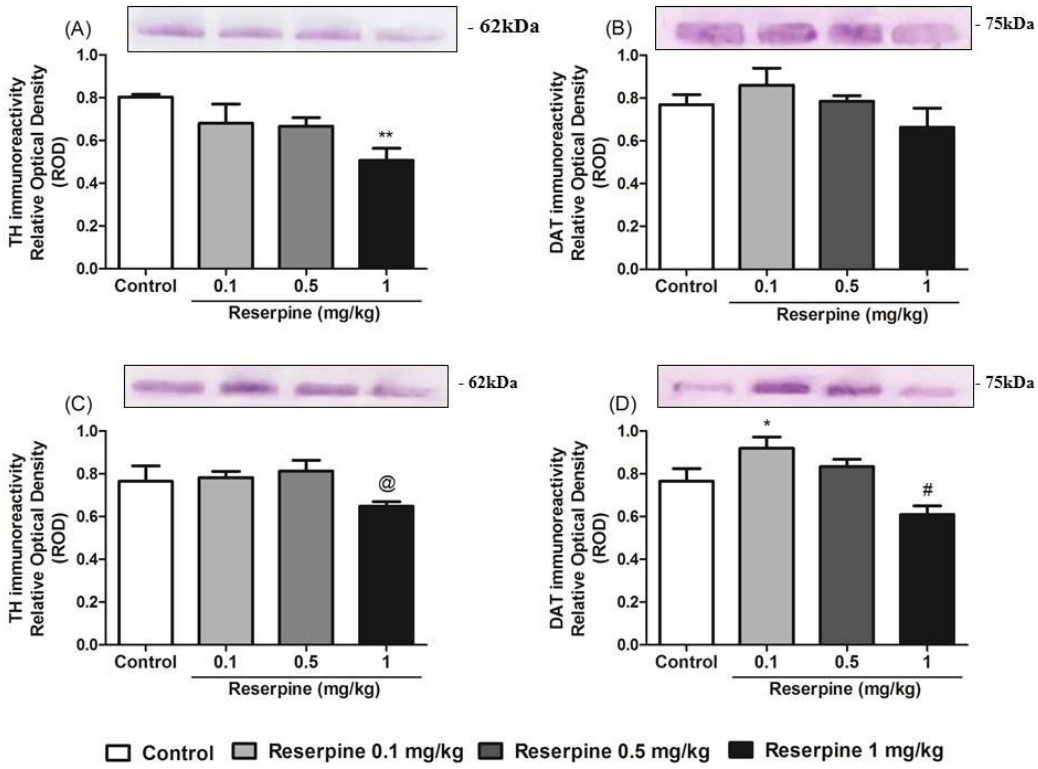

Table 1: Monoamine oxidase (MAO) activity in striatum and *substantia nigra* of mice treated with different doses of reserpine (0.1, 0.5, and 1mg/kg, s.c., for 4 days). Values are expressed as nmol of 4-HQ/mg protein/min.

		Day 6		Day 24	
		MAO-A	**MAO-B**	**MAO-A**	**MAO-B**
Control	Striatum	0.2903 ± 0.0256	0.6617 ± 0.0887	0.1783 ± 0.0034	0.5194 ± 0.0207
	Substantia nigra	0.3833 ± 0.0367	0.7307 ± 0.0818	0.3074 ± 0.0213	0.7049 ± 0.0605
Reserpine 0.1mg/kg	Striatum	0.2700 ± 0.0035	0.6080 ± 0.0278	0.1818 ±0.0195	0.4764 ± 0.0390
	Substantia nigra	0.4663 ± 0.0126	0.8450 ± 0.0372	0.2808 ± 0.0059	0.6199 ± 0.0191
Reserpine 0.5mg/kg	Striatum	0.3058 ± 0.0230	0.6445 ± 0.0467	0.1976 ±0.0078	0.5871 ± 0.0278$^{\#}$
	Substantia nigra	0.4315 ± 0.0185	0.7518 ± 0.0498	0.2908 ± 0.0194	0.6974 ± 0.0078
Reserpine 1mg/kg	Striatum	0.2998 ± 0.0269	0.6073 ± 0.0532	0.1991 ± 0.0041	0.5983 ± 0.0178$^{\#}$
	Substantia nigra	0.4625 ± 0.0124	0.7880 ± 0.0211	0.2169 ± 0.0541	0.4814 ± 0.1240

