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PAPEL DO MAGNÉSIO NA PREVENÇÃO E REVERSÃO DE DISTÚRBIOS MOTORES EXPERIMENTALMENTE INDUZIDOS

DISSERTAÇÃO DE MESTRADO

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Santa Maria, RS, Brasil

2014

PAPEL DO MAGNÉSIO NA PREVENÇÃO E REVERSÃO DE DISTÚRBIOS MOTORES EXPERIMENTALMENTE INDUZIDOS

por

Maikel Kronbauer

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Orientadora: Profª. Drª. Marilise Escobar Bürger

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elaborada por
Maikel Kronbauer

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

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Santa Maria, 31 de outubro de 2014.

Dedico esta dissertação à minha família

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RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
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PAPEL DO MAGNÉSIO NA PREVENÇÃO E REVERSÃO DE DISTÚRBIOS MOTORES EXPERIMENTALMENTE INDUZIDOS

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Data e Local: Santa Maria, 31 de outubro de 2014.

O tratamento crônico de distúrbios psicóticos está associado a efeitos adversos que afetam a função motora. Os distúrbios do movimento incluem o Parkinsonismo, acatisia, distonias e também discinesia tardia. O magnésio (Mg) é um mineral essencial para diversas funções fisiológicas no organismo e sua suplementação tem sido empregada em diversas doenças. O objetivo deste estudo foi investigar o efeito da suplementação de Mg sobre a prevenção e a reversão da discinesia orofacial (DO), designados como experimento 1 e 2, respectivamente, bem como o efeito sobre parâmetros de estresse oxidativo. Em ambos os experimentos foram utilizados ratos machos Wistar adultos. No experimento 1, os ratos foram divididos aleatoriamente em dois grupos, ambos suplementados oralmente com solução de aspartato de magnésio (40 mg/Kg/mL) ou água deionizada. Depois de 28 dias de suplementação, metade de cada grupo experimental foi tratada com uma solução de reserpina (0,7 mg/kg/mL; sc) (R e Mg + R grupos) ou veículo (C e Mg grupos) durante 3 dias (em dias alternados). Um dia (24 horas) após a última administração de R / veículo, todos os animais foram submetidos a avaliações comportamentais de DO, através da quantificação de movimentos de mascar no vazio (MMV), e tempo de catalepsia. No experimento 2 os ratos foram divididos aleatoriamente em dois grupos e tratados com solução de reserpina (0,7 mg/kg/mL; sc) (grupos R) ou veículo (grupo C) durante 3 dias (em dias alternados). Vinte e quatro horas após a última administração de R / veículo, o desenvolvimento de DO foi quantificada. Metade de cada grupo experimental foi suplementado imediatamente, uma vez por dia (por gavagem) com aspartato de magnésio (40 mg/kg/mL) (grupos Mg e R + Mg) ou água desionizada (grupos C e R). A DO foi quantificada durante os dias subsequentes (cada 48h). A suplementação de Mg foi mantida durante todo o tempo de avaliação comportamental (10 dias consecutivos). Após as avaliações comportamentais todos os animais foram eutanasiados por exsangüinação. O sangue foi retirado para análise dos níveis de lipoperoxidação (LP) eritrocitária. Os cérebros foram imediatamente dissecados para a separação da região do córtex, corpo estriado e *substantia nigra* para a determinação de espécies reativas (ER) e de proteína carbonil (PC). Os resultados mostraram que a suplementação de Mg antes da administração da reserpina foi suficiente para prevenir os distúrbios do movimento, observados pela frequência de MMV e tempo de catalepsia; bem como foi capaz de evitar a geração de ER e PC, tanto na região do córtex como na *substantia nigra,* também impedindo a LP nos eritrócitos. A suplementação de Mg após o tratamento com reserpina foi capaz de minimizar a frequência de MMV e o tempo de catalepsia, reduzir a geração de ER e os níveis de PC nas regiões do córtex e do corpo estriado, revertendo também o aumento do nível de LP nos eritrócitos. Nossos resultados ressaltam a importância da inclusão de terapias alternativas através da suplementação de substancias naturais essenciais, como o magnésio, as quais podem

prevenir ou atenuar distúrbios motores, frequentemente relacionados ao tratamento crônico de distúrbios psicóticos que até o momento não dispõe de um tratamento eficaz.

Palavras-chave: reserpina; distúrbios do movimento; discinesia orofacial; magnésio; estresse oxidativo.

ABSTRACT

Master Science Dissertation
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PREVENTION AND REVERSAL ROLE OF MAGNESIUM ON MOTOR DISORDERS EXPERIMENTALLY INDUCED

AUTHOR: MAIKEL KRONBAUER
ADVISOR: MARILISE ESCOBAR BÜRGER
Date and place: October 31, 2014, Santa Maria.

Chronic treatment of psychotic disorders is associated with adverse effects that affect motor function. Movement disorders include Parkinsonism, akathisia, dystonia and tardive dyskinesia. Magnesium (Mg) is an essential mineral for various physiological functions in the body and its supplementation is used in several diseases. The purpose of this study was investigate the effect of Mg supplementation on the prevention and reversal of orofacial dyskinesia (OD), designated as experiment 1 and 2, respectively, as well as the effect on oxidative stress parameters. In both experiments, male Wistar adult rats were used. In experiment 1, rats were randomly divided into two groups both supplemented with oral solution of magnesium aspartate (40 mg/Kg/mL) or deionized water. After 28 days of supplementation, half of each experimental group was treated with a reserpine solution (0.7 mg/kg/ml, sc) (Mg + R and R groups) or vehicle (C and Mg groups) for 3 days (every other day). One day (24 hours) after the last administration of R/vehicle, all animals were subjected to OD and catalepsy time behavioral assessments. In the experiment 2 rats were randomly divided into two groups and treated with a solution of reserpine (0.7 mg / kg / ml, sc) (R groups) or vehicle (Group C) for 3 days (every other day). Twenty-four hours after the last administration of R/vehicle, the development of OD was quantified. One-half of each experimental group was supplemented immediately once a day (by gavage) with magnesium aspartate (40 mg / kg / ml) (Mg, and groups R + Mg) or deionized water (groups C and R). The OD was measured during subsequent days (every 48 hours). Mg supplementation was maintained throughout the time of behavioral assessment (10 consecutive days). After behavioral evaluations, all animals were euthanized by exsanguination. Blood was drawn for analysis of erythrocytes lipid peroxidation (LP). The brains were immediately dissected for separating cortex, striatum and *substantia nigra* for determining reactive species (RS) and protein carbonyl (PC). The results showed that Mg supplementation before reserpine administration was sufficient to prevent movement disorder observed in the vacuous chewing movement frequency (VCM) and catalepsy time and also was able to prevent the generation of RS and PC, in both the cortex and the *substantia nigra* regions, also preventing LP in the erythrocytes. Supplementation of Mg after treatment with reserpine was able to minimize the frequency of VCM and catalepsy time, reduce the generation of RS and PC levels in both cortex and the corpus striatum regions and also reversing the increasing the level of LP in the erythrocytes. Our results underscore the importance of including alternative therapies through supplementation of essential natural substances, like magnesium, which can prevent or ameliorate motor disturbances, often related to chronic treatment of psychotic disorders that so far have no effective treatment.

Keywords: reserpine; movement disorders; orofacial dyskinesia; magnesium; oxidative stress.

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SUMÁRIO

APRESENTAÇÃO

Esta dissertação apresenta parte dos métodos e resultados embutidos em um manuscrito que se encontra na secção **MANUSCRITO CIENTÍFICO**, sob a formatação da revista para a qual foi submetido para publicação.

Ao fim desta dissertação encontram-se os itens **DISCUSSÃO, CONCLUSÕES** e **PERSPECTIVAS** nos quais há interpretações e comentários gerais sobre o manuscrito científico contido neste estudo, bem como propostas para a continuidade do estudo.

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

1 INTRODUÇÃO

O uso crônico de antipsicóticos típicos no tratamento sintomático de psicoses como a esquizofrenia, está diretamente relacionado com o desenvolvimento de distúrbios motores extrapiramidais tais como parkinsonismo e discinesia tardia (DT). Estes distúrbios são, muitas vezes, incapacitantes e irreversíveis além de serem associados ao estigma social, deficiências físicas e uma pior qualidade de vida. Eles também contribuem para a não-conformidade ao tratamento, dificultando a adesão, melhora clínica e o reengajamento à sociedade o que resulta em um aumento do risco de recaída psicótica (CHAPLIN; KENT, 1998; LAMBERT et al., 2004; CASEY, 2006). A esperança de que a nova geração de antipsicóticos atípicos não desenvolveria tais distúrbios parece improvável de ser cumprida (PELUSO et al., 2012; REMINGTON; HAHN, 2014).

Devido à complexidade destes distúrbios, diversas hipóteses têm sido sugeridas sobre sua etiologia e, consequentemente, estratégias farmacológicas para reduzir esses efeitos têm sido aplicadas, como: drogas colinérgicas (DESMARAIS et al., 2012), agonistas gabaérgicos (THAKER et al., 1990; ALABED et al., 2011), amantadina (PAPPA et al., 2010), porém, com pouca eficácia. Tem se observado a melhora clínica de pacientes com DT que fazem uso de substâncias antioxidantes (LERNER et al., 2007; ZHANG et al., 2011; ANDERSON; MAES, 2012) e em modelos animais a eficácia dessas substâncias também têm sido demonstrada tanto na prevenção, quanto na reversão de distúrbios motores extrapiramidais (NADE et al., 2010; BARCELOS et al., 2010; 2011; MACÊDO et al., 2011; BUSANELLO et al., 2011; TREVIZOL et al., 2011; PATIL et al., 2012; PEROZA et al., 2013).

O magnésio é um mineral essencial para os seres humanos, fundamental para regulação de uma grande variedade de sistemas enzimáticos (SARIS et al., 2000; SABATIER et al., 2002; WOLF; CITTADINI, 2003). Deste modo, o potencial terapêutico do magnésio tem sido empregado em diversas condições patológicas como eclampsia (EUSER; CIPOLLA, 2009), fibromialgia (PORTER et al., 2010), dor neuropática (PICKERING et al., 2011) e doenças cardiovasculares (MANRIQUE et al., 2010). Estudos recentes sugerem que alterações de algumas funções cerebrais em condições normais e patológicas podem estar ligados a alterações na homeostase de magnésio (SARTORI et al., 2012; WANG et al., 2012; GOÑI-DE-CERIO et al., 2012).

2 REVISÃO BIBLIOGRÁFICA

2.1 As doenças mentais e os fármacos antipsicóticos

As doenças mentais generalizadamente descritas como “psicoses” afetam cerca de 24 milhões de pessoas em todo o mundo, sendo consideradas um grave problema de saúde pública (WHO, 2011; FERRARI et al., 2013). Embora as psicoses afetem pessoas de ambos os sexos com igual frequência, sua manifestação é mais frequente no sexo masculino, a partir do início da adolescência e da fase adulta (ALEMAN et al., 2003; LAWRIE et al., 2004). As doenças mentais, de um modo geral, são multifatoriais, cuja etiologia pode ser influenciada por fatores genéticos, fatores ambientais e estilo de vida, medicamentos, infecção pré-natal e neonatal, histórico de uso de drogas, entre outros (MORTENSEN et al., 1999; BROMET et al., 1999; LEWIS et al., 2000; TIENARI et al., 2004).

Fármacos antipsicóticos são muito utilizados no tratamento das doenças mentais e foram introduzidos na pratica clínica na década de 1950, a partir do desenvolvimento da clorpromazina (LOPEZ-MUÑOZ; ALAMO, 2009). O objetivo primário desta terapia é melhorar a qualidade de vida das pessoas acometidas pelas doenças mentais, reduzindo a frequência e a gravidade dos episódios psicóticos (TANDON et al., 2010). Infelizmente, a eficácia clínica dos antipsicóticos não ultrapassa 70% dos pacientes, sendo os 30% restantes classificados como “resistentes ou refratários ao tratamento”, representando um sério problema na psiquiatria (RANG et al., 2004).

A partir da descoberta da clorpromazina, outros fármacos antipsicóticos foram desenvolvidos e classificados como antipsicóticos de 1ª geração, clássicos ou típicos, devido às semelhanças no mecanismo de ação, eficácia e perfil de efeitos adversos (GARVER, 2006). Os antipsicóticos de 1ª geração, especialmente a clorpromazina, flufenazina e o haloperidol, atuam bloqueando principalmente receptores dopaminérgicos D2 (CREESE et al., 1976; SNYDER, 1986; FLEISCHHACKER, 2005) na via dopaminérgica mesolímbica, a qual está intimamente envolvida com a fisiopatologia das psicoses. Entretanto, tais fármacos também são capazes de bloquear receptores de outras vias dopaminérgicas, tais como a via túbero-infundibular e a via nigro-estriatal. De particular importância, esta última apresenta neurônios dopaminérgicos que se projetam desde a *substantia nigra* até o estriado dorsal (núcleos da base) (HEIMER, 2003), conferindo um risco aumentado para o desenvolvimento de distúrbios extrapiramidais. Tais efeitos colaterais manifestam-se através de distúrbios motores ou do movimento, como distonia, parkinsonismo (rigidez, bradicinesia, tremores), acatisia e também discinesia tardia (DT), a qual

pode apresentar-se de forma incapacitante e irreversível (BALDESSARINI et al., 1980; CASEY, 1995; KANE, 1995; KULKARNI; NAIDU, 2001).

Com o decorrer das décadas, novos medicamentos passaram a ser desenvolvidas afim de reduzir os efeitos indesejados e melhorar sua eficácia sobre os sintomas negativos da doença mental, recebendo a classificação como antipsicóticos de 2ª geração ou atípicos (GEDDES et al., 2000; ALEXANDER et al., 2011). Os antipsicóticos de 2ª geração, como a clozapina, olanzapina, risperidona, entre outros, possuem a capacidade de atuar em outros receptores, como por exemplo, os receptores da serotonina (5-HT), além de atuar mais fracamente nos receptores dopaminérgicos (STAHL, 2003; GROSS et al., 2012). Este grupo farmacológico, em contraste aos antipsicóticos típicos, apresenta respostas mais satisfatórias nos sintomas negativos da doença mental, exercendo nenhuma ou pouca influência sobre o movimento, o qual é relacionado ao sistema dopaminérgico extrapiramidal (LEUCHT et al., 2009). Por outro lado, apesar de serem menos neurotóxicos, são considerados disruptores metabólicos, sendo implicados ao desenvolvimento de *diabetes melittus*, pancreatite, ganho de peso, desencadeando também graves discrasias sanguíneas. Contudo, pelo fato dos antipsicóticos atípicos apresentarem uma menor eficácia em relação aos sintomas positivos e também possuírem um maior custo, os antipsicóticos típicos, em especial o haloperidol, continuam sendo amplamente empregados no tratamento farmacológico das psicoses (SHARM et al., 2003; PONTO et al., 2010).

2.2 O uso de antipsicóticos típicos e os distúrbios motores

O uso crônico de antipsicóticos do tipo haloperidol e afins, está associado a um aumento da síntese de dopamina (DA) e também de seu metabolismo, o qual ocorre através da auto-oxidação e a partir da atividade da enzima monoaminoxidase (MAO) (LOHR, 1991; ANDREASSEN; JORGENSEN, 2000; ZHU, 2004), que oxida a DA. Tomados em conjunto, os metabólitos desaminados e quinonas de dopamina, produtos da auto-oxidação da DA, são precursores de radicais livres (RL) (LOHR et al., 1991;2003; ASANUMA et al., 2003), cujo acúmulo na fenda sináptica pode resultar em estresse oxidativo. Se o sistema de defesa antioxidante não for suficiente para controlar os insultos oxidativos, em busca de estabilidade química, estes RL são capazes de afetar lipídeos de membrana (LP), proteínas transmembrana (carbonilação), mitocôndrias e até mesmo o DNA intranuclear em diferentes regiões

dopaminérgicas (BECHELLI, 2000; BURGER et al., 2005b; BARCELOS et al., 2010; 2011), comprometendo suas funções. Somado aos eventos pró-oxidantes, o bloqueio dopaminérgico estriatal pode ainda desenvolver excitotoxicidade, já que um significante aumento do glutamato extracelular tem sido observado, contribuindo assim para o desenvolvimento dos danos oxidativos já referidos (TSAI et al., 1998; BURGER et al., 2005a; ALBENSI, 2007; ZHURAVLIOVA et al., 2007).

Ensaios experimentais desenvolvidos com fármacos antipsicóticos mostraram um aumento nos níveis de peroxidação lipídica (PL) e de carbonilação de proteínas (CP), além de uma redução na atividade de enzimas antioxidantes, tais como a superóxido dismutase (SOD), a catalase (CAT) e a glutationa peroxidase (GPx) além da redução da glutationa reduzida (GSH) e consequente aumento da glutationa oxidada (GSSH) (POST et al., 2002; NAIDU et al., 2003; ABILIO et al., 2003; BURGER et al., 2005 a; b; FARIA et al., 2005; PILLAI et al., 2007).

Considerando que os distúrbios motores consequentes ao uso de antipsicóticos típicos estão, pelo menos em parte, relacionados ao desenvolvimento de estresse oxidativo e excitotoxicidade (BURGER et al., 2005a; TEIXEIRA et al., 2009; TREVIZOL et al., 2011), pode ser hipotetizado que uma agravação desta condição pode resultar em DT. De fato, esta síndrome, a qual é essencialmente humana, pode ser desenvolvida após uso prolongado de antipsicóticos clássicos e caracteriza-se por movimentos involuntários, repetitivos e hipercinéticos da região orofacial, pescoço e tronco, podendo ser incapacitante e irreversível (JESTE; WYATT, 1982; KANE; LIEBERMAN, 1993). A prevalência da DT é de cerca de 20 a 25% da população psiquiátrica que usa antipsicóticos, porém este índice pode alcançar a faixa de 50% conforme o aumento da idade (ANDREASSEN; JORGENSEN, 2000). A DT apresenta uma incidência anual de novos casos de, aproximadamente, 3 a 5% e parece ocorrer de maneira irreversível, frequentemente cumulativa e incapacitante (KANE, 1995).

2.3 Modelos animais de efeitos extrapiramidais

Para o estudo dos possíveis mecanismos relacionados ao estresse oxidativo envolvidos na patofisiologia da DT, modelos animais de distúrbios do movimento são amplamente utilizados. Nos modelos animais, a DT é denominada discinesia orofacial (DO), os quais destacam-se os modelos agudos induzidos por antipsicóticos como o haloperidol e o modelo de DO induzido por reserpina.