Data are expressed as means ± SEM of three to four animals per group. $^{\#}p < 0.05$ compared with group 0.1 mg/kg reserpine (ANOVA with repeated measures followed by Duncan's multiple range test)

4 CONCLUSÕES ESPECÍFICAS

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

- O bloqueio farmacológico do TVMA-2 pela reserpina na dose de 1mg/kg causou alterações comportamentais, observadas por um aumento dos MMVs e por diminuição da atividade locomotora, e estas alterações permaneceram por pelo menos 20 dias após a retirada do tratamento, sugerindo que a depleção de monoaminas causa um dano que não é facilmente revertido.

- As alterações motoras causadas pela reserpina estão relacionadas com uma redução na atividade de proteínas dopaminérgicas na via nigroestriatal observada pela diminuição nos níveis de TH e TDA causada provavelmente devido a uma interferência na homeostase da dopamina.

- A reserpina não causou alterações significativas na atividade tanto da MAO-A quanto da MAO-B no estriado e na região contendo a *substantia nigra*, demonstrando que as alterações motoras e neuroquímicas observadas não estão diretamente relacionadas com a atividade da MAO.

5 CONCLUSÕES FINAIS

Este estudou demonstrou que o bloqueio farmacológico do TVMA2 pela dose mais alta de reserpina aqui avaliada causou alterações comportamentais que foram acompanhadas por uma diminuição na atividade do TDA e da TH. Dessa forma, a reserpina causa uma redução na atividade dos neurônios dopaminérgicos na via nigroestriatal que provavelmente conduz as alterações motoras. Todavia, é preciso mais estudos para elucidar os mecanismos relacionados com as alterações nos níveis do TDA e da TH e se intervenções farmacológicas nessas proteínas poderiam aliviar os sintomas motores tanto na DP quanto na DT.

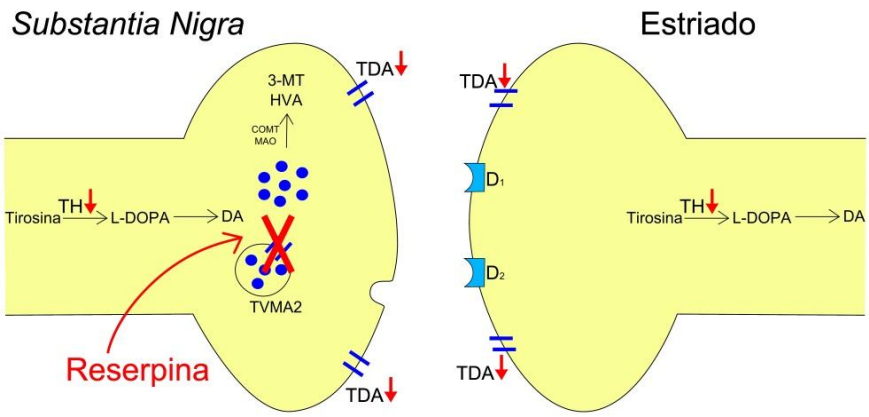

Figura 5 – Representação esquemática das alterações causadas pelo tratamento com reserpina nas proteínas do sistema dopaminérgico no estriado e *substantia nigra* de camundongos. O bloqueio farmacológico da TVMA2 pela reserpina na dose de 1 mg/kg causou uma alteração na homeostase da dopamina o que levou a uma redução nos níveis do TDA e da TH tanto no estriado quanto na *substantia nigra* 20 dias após a retirada da reserpina.

6 PERSPECTIVAS

Com base nos resultados obtidos no presente trabalho, temos como perspectivas:

- Analisar a possível participação da apoptose nos efeitos da reserpina nestes modelos verificando o envolvimento de parâmetros apoptóticos: alterações na expressão proteíca das caspases 3 e 9.

- Investigar os efeitos da reserpina em marcadores inflamatórios: alterações na expressão proteica NF-κb e alterações nos níveis de interleucinas (IL-2, IL-6, IL-10 e TNF-α).