2.3.1 Distúrbios do movimento induzido por antipsicóticos

Antipsicóticos típicos, como o haloperidol, são também reconhecidos por sua atividade neuroléptica, em decorrência da sua capacidade de causar distúrbios motores extrapiramidais. Neste sentido, animais tratados com neurolépticos manifestam catalepsia e DO, que têm sido quantificadas através da frequência dos MMV e tremores da mandíbula, acompanhados de danos oxidativos em áreas dopaminérgicas cerebrais (NAIDU et al., 2003; BURGER et al., 2006; FACHINETTO et al., 2007; COLPO et al., 2007; BARCELOS et al., 2010; TREVIZOL et al., 2011; TEIXEIRA et al., 2011; BENVEGNU et al., 2012; REIS et al., 2013; ROPKE et al., 2014). Tais eventos têm sido associados a alterações morfológicas nas regiões cerebrais dos núcleos da base, como o corpo estriado e a região da *substantia nigra* (MESHUL; TAN, 1994; BARCELOS et al., 2010;2011).

2.3.2 Distúrbios do movimento induzido por reserpina

A reserpina, um alcaloide purificado da planta *Rauwolfia serpentina*, foi uma das primeiras drogas eficazes amplamente utilizada no tratamento da hipertensão. Atualmente é raramente utilizada para este fim, devido ao desenvolvimento de efeitos colaterais como sedação, incapacidade de se concentrar ou executar tarefas complexas e principalmente por levar a diversos casos de suicídio devido ao desenvolvimento de depressão. Na década de 50, foi utilizada para o tratamento de pessoas com esquizofrenia (BLEULER; STOLL, 1955). A reserpina se liga firmemente a vesículas de armazenamento adrenérgicos em neurónios adrenérgicos centrais e periféricos e inibe o transportador de monoamina vesicular (VMAT-2)

que facilita o armazenamento de noraepinefrina (NE). Assim, as terminações nervosas perdem a sua capacidade de concentração e armazenamento das monoaminas NE, DA e 5-HT, acumulam-se no citoplasma, onde são metabolizados pela MAO, de tal modo que pouco ou nenhum transmissor ativo é descarregada a partir de terminações nervosas após despolarização. A recuperação da função simpática requer síntese de novas vesículas de armazenamento, o que leva dias ou semanas após a descontinuação da droga. Uma vez que a reserpina esgota as aminas no SNC, bem como em neurónios adrenérgicos periféricos, os seus efeitos anti-hipertensivos são, provavelmente, relacionados com ambas as ações centrais e periféricas (KATZUNG, 2006; BRUNTON; CHABNER; KNOLLMAN, 2011)

A reserpina vem sendo amplamente utilizada experimentalmente na indução de distúrbios do movimento, tal como a DO, a qual tem sido relacionada à geração de RL e desenvolvimento de estresse oxidativo (DUTRA et al., 2002; ABILIO et al., 2003; BURGER et al., 2003;2004;2005; PEREIRA et al., 2011). Por sua capacidade de prevenir a estocagem de DA nas vesículas neuronais, desde que exerce um bloqueio no transporte vesicular de monoaminas, causando consequentemente uma aceleração do catabolismo de DA citosólica através da enzima MAO, seguido da formação simultânea de metabólitos acídicos e peróxido de hidrogênio, os quais são associados ao desenvolvimento de danos oxidativos (ABILIO et al., 2002; BURGER et al., 2003; NAIDU et al., 2004; SADAN et al., 2005; BILSKA et al., 2007). Semelhantemente à descrição acima, a auto-oxidação da DA contribui para a formação do aminocromo, o qual é precursor de RL. Como destino, este metabólito pode contribuir para a formação de subprodutos (neuromelanina, leucoaminocromo), descritos como fontes geradoras de ER endógenas envolvidas nos processos degenerativos (ARRIAGADA et al., 2004).

2.4 Magnésio

O magnésio (Mg) é o segundo cátion intracelular mais abundante e o quarto cátion mais abundante no organismo. Este íon é um importante cofator para mais de 300 enzimas e possui um papel fisiológico essencial em uma grande variedade de reações metabólicas essenciais, dentre as quais incluem a glicólise e o metabolismo protéico e lipídico. O papel do Mg na regulação da via glicolítica é particularmente importante, visto que na primeira das quatro reações de oxidação-redução desta via, onde o isocitrato é convertido em α-cetoglutarato pela isocitrato desidrogenase, enzima que necessita de Mg para sua atividade (BRILLA;

LOMBARDI, 1995) como por exemplo, a geração de energia aeróbica e anaeróbica (RYAN, 1991; ESKES et al., 1998; NORONHA; MATUSCHAK, 2002; NELSON; COX, 2008).

2.4.1 Magnésio no organismo: distribuição, absorção e excreção.

Fisiologicamente, encontra-se cerca de 21 a 28 g de Mg amplamente distribuído no organismo de uma pessoa adulta. Aproximadamente 60-65% do total de reservas de Mg encontram-se no compartimento ósseo, 27% no músculo, 19% nos tecidos moles, 0,5% nos eritrócitos e 0,3% no soro (VORMANN, 2003). Dentro da célula 90% do íon está ligado, principalmente, à ácidos nucleicos, ATP, fosfolipídios carregados negativamente e proteínas, e os 10% restantes, encontram-se livre (WOLF; CITTADINI, 2003). Embora pouco Mg esteja sob a forma ionizada, esta é a forma essencial para que haja a regulação da homeostase intracelular. A concentração sérica de magnésio é resultado de um balanço na ingestão/absorção do íon, biodistribuição e excreção.

A absorção de Mg ocorre principalmente na porção distal do intestino (íleo e cólon), por difusão passiva e são dependentes da quantidade de Mg dietética, do estado nutricional e da presença de agentes dietéticos promotores e inibidores de sua absorção. (HARDWICK, 1991; KAYNE; LEE, 1993; BOHL; VOLPE, 2002; VORMANN, 2003). Os principais órgãos excretores envolvidos na homeostasia do Mg são os rins, que atuam no processo de filtração e reabsorção do mineral. Mediante uma baixa ingestão oral de Mg, os rins são capazes de reduzir a excreção diária do mesmo. As demais formas de excreção do magnésio são as fezes e o suor (QUAMME, 1997; SARIS et al., 2000; BOHL; VOLPE, 2002).

Os termos deficiência de Mg e hipomagnesemia são corriqueiramente utilizados como sinônimos. Contudo, a deficiência de Mg pode estar presente mesmo mediante concentrações normais de Mg sérico, que são comumente mantidas às custas de mobilização do íon a partir de outros compartimentos. Em contrapartida, o termo hipomagnesemia refere-se a baixas concentrações séricas de Mg (SWAMINATHAN, 2003).

2.4.2 Patologias e o magnésio

Muitas condições patológicas relevantes são associadas com a redução da disponibilidade de Mg e/ou o aumento da excreção, seja em nível sistêmico ou em tecidos específicos. Tais condições incluem doenças cardiovasculares (ALTURA et al., 1984; TOUYZ, 2004), diabetes mellitus (RESNICK et al, 1993; BARBAGALLO et al, 2007), síndrome metabólica (BELIN et al., 2007), citopatias mitocondriais (BARBIROLI et al, 1999) e distúrbios neuropsiquiátricos (HELPERN et al, 1990; BARRA et al., 2007).

No sistema nervoso central (SNC), o papel crucial de cátions, incluindo o Mg, tornou-se evidente, apesar de incompreendido em nível de mecanismo. Estudos evidenciam que alterações na função cerebral em condições normais ou patológicas como nas doenças psiquiátricas da depressão e esquizofrenia podem estar ligadas a alterações na concentração de Mg no plasma (WIDMER et al., 1995; JOFFE et al., 1996; NECHIFOR, 2009). De fato, tem se estabelecido o papel importante do Mg no SNC devido a sua influência na estabilização elétrica das membranas celulares sendo essencial na condução sináptica (FREEDMAN et al., 1992), e na modulação dos receptores N-metil D-Aspartato (NMDA) (SOBOLEVSKII; KHODOROV, 2002; GATHWALA, 2010).

Nesse sentido, o efeito benéfico da administração de Mg em condições patológicas do cérebro também tem sido investigado. Vários estudos averiguaram a eficácia clínica da terapia de Mg em modelos animais de lesão cerebral traumática, mostrando que a sua administração pré- e pós-lesão favorecem a recuperação dos déficits cognitivos (HOANE, 2007; HOANE et al., 2008).

Além disso, o potencial antioxidante do Mg tem sido demonstrado (ARIZA et al., 2005; TURKOGLU et al., 2008). Em culturas de células neuronais, a depleção Mg aumenta a morte celular gerada por dano oxidativo (ALTURA et al., 2003) enquanto que a sua elevação mostrou ação citoprotetora (REGAN et al, 1998.). Ratos submetidos a uma baixa ingestão de Mg apresentaram um aumento da susceptibilidade ao estresse oxidativo, observando-se o aumento da LP e oxidação protéica (KUZNIAR et al, 2003; BOPARAI et al., 2007).

A atividade antioxidante do Mg também está relacionada com a capacidade de bloquear de maneira voltagem-dependente receptores NMDA reduzindo, consequentemente, o influxo de Ca^{2+} (Figura 1) (MAYER et al., 1984; BEKKERS; STEVENS, 1993; DINGLEDINE et al., 1999) que está relacionado ao desenvolvimento de citotoxicidade e disfunção mitocondrial,

com uma subsequente liberação de fatores apoptóticos, eventos que culminam em morte celular (TRUMP; BEREZESKY, 1995; NORBERG et al, 2008; LEMASTERS et al, 2009).

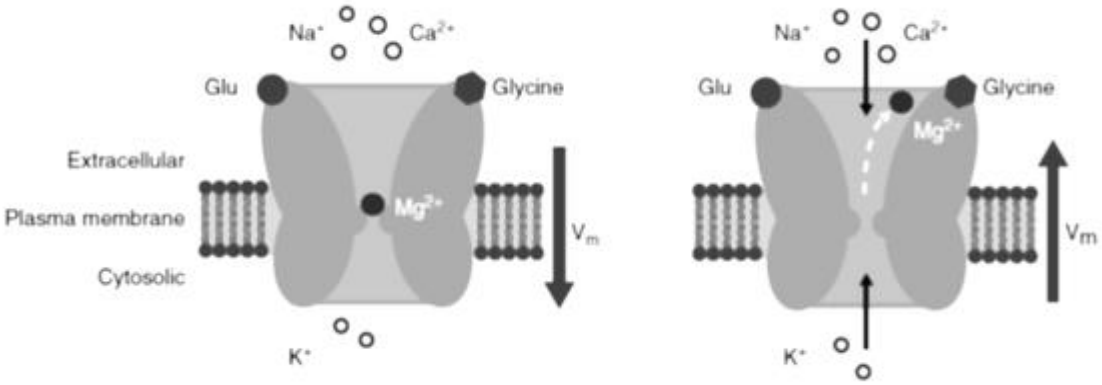

Figura 1 - Esquema do mecanismo de bloqueio do magnésio. No potencial de repouso o poro do canal do receptor NMDA está bloqueado pelos íons de magnésio. Após a despolarização, os íons de magnésio são removidos do poro e a corrente pode passar pelo canal.

3 JUSTIFICATIVA

A reserpina constitui um modelo animal agudo de distúrbios motores e está relacionada à geração de ER e de danos oxidativos em áreas dopaminérgicas extrapiramidais do SNC. Sabendo-se que o Mg desempenha funções fisiológicas importantes, atuando como um cofator enzimático capaz de modificar a plasticidade sináptica e modular as defesas antioxidantes, torna-se fundamental o estudo do papel deste mineral nestes modelos animais de distúrbio do movimento, visto que esta possível atividade neuroprotetora ainda carece de estudos.

4 OBJETIVOS

4.1 Objetivo geral

Avaliar a influência da suplementação de magnésio como agente de prevenção e reversão no desenvolvimento de distúrbios extrapiramidais induzidos pelo tratamento agudo com reserpina.

4.2 Objetivos específicos

- Avaliar o possível papel preventivo do Mg sobre o modelo animal agudo de distúrbio do movimento induzido por reserpina;
- Avaliar o possível papel do Mg na reversão de distúrbio do movimento induzido previamente por reserpina;
- Avaliar o possível papel preventivo e protetor (modelos de prevenção e reversão) da suplementação oral de Mg sobre o status oxidativo de áreas dopaminérgicas cerebrais (córtex, estriado e *substantia nigra*), consequente à administração aguda de reserpina.

5 MANUSCRITO CIENTÍFICO

Magnesium supplementation prevents and reverses experimentally induced movement disturbances in rats: Biochemical and behavioral parameters

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Abstract

Reserpine is a predictable animal model of orofacial dyskinesia (OD) which has been largely used to access movement disturbances related to extrapyramidal oxidative damage. Here, OD was acutely induced by reserpine (two doses of 0.7 mg/kg sc, every other day for 3 days), which was administered after (Experiment 1) and before (Experiment 2) magnesium (Mg) supplementation (40 mg/kg/mL, po). In Experiment 1, Mg was administered for 28 days before reserpine treatment, while in Experiment 2 it was initiated 24h after the last reserpine administration and was maintained for 10 consecutive days. Experiment 1 (prevention) showed that Mg supplementation was able to prevent reserpine-induced OD and catalepsy development. Mg was also able to prevent reactive species (RS) generation, thus preventing increase of protein carbonyl (PC) levels in both cortex and *substantia nigra*, but not in *striatum*. Experiment 2 (reversion) showed that Mg was able to decrease OD and catalepsy at all times assessed. In addition, Mg was able to decrease RS generation, with lower levels of PC in both cortex and *striatum*, but not in *substantia nigra.* These outcomes indicate that Mg is an important trace metal that should be present in the diet, , since its intake is able to prevent and minimize the development of movement disorders closely related to oxidative damage in the extrapyramidal brain areas, such as orofacial dyskinesia.

Keywords: Orofacial dyskinesia, acute reserpine, magnesium, oxidative damage, extrapyramidal brain area

1. Introduction

Chronic use of typical antipsychotic drugs to treat psychotic symptoms has been related to development of movement disorders, which are manifested by repetitive involuntary movements, parkinsonism and tremors [1, 2]. Often, these extrapyramidal symptoms can be incapacitating, while their prevention or reversion remains limited.

Reserpine-induced orofacial dyskinesia (OD) is a well-known experimental animal model [3-11], which may be quantified by orofacial movements and catalepsy [7, 12-14]. It has been shown that reserpine is able to deplete catecholamines such as dopamine (DA) by exerting a blockade in the vesicular monoamine transporter (VMAT), thus affecting neuronal transmission or storage. The consequent increase of DA in the cytosol promotes its auto-oxidation and catabolism by monoamine oxidase (MAO) [15], events that are closely related to development of oxidative stress (OS) [16, 17]. Therefore, extrapyramidal symptoms have been linked to reactive species generation and oxidative damage in the basal ganglia of the central nervous system (CNS) [18, 19].

While most studies have focused on antioxidant compounds [3, 20, 21], trace metals such as magnesium (Mg) are present in vegetables, bread and cold cereals, and milk [22] and their therapeutic potential has been applied clinically to treat asthma [23], fibromyalgia [24], pain [25], eclampsia [26] and cardiac arrhythmias [27]. In fact, Mg is the fourth most abundant monovalent cation and the second most abundant intracellular cation of the body. This metal exerts critical regulation of cellular and enzymatic functions, thus affecting ion channels, metabolic cycles, and signaling pathways [28-30].

While a growing number of evidences indicate that neuronal death may be closely related to both acute and chronic degenerative disorders, Mg levels have been found to be decreased in some of these disorders [31,32]. As a result, considerable research efforts have been directed toward establishing the mechanisms of such decline and the potential neuroprotective role for Mg [33, 34]. In addition, changes in Mg homeostasis and oxidative damage are closely correlated, suggesting a common mechanism involved in the pathogenesis of different disorders. Some studies have reported that Mg deficiencies may be related to increased susceptibility to *in vivo* and *in vitro* oxidative stress such as lipid peroxidation (LP), thus promoting immune-inflammatory response and reduced antioxidant defense systems such as glutathione (GSH), superoxide dismutase (SOD) and ascorbate [35-39].

Considering that acute reserpine is an extensively used animal model of movement disorder, here we propose to evaluate if Mg is able to prevent or minimize the development of reserpine-induced OD, as well as its beneficial effects on the oxidative damage in different brain areas such as cortex, striatum and *substantia nigra*.

2. Material and methods

2.1. Animals

Male Wistar rats weighing 250-320g (about 3 months old) were used. Groups of three (±1) animals were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (22-23°C) and on a 12h-light/dark cycle with lights on at 7:00 a.m. The experimental protocol was approved by the Animal Ethics Committee (Universidade Federal de Santa Maria – UFSM 064/2013), which is affiliated to the Council of Animal Experiments (CONCEA), following international norms of animal care and maintenance.

2.2. Drugs

Reserpine (methyl reserpate 3,4,5-trimethoxybenzoic acid ester-Sigma Chemical, St. Louis, MO) was dissolved in glacial acetic acid and then diluted to a final concentration of 0.1% acetic acid with distilled water. The vehicle consisted of a 0.1% acetic acid solution. Magnesium aspartate (Fragon do Brasil Farmacêutica Ltda) was dissolved in deionized water.

2.3. Experimental procedure

Experiment 1: Preventive effects of magnesium supplementation on the development of acute orofacial dyskinesia induced by reserpine – Twenty-eight rats were randomly divided in two groups of fourteen animals each and orally supplemented with magnesium aspartate solution (40 mg/Kg/mL) [40] or deionized water. After 28 days of supplementation, one half of each experimental group was treated with reserpine solution (0.7 mg/kg/mL; sc) (R and Mg+R groups) or vehicle (C and Mg groups) for 3 days (every other day). One day (24h) after the last administration of R/vehicle, all animals were submitted to behavioral evaluations as described in section 2.4.

Experiment 2: *Effects of supplementation with magnesium aspartate on the reversal of acute orofacial dyskinesia induced by reserpine* – Twenty-eight rats were randomly divided in two groups of fourteen animals each and treated with reserpine solution (0.7 mg/kg/mL; sc) (R groups) or vehicle (C groups) for 3 days (every other day). Twenty-four hours after the last administration of R/vehicle, the development of orofacial dyskinesia was quantified. One half of each experimental group was immediately supplemented once a day (by gavage) with magnesium aspartate (40mg/Kg/mL)

(groups Mg and R+Mg) or deionized water (groups C and R). Orofacial dyskinesia was quantified during the subsequent days (each 48h). Mg supplementation was maintained throughout the behavioral assessment period (10 consecutive days).

2.4. Behavioral testing

2.4.1. Orofacial dyskinesia (OD): Rats were placed individually in cages (20x20x19 cm) containing one mirror under the floor and one behind the back wall of the cage to allow behavioral quantification when the animal was facing away from the observer. To quantify the occurrence of OD, the incidence of vacuous chewing movement (VCM) was recorded for three sets of 6 min with 5-min intervals , totaling 18 min of observation. Observers were blind to the drug treatment. In a preliminary study (using 5 control and 10 reserpine-treated rats) of inter-rater reliability, we found that the use of this method of observation and parameter definition usually results in >91% agreement between the 3 different observers. All the calculated *p* values were significant for $p<0.05$.