REFERÊNCIAS BIBLIOGRÁFICAS

AARSLAND, D. et al. The rate of cognitive decline in Parkinson disease. **Archives of Neurology,** v. 61, p. 1906–1911, 2004.

ABÍLIO, V. C. et al. Important role of striatal catalase in aging- and reserpine-induced oral dyskinesia. **Neuropharmacology**, v. 47, p. 263–272, 2004.

ABÍLIO, V. C. et al. Vitamin E attenuates reserpine-induced oral dyskinesia and striatal oxidized glutatione/reduced glutatione ratio (GSSG/GSH) enhancement in rats. **Progress in Neuro-Psychophamarmacology & Biological Psychiatry**, v. 27, p. 109–114, 2003.

AGID, Y. Parkinson's disease: pathophysiology. **Lancet**, v. 337, p. 1321–1323, 1991.

AHLSKOG, J. E.; MUENTER, M. D. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. **Movement Disorders**, v. 16, p. 448–458, 2001.

ALACHKAR, A. et al. Locomotor response to L-DOPA in reserpine-treated rats following central inhibition of aromatic l-amino acid decarboxylase: Further evidence for non-dopaminergic actions of l-DOPA and its metabolites. **Neuroscience Research**, v. 68, p. 44–50, 2010.

ALBIN, R. L.; YOUNG, A. B.; PENNEY, J. B. The functional anatomy of basal ganglia disorders. **Trends in Neurosciences**, v. 12, n. 10, p. 366–375, 1989.

AL-BLOUSHI, S. et al. Green tea modulates reserpine toxicity in animal models. **The Journal of Toxicological Sciences**, v. 34, p. 77–87, 2009.

ALUF, Y. et al. Alterations in striatal oxidative stress level produced by pharmacological manipulation of dopamine as shown by a novel synthetic marker molecule. **Neuropharmacology**, v. 61, p. 87–94, 2011.

ALVES, G. et al. Epidemiology of Parkinson's disease. **Journal of Neurology**, v. 255 [Suppl 5], p. 18–32, 2008.

AMARA, S. G.; KUHAR, M. J. Neurotransmitter transporters: recent progress**. Annual Review of Neuroscience**, v. 16, p. 73–93, 1993.

ANDREASSEN, O. A. et al. Oral dyskinesias and histopathological alterations in substantia nigra after long term haloperidol treatment of old rats. **Neuroscience**, v. 122, p. 717–725, 2003.

ANDREASSEN, O. A. et al. Oral dyskinesias and striatal lesions in rats after long-term co-treatment with haloperidol and 3-nitropropionic acid. **Neuroscience**, v. 87, p. 639–648, 1998.

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. **British Journal of Pharmacology**, v. 119, p. 751–757, 1996.

ANDREASSEN, O. A.; JORGENSEN, H. A. Neurotoxicity associated with neurolepticinducedoral dyskinesias in rats. Implications for tardive dyskinesia? **Progress in Neurobiology**, v. 61, p. 525–541, 2000.

ANTONY, P. M. A.; DIEDERICH, N. J.; BALLING, R. Parkinson's disease mouse models in translational research**. Mammalian Genome**, v. 22, p. 401–419, 2011.

BEAL, M. F.; LANG, A. E.; LUDOLPH, A. C. **Neurodegenerative Diseases**: Neurobiology, Pathogenesis and Therapeutics. Cambridge, England: Cambridge University Press, 2005.

BECKMAN, M. L.; QUICK, M. W. Neurotransmitter transporters: regulators of function and functional regulation. **The Journal of Membrane Biology**, v. 164, p. 1–10, 1998.

BERTLER, A. Effect of reserpine on the storage of catechol amines in brain and other tissues. **Acta Physiologica Scandinavica**, v. 51, p. 75–83, 1961.

BILSKA, A.; DUBIEL, M. Alpha-lipoic acid differently affects the reserpine induced oxidative stress in the striatum and prefrontal cortex of rat brain. **Neuroscience**, v. 146, p. 1758–1771, 2007.

BLACKSTONE, C. Infantile parkinsonism-dystonia: a dopamine "transportopathy". **The Journal of Clinical Investigation**, v. 119, p. 1455–1458, 2009.

BRONNICK, K. et al. Attentional deficits affect activities of daily living in dementia-associated with Parkinson's disease. **Journal of Neurology, Neurosurgery and Psychiatry**, v. 77, p. 1136–1142, 2006.

BURGER, M. E. et al. Acute reserpine and subchronic haloperidol treatments change synaptosomal brain glutamate uptake and elicit orofacial dyskinesia in rats. **Brain Research**, v. 1031, p. 202–210, 2005b.

BURGER, M. E. et al. Ebselen attenuates haloperidol-induced orofacial dyskinesia and oxidative stress in rat brain. **Pharmacology Biochemistry and Behavior**, v. 81, p. 608–615, 2005a.

BURGER, M. E. et al. Ebselen attenuates reserpine-induced orofacial dyskinesia and oxidative stress in rat striatum. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 27, p. 135–140, 2003.

BURGER, M. E. et al. Effects on age on orofacial dyskinesia reserpine-induced and possible protection of diphenyl-diselenide. **Brain Research Bulletin**, v. 64, p. 339-345, 2004.

BUSANELLO, A. et al. Resveratrol reduces vacuous chewing movements induced by acute treatment with fluphenazine. **Pharmacology, Biochemistry and Behavior**, v. 101, p. 307–310, 2012.

BUSANELLO, A. et al. Resveratrol protects against a model of vacuous chewing movements induced by reserpine in mice. **BehaviouralPharmacology**, v. 22, p. 71–75, 2011.

CARLSSON, A.; LINDQVIST, M.; MAGNUSSON, T. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. **Nature**, v. 180, p. 1200, 1957.

CASEY, D. E. Tardive dyskinesia: reversible and irreversible. **Psychopharmacology**, v. 2, p. 88–97, 1985.

CASTRO, J. P. et al. Effects of baclofen on reserpine-induced vacuous chewing movements in mice. **Brain Research Bulletin**, v. 68, p. 436–441, 2006.

COLPAERT, F. C. Pharmacological characteristics of tremor, rigidity and
COOKSON, M. R. The biochemistry of Parkinson's disease. **Annual Review Biochemistry,** v. 74, p. 29–52, 2005.

COTZIAS, G. C.; VAN WOERT, M. H.; SCHIFFER, L. M. Aromatic amino acids and modification of parkinsonism. **The New England Journal of Medicine**, v. 276, p. 374–379, 1967.

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

DARLING, H. F. Fluphenazine: a preliminary study. **Diseases of Nervous System**, v. 20, p. 167–170, 1959.

DAUER, W.; PRZEDBORSKI, S. Parkinson's Disease: mechanisms and models. **Neuron**, v. 39, p. 889–909, 2003.

DAWSON, L. et al. The group II metabotropic glutamate receptor agonist, DCG-IV, alleviates akinesia following intranigral or intraventricular administration in the reserpine treated rat. **British Journal of Pharmacology**, v. 129, p. 541–546, 2000.

DAWSON, T. M.; DAWSON, V. L. Molecular pathways of neurodegeneration in Parkinson's disease. **Science**, v. 302, p. 819–822, 2003.

DOYLE, A. E.; MCQUEEN, E. G.; SMIRK, F. H. Treatment of hypertension with reserpine, with reserpine in combination with pentapyrrolidinium, and with reserpine in combination with veratrum alkaloids. **Circulation**, v. 11, p. 170–181, 1955.

DUTRA, R. C. et al. Behavioral effects of MK-801 on reserpine-treated mice. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 26, p. 487–495, 2002.

EGAN, M. F. et al. Neuroleptic-induced vacuous chewing movements in rodents: incidence and effects of long-term increases in haloperidol dose. **Psychopharmacology (Berl)**, v. 117, p. 74–81, 1995.

ELLENBROEK, B. A. Treatment of schizophrenia: a clinical and preclinical evaluation of neuroleptic drugs. **Pharmacology & Therapeutics**, v. 57, p. 1–78, 1993.

FACHINETTO, R. et al. Diphenyl diselenide decreases the prevalence of vacuous chewing movements induced by fluphenazine in rats. **Psychopharmacology (Berl)**, v. 194, p. 423–432, 2007b.