2.4.2. Catalepsy time: Catalepsy was measured immediately after OD observation in rats submitted to Experiments 1 and 2 using a wire grid (25 X 30 cm^2) inclined 45° relative to the bench top. Each rat was placed with its forepaws near the edge of the grid and the amount of time spent in this atypical position (motionless) was recorded for three times, with a 5-min interval between them. All of the rats treated with reserpine (R and Mg + R of both experiments) were individually placed on the inclined grid and observed for 60 s. At the end of the three replications, the mean time spent by the rat without moving was calculated for each test. This behavioral test was adapted from Rocha [41].

2.5. *Biochemical assays*

After behavioral evaluations all animals were anesthetized with sodium thiopental (50mg/kg body weight; ip) and euthanized by exsanguinations. Brains were immediately removed and cut coronally at the caudal border of the olfactory tubercle. Cortex, striatum and *substantia nigra* were dissected according to Paxinos and Watson [42], and homogenized in 10 volumes (w/v) of 10mM Tris–HCl buffer (pH7.4) for determination of reactive species (RS) generation and protein carbonyl (PC).

2.5.1 *Reactive species (RS) generation with DCH (dichlorofluorescein-reactive species, DCH–RS)*

RS levels were measured using the oxidant sensing fluorescent probe, 2',7'-dichlorofluorescein diacetate (DCHF-DA) [43]. Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 2, 7-dichloro dihydrofluorescein diacetate, 5 (and 6) - carboxy-2, 7-dichloro dihydrofluorescein diacetate, and dihydrorhodamine. Theoxidation (DCHF-DA) to fluorescent dichlorofluorescein (DCF) was determined at 488 nm for excitation and 525 nm for emission. After tissue homogenization in 10 volumes (w/v) of 10 mMTris–HCl buffer pH 7.4 and centrifuged (15 min, 3.500 rpm), 3 mL of the same buffer was added. After 10 s, 10 µM (DCHF-DA) (prepared in ethanol) was added to the mixture, and the fluorescence intensity from DCF was measured for 300 s and expressed as a percentage of the untreated control group. The protein content was normalized by quantification according to Lowry [44].

2.5.2 Protein carbonyl (PC) quantification

PC was quantified by the method of Levine [45], with some modifications. Soluble protein was mixed with 2,4-dinitrophenylhydrazine (DNPH; 10 mMin 2 M HCl) or HCl (2 M) and incubated at room temperature for 1 h. Denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, with 3% sodium dodecyl sulfate), ethanol (99.8%) and hexane (99.5%) were added, mixed by shaking and centrifuged. The protein isolated from the interface was washed twice with ethyl acetate/ethanol 1:1 (v/v) and suspended in denaturing buffer. Each DNPH sample was read at 370 nm in a spectrophotometer against the corresponding HCl sample (blank). The results were expressed as nmol carbonyl/g tissue.

2.5.3 Lipid peroxidation (LP) estimation

LP of erythrocytes was determined by measuring the accumulation of thiobarbituric acid reactive substances (TBARS) as described by Ohkawa [46], and expressed as nmol MDA/mL.

2.6 Statistical analysis

In Experiment 1, while orofacial dyskinesia (OD) was analyzed by two-way ANOVA 2 (control/ Mg) X 2 (control/ reserpine), catalepsy time was analyzed by the Student's T-test, because this behavior was observed only in the reserpine-treated groups. In Experiment 2, the Student's T test was used in the first assessment of OD (vehicle/reserpine only), which was quantified 24h after the last reserpine administration. From the 2nd day on (when Mg supplementation was initiated), three-way ANOVA was applied [2 (control/ reserpine) X 2 (control/ Mg) X 5 behavioral quantifications]. This last factor was considered as a repeated measure followed by

pair-wise comparisons. Catalepsy time was analyzed by two-way ANOVA [2 (control/ Mg) X 5 behavioral quantifications] and by pair-wise test, considering the behavioral quantification as a repeated measure. Biochemical data from both Experiments 1 and 2 were analyzed by two-way ANOVA 2 (control/ Mg or reserpine) x (control/ reserpine or Mg) for each analyzed tissue (cortex, striatum, *substantia nigra*, erythrocytes). All the comparisons were followed by unvitiated analysis and Duncan's multiple range test when appropriate (software Statistica 8.0 for Windows was used). Values of $P<0.05$ were considered as statistically significant for all comparisons made.

3. Results

Experiment 1: *Preventive effects of magnesium supplementation on the development of acute orofacial dyskinesia and oxidative damage induced by reserpine*

3.1. Preventive effects of Mg on orofacial dyskinesia (OD) development and catalepsy time are shown in Figure 1:

Two-way ANOVA of vacuous chewing movements (VCM) revealed a main effect of supplementation, drug and a significant supplementation x drug interaction [$F(1,24)=40.09$; 67.22 and 26.31, $P<0.001$, respectively].

While Mg supplementation did not change orofacial parameters, reserpine treatment was related to OD development, which was partially prevented by Mg (Fig. 1A). Similarly, independent T-test showed that reserpine-treated rats showed catalepsy, while previous Mg supplementation was able to reduce the time of this behavior (Fig. 1B).

3.2 Preventive effects of Mg on oxidative status in cortex, striatum and substantia nigra are shown in Figure2.

Two-way ANOVA of reactive species (RS) generation revealed a significant main effect of supplementation in cortex and *substantia nigra* [F(1,24)=44.28 and 90.52, *P*<0.001, respectively], as well as a significant main effect of drug in cortex, striatum and *substantia nigra* [F(1,24)=5.37, *P*<0.05; 45.65, *P*<0.001 and 6.24, *P*<0.05, respectively].

Two-way ANOVA of protein carbonyl (PC) levels revealed a significant main effect of supplementation in cortex and *substantia nigra* [F(1,24)=9.23, *P*<0.05 and 49.42, *P*<0.001, respectively], as well as a significant main effect of drug in striatum and *substantia nigra* [F(1,24)=17.47, *P*<0.001 and 27.79, *P*<0.001, respectively].

Post-hoc test showed that while Mg decreased RS generation and PC level *per se* in both cortex (Fig. 2A) and *substantia nigra* (Fig. 2C), reserpine treatment was able to increase these oxidative parameters in all evaluated brain areas (Fig. 2A,B,C). Reserpine administration increased RS generation in all evaluated brain areas (Fig. 2 D,E,F), also increasing PC levels in both striatum (Fig. 2E) and *substantia nigra* (Fig. 2F). Previous supplementation of Mg was able to prevent reserpine-induced RS generation in both cortex (Fig. 2A) and *substantia nigra* (Fig. 2C), while the increase of PC levels was prevented in cortex (Fig. 2D) and attenuated in *substantia nigra* (Fig. 2F). In fact, Mg did not exertprotective influence on RS generation and PC levels in striatum (Fig. 2B,E), whose values were comparable to those of the reserpine-treated group.

3.3 Influence of Mg supplementation prior to reserpine administration on lipid peroxidation (LP) in erythrocytes is shown in Table 1:

Two-way ANOVA of LP revealed a significant main effect of supplementation, drug [F(1,24)=35.48; and 59.67, *P*<0.001, respectively] and a significant supplementation x drug interaction [F(1,24)=57.33, *P*<0.001] in erythrocytes.

Post-hoc test showed that Mg did not affect LP *per se* in erythrocytes, but this supplementation was able to prevent reserpine-induced increase of this oxidative marker. In fact, reserpine-treated rats that did not receive Mg previously showed a significant increase of LP in erythrocytes (Table 1).

Experiment 2: *Effects of magnesium supplementation on the development of acute orofacial dyskinesia and oxidative damage previously induced by reserpine*

3.3. Reversion of reserpine-induced orofacial dyskinesia (OD) and catalepsy time is shown in Figure 3:

Three-way ANOVA with repeated measure (2x2x5) of VCM revealed a significant main effect of drug [F(1,24)= 72.50; *p*<0.001)], supplementation [F(1,24)= 5.69; *p*<0.05)], repeated measure [F(4,108)= 5.10; *p*<0.001], a significant drug x supplementation [F(4,108)= 12.83; *p*<0.001)], a significant repeated measure x drug [F(4,108)= 11.89; *p*<0.001)] and a significant repeated measure x supplementation [F(4,108)= 2.86; *p*<0.05)] interaction.

Two-way ANOVA with repeated measure (2x5) of catalepsy time revealed a significant main effect of supplementation [F(1,24)= 33.07; *p*<0.001)], repeated measure [F(4,108)= 14.71; *p*<0.001] and a significant repeated measure x supplementation [F(4,108)= 4.35; *p*<0.001)] interaction.

Student's T-test showed increased VCM frequency 24h after the last reserpine administration (Fig. 3A). After Mg supplementation was initiated, paired test comparisons indicated that while VCM frequency remained increased in all assessments in the R group, the R+Mg group showed a significant and progressive decrease of VCM frequency from day 2 until day 10. As expected, control and Mg-treated groups showed unchanged VCM number at all times observed (Fig. 3B). Duncan's post-hoc tests showed increased VCM frequency in R, which was higher than in R+Mg in all assessments. In fact, this last experimental group (R+Mg) was able to reverse the higher frequency of VCM induced by reserpine at days 6 and 10, minimizing this behavior at days 2, 4 and 8, as at these times Mg and R+Mg showed significant differences from each other (Fig. 3B).