FACHINETTO, R. et al. *Valeriana officinalis* does not alter the orofacial dyskinesia induced by haloperidol in rats: role of dopamine transporter. **Progress in Neuropsychopharmacology & Biological Psychiatry**, v. 31, p. 1478–1486, 2007a.

FAHN, S. Description of Parkinson's disease as a clinical syndrome. **Annals of New York Academy of Sciences**, v. 991, p. 1–14, 2003.

FARIA, R. R. et al. Beneficial effect of vitamin C and vitamin E on reserpine-induced oral dyskinesia in rats: Critical role of striatal catalase activity. **Neuropharmacology**, v. 48, p. 993–1001, 2005.

FERNANDES, V. S. et al. Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's disease. **Behavioural Brain Research**, v. 231, p. 154–163, 2012.

GERLACH, M.; RIEDERER, P. Animal models of Parkinson's disease: an empirical comparison with the phenomenology of disease in man. **Journal of Neural Transmission**, v. 103, p. 987–1041, 1996.

GLAZER, W. M. et al. Predictors of improvement in tardive dyskinesia following discontinuation of neuroleptic medication. **The British Journal of Psychiatry**, v. 157, p. 585–592, 1990.

GOODMAN, L. S.; GILMAN, A. **Manual de farmacologia e terapêutica**. Rio de Janeiro: Mc Graw Hill, 2010.

HARRINGTON, K. A. et al. Dopamine transporter (Dat) and synaptic vesicle amine transporter (VMAT2) gene expression in the substantia nigra of control and Parkinson's disease. **Brain Research: Molecular Brain Research**, v. 36, p. 157–162, 1996.

HENDERSON, D. C. Diabetes mellitus and other metabolic disturbances induced by atypical and antipsychotic agents. **Current Diabetes Reports**, v. 2, p. 135–140, 2002.

HIRTH, F. *Drosophila melanogaster* in the study of human neurodegeneration. **CNS & Neurological Disorders - Drug Targets**, v. 9, p. 504–523, 2010.

HORNYKIEWICZ, O. L-DOPA: from a biologically inactive amino acid to a successful therapeutic agent. **Amino acids**, v. 23, p. 65–70, 2002.

JELLINGER, K. A. Pathology of Parkinson's syndrome. In: CALNE, D. B. (Ed.). **Handbook of experimental pharmacology**, v. 88. Springer, New York, p. 47–112, 1988.

JORGENSEN, H. A.; ANDREASSEN, O. A.; HOLE, K. The relationship between motor effects in rats following acute and chronic haloperidol treatment. **Psychopharmacology (Berl)**, v. 116, p. 89–92, 1994.

KAHLIG, K. M.; GALLI, A. Regulation of dopamine transporter function and plasma membrane expression by dopamine, amphetamine, and cocaine. **European Journal of Pharmacology,** v. 479, p. 153–158, 2003.

KANE, J. et al. Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. **Archives of General Psychiatry**, v. 45, p. 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**, v. 39. Raven Press, Ney York, p. 1485–1495, 1995.

KLAWANS, J. R. H. L.; RUBOVITS, R. An experimental model of tardive dyskinesia. **Journal of Neural Transmission**, v. 33, p. 235–246, 1972.

KLOCKGETHER, T. Parkinson's disease: clinical aspects**. Cell and Tissue Research,** v. 318, p. 115–120, 2004.

KULKARNI, S. K.; NAIDU, P. S. Pathophysiology and drug therapy of tardive dyskinesia: current concepts and future perspectives. **Drugs of Today**, v. 39, p. 19–49, 2003.

KUROKI, T.; NAGAO, N.; NAKAHARA, T. Neuropharmacology of second-generation antipsychotic drugs: a validity of the serotonin-dopamine hypothesis. **Progress in Brain Research**, v. 172, p. 199–212, 2008.

LEUCHT, S. et al. A meta-analysis of head-to-head comparisons of second-generation antipsychotics in the treatment of schizophrenia. **American Journal of Psychiatry**, v. 166, p. 152–163, 2009.

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

LIMA, M. M. S. et al. Paradoxical sleep deprivation modulates tyrosine hydroxylase expression in the nigrostriatal pathway and attenuates motor deficits induced by dopaminergic depletion. **CNS & Neurological Disorders – Drug Targets**, v. 11, p. 359–68, 2012.

LIMONGI, J. C. P. Doença de Parkinson: Aspectos diagnósticos. In: Congresso Neuropsiquiatria dos gânglios da base. Protoc, HCFM-USP, São Paulo. **Anais**. v. 1, p. 194–195, 1995.

LINDNER, M. D. et al. Incomplete nigrostriatal dopaminergic cell loss and partial reductions in striatal dopamine produce akinesia, rigidity, tremor and cognitive deficits in middle-aged rats. **Behavioural Brain Research**, v. 102, p. 1–16, 1999.

LLORCA, P. et al. Tardive dyskinesias and antipsychotics: a review. **European Psychiatry**, v. 17, p. 129–138, 2002.

LUNDBLAD, M. et al. Pharmacological validation of a mouse model of L-DOPA-induced dyskinesia. **Experimental Neurology**, v. 194, p. 66–75, 2005.

MAHIEUX, F. et al. Neuropsychological prediction of dementia in Parkinson's disease. **Journal of Neurology, Neurosurgery and Psychiatry**, v. 64, p. 178–183, 1998.

MENESES, M. S.; TEIVE, H. A. G. **Doença de Parkinson**. **Aspectos Clínicos e cirúrgicos**. Guanabara Koogan, Rio de Janeiro, 1996.

MENZAGHI, F.; WHELAN, K. T.; RISBROUGH, V. B.; RAO, T. S.; LLOYD, G. K. Interactions between a novel cholinergic ion channel agonist, SIB-1765F and L-DOPA in the reserpine model of Parkinson's disease in rats. **The Journal of Pharmacology and Experimental Therapeutics**, v. 280, p. 393–401, 1997.

METZGER, R. R. et al. Inhibitory effect of reserpine on dopamine transporter function. **European Journal of Pharmacology**, v. 456, p. 39–43, 2002.

MOORE, D. J. et al. Molecular pathophysiology of Parkinson's disease. **Annual Review of Neuroscience**, v. 28, p. 57–87, 2005.

NAGATSU, T.; SAWADA, M. L-DOPA therapy for Parkinson's disease: Past, present, and future. **Parkinsonism and Related Disorders**, v. 15, p. 3–8, 2009.

NAIDU, P. S. et al. Quercetin, a bioflavonoid, attenuates haloperidol-induced orofacial dyskinesia. **Neuropharmacology**, v. 44, p. 1100–1106, 2003.

NAIDU, P. S.; SINGH, A.; KULKARNI, S. K. Reversal of reserpine-induced orofacial dyskinesia and cognitive dysfunction by quercetin. **Pharmacology**, v. 70, p. 59–67, 2004.

NEISEWANDER, J. L.; CASTAÑEDA, E.; DAVIS, D. A. Dose-dependent differences in the development of reserpine-induced oral dyskinesia in rats: support for a model of tardive dyskinesia. **Psychopharmacology**, v. 116, p. 79–84, 1994.

NEISEWANDER, J. L.; LUCKI, I.; MCGONIGLE, P. Neurochemical changes associated with the persistence of spontaneous oral dyskinesia. **Brain Research**, v. 558, p. 27–35, 1991.

Neuropsiquiatria dos gânglios da base. Protoc, HCFM-USP, São Paulo. **Anais**. v. 1, p. 194–195, 1995.

OBESO, J. A. et al. Pathophysiology of the basal ganglia in Parkinson's disease. **Trends in Neurosciences** (Suppl.), v. 23, p. S8–S19, 2000.

PEREIRA, R. P. et al. *Valeriana officinalis* ameliorates vacuous chewing movements induced by reserpine in rats. **Journal of Neural Transmission**, v. 118, p. 1547–1557, 2011.

PEROZA, L. R. et al. *Bauhinia forficata* prevents vacuous chewing movements induced by haloperidol in rats and has antioxidant potential in vitro. **Neurochemical Research**, v. 38, p. 789–796, 2013.