Student's T-test showed increased catalepsy time 24h after the last reserpine administration (Fig. 4). After Mg supplementation was initiated, paired test comparisons indicated that while catalepsy time remained increased in all assessments in the R group, the R+Mg group showed decreased cataleptic behavior from day 6 to day 10. Duncan's post-hoc tests showed that Mg supplementation (R+Mg group) decreased catalepsy time at days 2, 8 and 10 after the last reserpine injection. In fact, at days 4 and 6, both experimental groups (R and R+Mg) showed similar catalepsy time (Fig. 4).

3.4 The effect of Mg supplementation on oxidative status in cortex, striatum and substantia nigra is shown in Figure 5:

Two-way ANOVA of reactive species (RS) generation revealed a significant main effect of supplementation in striatum [$F(1,24)=18.42$, $P<0.001$], a significant main effect of drug in cortex, striatum and *substantia nigra* [$F(1,24)=10.06$, $P<0.05$; 5.93, $P<0.05$ and

94.43, *P*<0.001, respectively] and a significant drug x supplementation interaction in all evaluated brain areas [F(1,24)=23.69, *P*<0.001; 18.93, *P*<0.001 and 6.76, *P*<0.05, respectively].

Two-way ANOVA of protein carbonyl (PC) levels revealed a significant main effect of drug in striatum [F(1,24)= 4.74, *P*<0.05], as well as a significant main effect of supplementation and a significant drug x supplementation interaction in cortex [F(1,24)= 30.12, *P*<0.001; 4.57, *P*<0.05, respectively].

Post-hoc test showed that while reserpine increased RS generation in all evaluated brain areas (Fig. 5A,B,C), this treatment increased PC levels in both cortex (Fig. 5D) and striatum (Fig. 5E), but not in *substantia nigra* (Fig. 5F). Mg supplementation was able to decrease RS generation in both cortex (Fig. 5A) and striatum (Fig. 5B), but not in *substantia nigra* (Fig. 5C). PC levels were also reduced by Mg supplementation in cortex (Fig. 5D) and striatum (Fig. 5E), whose values were similar to those of control and Mg-treated groups. In fact, Mg did not reduce PC levels in the *substantia nigra* (Fig. 5F), for in this brain area reserpine did not increase the levels of this oxidative marker.

3.5 Influence of Mg supplementation after reserpine administration on lipid peroxidation in erythrocytes is shown in Table 1:

Post-hoc test showed that reserpine administration was able to increase LP *per se* in erythrocytes, whose levels were decreased by Mg supplementation. Mg per se did not change the levels of this oxidative marker, whose value was comparable to that of the control group (Table 1).

4. Discussion

The current findings showed that: i) Mg supplementation before reserpine administration was sufficient to prevent movement disturbances, as quantified by VCM frequency and catalepsy time; ii) Mg supplementation was able to prevent RS generation and PC levels in both cortex and *substantia nigra*, also preventing LP development in erythrocytes; iii) Mg supplementation following reserpine treatment was able to minimize reserpine-induced VCM frequency and catalepsy time; iv) Mg supplementation was able to reduce RS generation and PC levels in both cortex and *striatum*, thus reverting the increased level of LP in erythrocytes, which were increased in the reserpine-treated group.

Reserpine is a reliable animal model of movement disturbance, whose mechanism of action is related to dopamine metabolism, excitotoxicity and neurodegeneration [15]. In fact, reserpine depletes monoamines storage, mainly by blocking their vesicular transporter, thus favoring an excess of the neurotransmitter in the cytosol and consequently in the synaptic cleft. In such dopaminergic structures as cortex, *striatum* and *substantia nigra*, DA itself can be a major contributor for oxidative damage, especially due to dopamine-quinones and hydrogen peroxide generation, as described elsewhere [47-49]. Experimentally, OD has been related to increased oxidative damage in extrapyramidal brain areas [5, 19, 50-54], as also observed here. In the current study, Experiment 1 showed that extrapyramidal disorder occurred together with an increased generation of reactive species and a consequent oxidation of proteins in dopaminergic brain areas. Interestingly, magnesium supplementation was able to prevent and partially reverse both movement disturbance and oxidative events, which were quantified in brain areas and erythrocytes. In fact, increased VCM and catalepsy time were observed 24h after reserpine treatment, which were

decreased at days 2 and 6, respectively, of magnesium supplementation. It should be noted that the beneficial properties of magnesium supplementation were more significant when it was initiated prior to reserpine treatment, as in the reversion assay reserpine-induced behavioral and oxidative damage was more subtly declined. Such evidence was somewhat expected, as it is more difficult to reverse damage already done. Nevertheless, this study confirmed the beneficial effects of magnesium, which were also observed after the development of such damage.

Of particular importance for our findings, current eating habits in Western countries include processed foods, whose chronic consumption has been related to an inadequate supply of micronutrients like vitamins, essential fatty acids and trace minerals, including Mg. In fact, a decreased dietary provision of Mg was experimentally related to cataleptic behavior in rodents, while antiparkinson drugs were able to inhibit this extrapyramidal disturbance, indicating that this metal exerts a pivotal role in movement disorders [55]. More exactly, these authors suggested a relationship between dopaminergic hypofunction and cataleptic behavior as a consequence of a low Mg and calcium intake. Additionally, neurodegeneration of nigroestriatal dopaminergic brain area was linked to longer catalepsy [56]. Our attention on Mg was fueled by its important physiological role in regulating cellular and enzymatic functions pertaining to ion channels, metabolic cycles, and signaling pathways. In fact, abnormalities in Mg homeostasis may lead to biochemical dysregulation and thus contribute to the development of neurological disorders, including depression [57-59], Parkinson’s Disease [60], energetic stress induced by hypoxia, ischemic and traumatic brain injury [32, 61, 62], among others. Indeed, decreased levels of Mg may influence glutathione levels, especially in erythrocytes [63], where this metal trace is an essential cofactor for synthesis of this antioxidant agent [64]. In this sense, rats submitted to a

low magnesium intake showed an increased susceptibility to oxidative stress as observed by an increased lipid peroxidation in plasma and liver [63, 65]. So, Mg supplementation is able to modulate the oxidative/antioxidant status [36, 38] and contribute to different pharmacotherapies for disorders involving oxidative damage. Furthermore, in cultures of neuronal cells, while Mg depletion enhances cell death generated by oxidative damage, its supplementation showed a cytoprotective effect [66]. The use of antioxidant substances seems to be effective to reduce experimentally induced movement disorders, as previously reported by our group [3, 4, 67] and other research groups [53, 68-71].

In the current study, when supplemented before reserpine, Mg seems to have exerted a protective action against the generation of reactive species and protein oxidation in the cortex and *substantia nigra*, preventing as well lipid peroxidation in erythrocytes. Our findings are therefore consistent with previous studies showing that reserpine is able to negatively affect the oxidative status in brain areas involved in movement control [4, 19, 21, 72]. Moreover, besides dopamine metabolism, a negative relationship between glutamate transporter and OD manifestation in rats exposed to reserpine or haloperidol was reported [5], strengthening relationships between oxidative stress, excitotoxicity and movement disorders. Concerning the aim of the current study, Mg exerts neuroprotective effects on the central nervous system (CNS). Its action mechanism has been related to a decreased presynaptic release of glutamate, an important excitatory neurotransmitter of the CNS [73]. Moreover, a blockade of glutamatergic N-methyl-D-aspartate (NMDA) receptor has also been associated with Mg [74-76]. In fact, Mg is able to block NMDA-glutamate receptor ion channels, preventing ionic flow at typical neuronal resting potentials, thus decreasing activation of voltage-gated channels and reducing neuronal excitability. Of particular

importance for our findings, continuous stimulation of NMDA receptor could induce a massive influx of Ca^{2+} into the cells, promoting cytotoxicity and mitochondrial dysfunction together with a subsequent release of apoptotic factors, which are precursors of cell death [77-80]. In this sense, Ca^{2+} dysregulation is decisive for neural death and degeneration following ischemic stroke, Parkinson's disease [81] and Huntington's disease [82]. In addition, experimental research on spinal cord injuries have shown that Mg was able to inhibit apoptosis, decreasing reactive species generation, lipid peroxidation and caspases activation by blocking NMDA receptors [83, 84]. Based on all these evidences, it is possible to propose that Mg supplementation may act as an antioxidant modulator, whose action mechanism is mediated by NMDA receptor blockade, thus reducing excitotoxicity from glutamate. However, molecular studies involving the NMDA receptor and glutamate cascade should be conducted.

The present study indicates the beneficial influence of Mg supplementation, as observed by both prevention and reversion of reserpine-induced orofacial dyskinesia and catalepsy. These experimental protocols may contribute to increase our understanding of the pathophysiology of movement disorders and possibly to a preventive treatment.

In conclusion, our study showed that movement disorders may be prevented or attenuated by dietary Mg or by its supplementation. These findings reinforce the validity of this animal model as a fundamental tool to study motor diseases, including parkinsonism, dystonias and akathisia related to antipsychotic treatment, whose pathophysiology has also been related to oxidative damage and neurotoxicity.

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Figure captions

Fig. 1 Influence of magnesium supplementation (Mg, 40 mg/kg/mL, p.o., for 28 days) on the prevention of orofacial dyskinesia (A) and catalepsy time (B) of rats subsequently injected with reserpine (R, two doses of 0.7 mg/kg/mL, s.c., every other day). Data are expressed as mean ± S.E.M. *****Indicates significant difference from vehicle group; Different lowercase ^{a,b,c} indicates significant difference between reserpine-treated groups.

Fig. 2 Influence of magnesium supplementation (Mg, 40 mg/kg/mL, p.o., for 28 days) on the prevention of reactive species (RS) generation (A, B, C) and protein carbonyl (PC) levels (D, E, F) in cortex, *striatum* and *substantia nigra*, respectively, of rats subsequently injected with reserpine (R, two doses of 0.7 mg/kg/mL, s.c., every other day). Data are expressed as mean ± S.E.M. *****Indicates significant difference from

vehicle group; Different lowercase **a,b,c** indicates significant difference between reserpine-treated groups.

Fig. 3 Influence of reserpine (R, two doses of 0.7 mg/kg/mL, s.c., every other day) injection on development of orofacial dyskinesia (OD) (A). Influence of magnesium (Mg,40 mg/kg/mL, p.o.) supplementation or vehicle on OD development in rats previously treated with reserpine. Daily Mg supplementation was initiated immediately after the first behavioral assessment (basal), and maintained at day 10 (B). *Indicates a significant difference from control group; #Indicates a significant difference from reserpine + Mg (RM) group.

Fig. 4 Influence of magnesium supplementation (Mg, 40 mg/kg/mL, p.o.) or vehicle on reserpine-induced catalepsy time, observed every two days during its administration. Data are expressed as means±S.E.M. *Indicates a significant difference from control group; #Indicates a significant difference from baseline.

Fig. 5 Influence of magnesium supplementation (Mg,40 mg/kg/mL, p.o.) or vehicle on reactive species (RS) generation (A, B, C) and protein carbonyl (PC) levels (D, E, F) in cortex, *striatum* and *substantia nigra*, respectively, of rats previously injected with reserpine (R, two doses of 0.7 mg/kg/mL, s.c., every other day). Data are expressed as mean ± S.E.M. *****Indicates significant difference from vehicle group; Different lowercase **a,b,c** indicates significant difference between reserpine-treated groups.

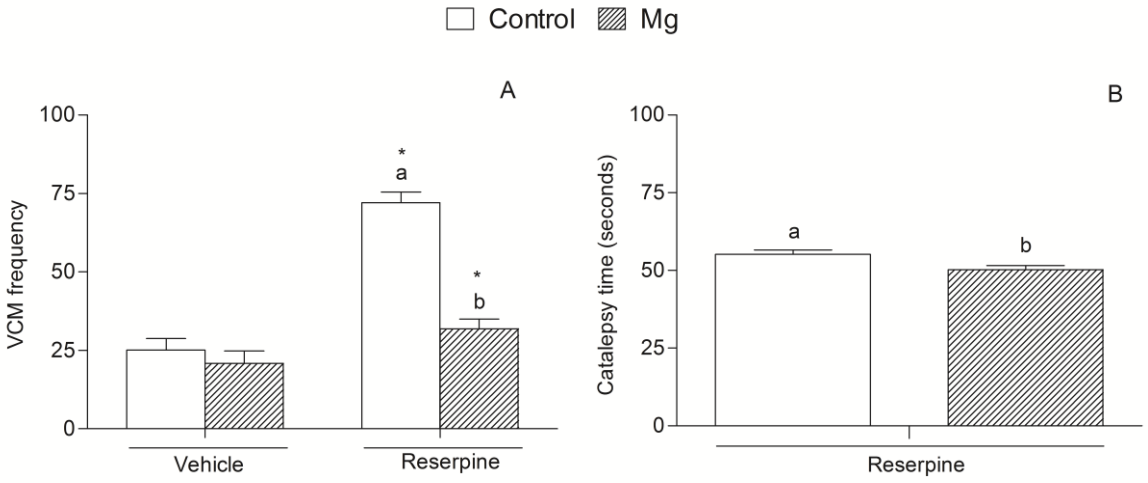
Control
Mg
A
B
VCM frequency
100
75
50
25
0
*
a
*
b
Vehicle
Reserpine
Catalepsy time (seconds)
a
b
Reserpine

Fig 1

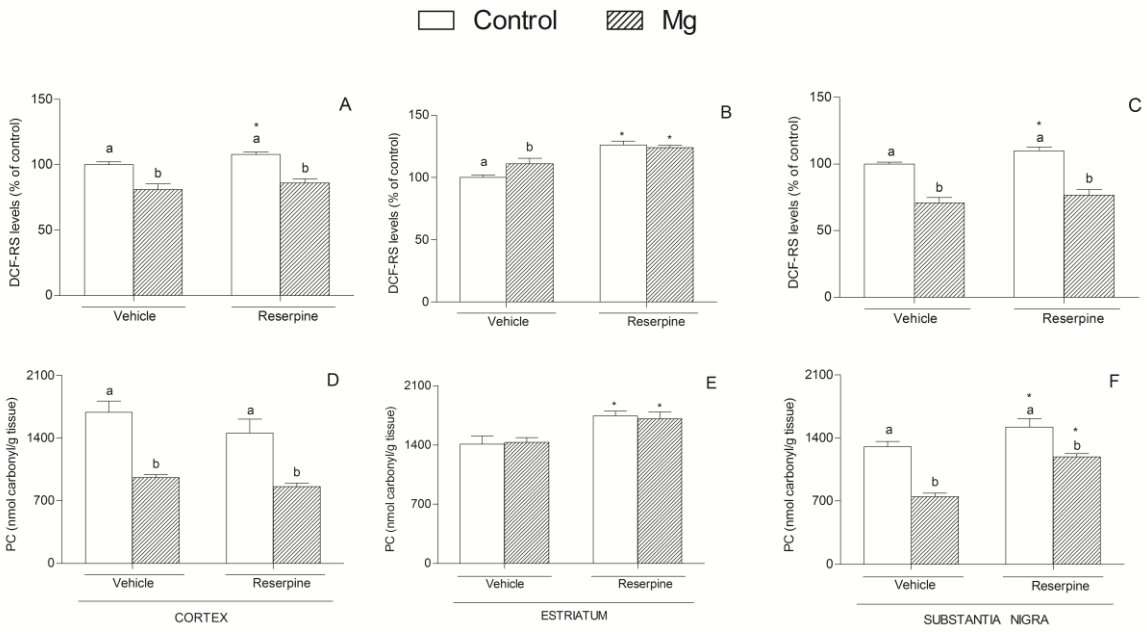
Control
Mg
A
B
C
D
E
F
DCF-RS levels (% of control)
PC (nmol carbonyl/g tissue)
Vehicle
Reserpine
CORTEX
ESTRIATUM
SUBSTANTIA NIGRA

Fig 2

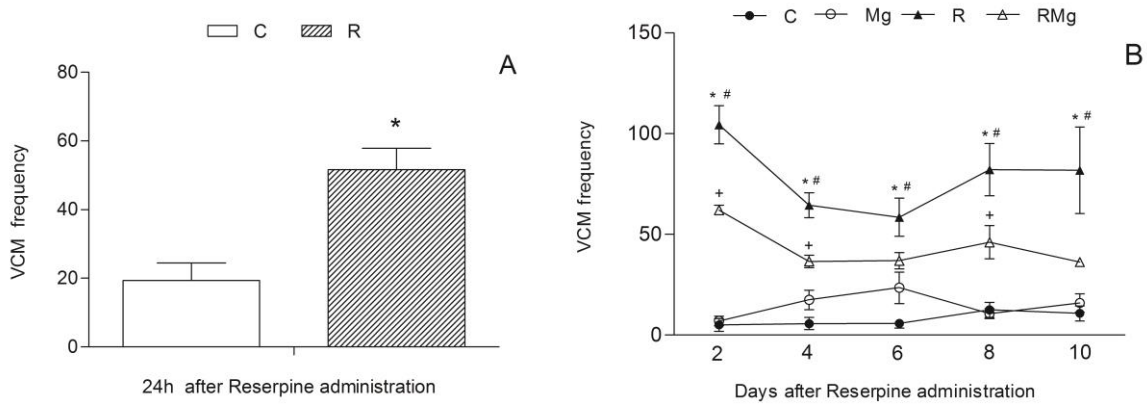
C
R
A
80
60
40
20
0
VCM frequency
*
24h after Reserpine administration
C
Mg
R
RMg
B
150
100
50
0
VCM frequency
* #
+
2
4
6
8
10
Days after Reserpine administration

Fig 3

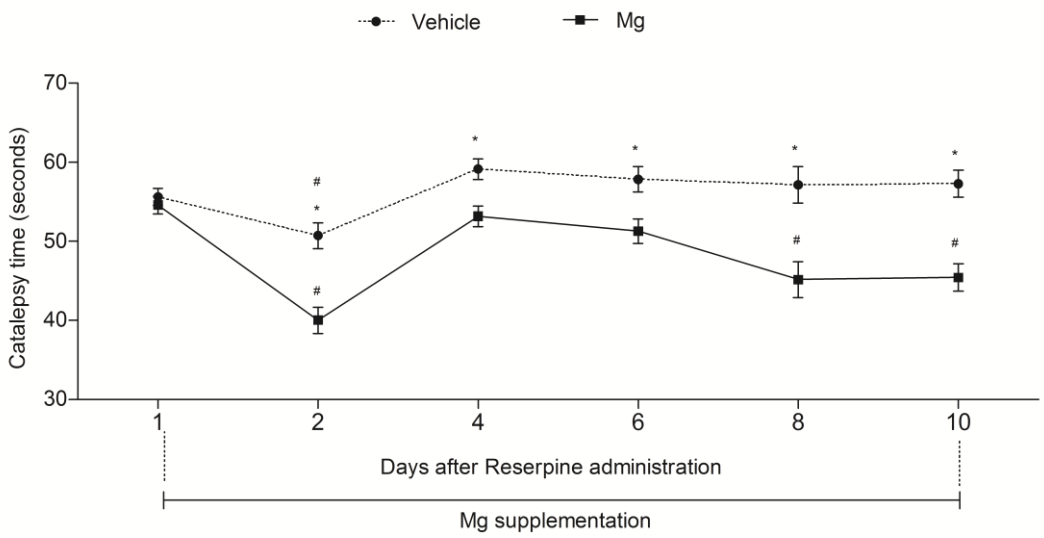
Vehicle
Mg
Catalepsy time (seconds)
70
60
50
40
30
1
2
4
6
8
10
Days after Reserpine administration
Mg supplementation