POST, A. et al. Mechanisms underlying the protective potential of alpha-tocopherol (vitamin E) against haloperidolassociated neurotoxicity. **Neuropsychopharmacology**, v. 26, p. 397–407, 2002.

POSTLE, B. R. et al. The time course of spatial and object learning in Parkinson's disease. **Neuropsychologia**, v. 35, p. 1413–1422, 1997.

QI, Z.; MILLER, G. W.; VOIT, E. O. Computational systems analysis of dopamine metabolism. **Plos One**, v. 3, p. 1–10, 2008.

RANG, H. P. et al. **Farmacologia**. 7. ed., Rio de Janeiro: Elsevier, 2012.

RECKZIEGEL, P. et al. Gallic acid decreases vacuous chewing movements induced by reserpine in rats. **Pharmacology, Biochemistry and Behavior**, v. 104, p. 132–137, 2013.

RUBINSTEIN, M. et al. Adaptative mechanisms of striatal D1 and D2 dopamine receptors in response to a prolonged reserpine treated mice. **Journal of Pharmacology and Experimental Therapeutics**, v. 252, p. 810–816, 1990.

SADAN, O. et al. A novel brain-targeted antioxidant (AD4) attenuates haloperidol-induced abnormal movement in rats: implications for tardive dyskinesia. **Clinical Neuropharmacology**, v. 28, p. 285–288, 2005.

SALAMONE, J.; BASKIN, P. Vacuous jaw movements induced by acute reserpine and low-dose apomorphine: possible model of parkinsonian tremor. **Pharmacology, Biochemistry and Behavior**, v. 53, p. 179–183, 1996.

SANTOS, J. R. et al. Cognitive, motor and tyrosine hydroxylase temporal impairment in a model of parkinsonism induced by reserpine. **Behavioural Brain Research**, v. 253, p. 68–77, 2013.

SCHERMAN, D. et al. Striatal dopamine deficiency in Parkinson's disease: role of aging. **Annals of Neurology**, v. 26, p. 551–557, 1989.

SCHMITT-ELIASSEN, J. et al. Feedback-based versus observational classification learning in healthy aging and Parkinson's disease. **Brain Research**, v. 1142, p. 178–188, 2007.

SILVA, R. S. A. **O stress oxidativo na toxicidade das catecolaminas.** 2006/2007. 106 f. Seminário (Mestrado em Toxicologia Analítica, Clínica e Forense) – Faculdade de Farmácia da Universidade do Porto. Porto: 2007.

STANDAERT, D. G.; ROBERSON, E. D. Tratamento dos distúrbios degenerativos do sistema nervoso central. In: BRUNTON, L. L.; CHABNER, B. A.; KNOLLMANN, B. C. (Ed). **As bases farmacológicas da terapêutica de Goodman & Gilman**. 12. ed., Porto Alegre: AMGH, 2012, cap. 22, p. 609–628.

TADAIESKY M. T. et al. Emotional, cognitive and neurochemical alterations in a premotor model of Parkinson's disease. **Neuroscience**, v. 156, p. 830–840, 2008.

TADAIESKY, M. T.; ANDREATINI, R.; VITAL, M. A. B. F. Different effects of 7-nitroindazole in reserpine-induced hypolocomotion in two strains of mice. **European Journal of Pharmacology**, v. 535, p. 199–207, 2006.

THOMAS, B.; BEAL, M. F. Parkinson's disease. **Human Molecular Genetics**, v. 16, p. R183–R194, 2007.

TRONCI, E. et al. Amphetamine-induced rotation and l-DOPA-induced dyskinesia in the rat 6-OHDA model: A correlation study. **Neuroscience Research**, v. 73, p. 168–172, 2012.

VERBAAN, D. et al. Cognitive impairment in Parkinson's disease. **Journal of Neurology, Neurosurgery and Psychiatry**, v. 78, p. 1182–1187, 2007.

YAHR M. D. et al. Treatment of parkinsonism with levodopa. **Archives of Neurology**, v. 21, p. 343–354, 1969.

YODER, K. K. et al. Dopamine transporter density in schizophrenic subjects with and without tardive dyskinesia. **Schizophrenia Research**, v. 71, p. 371–375, 2004.