Fig 4

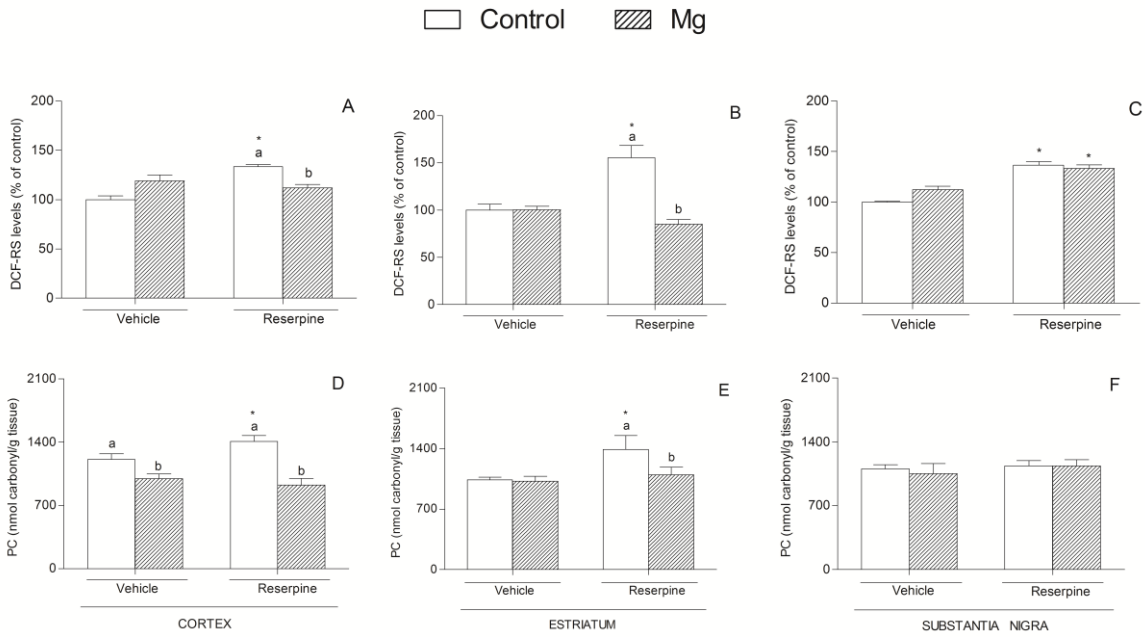
Control
Mg
A
B
C
D
E
F
DCF-RS levels (% of control)
PC (nmol carbonyl/g tissue)
Vehicle
Reserpine
CORTEX
ESTRIATUM
SUBSTANTIA NIGRA

Fig 5

Table 1. Estimation of lipid peroxidation (LP) in erythrocytes of rats, which received Mg supplementation before and after reserpine administration.

	Vehicle		Reserpine	
	C	**Mg**	**C**	**Mg**
Prevention	11,46 ± 0,65	12,79 ± 0,34	24,08 ±1,22^{*,a}	12,92 ± 0,82 ^{b}
Reversion	28,28 ± 1,47	30,78 ± 0,82	36,29 ±0,75^{*,a}	31,23 ± 0,47 ^{b}

Abbreviations: C-control; Mg-magnesium. Data are expressed as mean±S.E.M. *indicates difference from vehicle control group ($P < 0.05$); ^{a,b} indicates difference between supplementations (Mg / vehicle) in the same treatment ($P < 0.05$).

6 DISCUSSÃO

Nesta dissertação tivemos por objetivo avaliar os possíveis efeitos benéficos do Mg na prevenção e reversão de distúrbios motores induzidos em um modelo animal de DO induzido por reserpina. Experimentalmente, este modelo tem sido amplamente empregado para o estudo dos transtornos do movimento relacionados ao EO, como também à doenças neurodegenerativas (TREVIZOL et al., 2011; BARCELOS et al., 2011; BUSANELLO et al., 2011; PEREIRA et al., 2011; RECKZIEGEL et al., 2013) e diferentes grupos de pesquisa têm mostrado o desenvolvimento de danos oxidativos em regiões cerebrais envolvidas no controle motor (ABILIO et al. 2003; BURGER et al. 2003; ANDREASSEN et al., 2003; NAIDU et al., 2003; 2004; FARIA et al., 2005; BARCELOS et al., 2011; PEROZA et al., 2013). Deste modo, o modelo animal utilizado no presente estudo confirma sua previsibilidade, a qual foi evidenciada pelo aumento da frequência de MMV e pelo tempo de catalepsia. Estas alterações comportamentais foram relacionadas a um aumento na geração de ER e uma elevada oxidação de proteínas, quantificadas através da formação de grupos carbonila no córtex, estriado e *substantia nigra*. Tais eventos oxidativos ocorreram conjuntamente com um aumento da LP nos eritrócitos, confirmando assim o desenvolvimento de danos oxidativos induzidos pela reserpina em ambos modelos de prevenção e de reversão adotados aqui.

Os distúrbios do movimento induzidos por neurolépticos típicos ou por reserpina têm sido objeto de vários estudos e pesquisas que buscam minimizá-los ou revertê-los (BURGER et al., 2003; 2005; BARCELOS et al., 2011; TREVIZOL et al., 2011; BUSANELLO et al., 2011; REIS et al., 2013; ROPKE et al., 2014). Os agentes que podem reduzir o EO, atuando tanto como antioxidante (LERNER et al., 2007; BARCELOS et al., 2011; BUSANELLO et al., 2011; PEREIRA et al., 2011; TEIXEIRA et al, 2011; TREVIZOL et al., 2011; ZHANG et al., 2011; ANDERSON e MAES, 2012; RECKZIEGEL et al., 2013;), quanto como uma nova forma farmacêutica (BENVEGNU et al., 2012), podem minimizar os danos cerebrais induzidos pelo tratamento de psicoses com neuroléptico típico, além de contribuir para uma maior compreensão da fisiopatologia de um dos maiores problemas da psiquiatria, a DT (ABILIO et al., 2003; BURGER et al., 2003, 2005; FARIA et al., 2005), cuja gravidade pode ser incapacitante e irreversível (KANE, 1995).

A suplementação de Mg tem sido utilizada no co-tratamento de diferentes situações clínicas, as quais incluem condições cardiovasculares, metabólicas e neurológicas (HEIDEN et al., 1999; SOAVE et al., 2009; PORTER et al., 2010; MANRIQUE et al., 2010; PICKERING et al., 2011) e a carência nutricional do metal relaciona-se ao desenvolvimento de diferentes

patologias (OYANAGI et al., 2006; VINK et al, 2009; SARTORI et al., 2012; WANG et al., 2012; GONI-DE-CERIO et al., 2012). Adicionalmente, a carência de Mg também tem sido associada ao desenvolvimento de distúrbios relacionados aos danos oxidativo demonstrando que o metal tem participação na modulação das defesas antioxidantes (KUZNIAR et al., 2003; ARIZA et al., 2005; BOPARAI et al., 2007; TURKOGLU et al., 2008). Neste sentido, o presente estudo pode confirmar o importante papel da suplementação de Mg em condições envolvidas com danos oxidativos na área extrapiramidal e o consequente distúrbio do controle motor. Deste modo, é possível sugerir que uma alimentação rica em sais de Mg ou mesmo a sua suplementação poderia retardar ou mesmo prevenir o desenvolvimento de distúrbios do movimento induzidos por medicamentos antipisicóticos típicos, uma vez que o uso clínico tem sido relacionado aos danos oxidativos da região extrapiramidal.

Por outro lado, distúrbios do movimento resultantes do desenvolvimento de EO induzido por reserpina ou por drogas neurolépticas como o haloperidol foram também relacionados a excitotoxicidade mediada por glutamato (GUNNE; ANDRÉN, 1993; BURGER et al., 2005a; SEKIGUCHI et al., 2012). A ação neuroprotetora no SNC atribuída ao Mg tem sido estudada por diferentes grupos de pesquisa, cujo alvo envolve a redução da liberação pré-sináptica de glutamato, como também o bloqueio de receptores NMDA (MAYER et al., 1984; BEKKERS; STEVENS, 1993; DINGLEDINE et al., 1999; LIN et al., 2002). Uma estimulação significativa destes receptores glutamatérgicos desencadeia um influxo massivo de íons Ca^{2+} para o interior das células, o que pode promover citotoxicidade e disfunções mitocondriais. Tais eventos podem ser acompanhados de uma subsequente liberação de fatores apoptóticos, os quais são reconhecidamente precursores de morte celular (TRUMP; BEREZESKY, 1995; NORBERG et al, 2008; LEMASTERS et al, 2009; SEO et al, 2012). O possível papel do Mg como bloqueador dos receptores NMDA foi também relacionado à redução da geração de ER, modificando parâmetros de LP e de ativação das caspases em estudos de lesões de medula espinal (SOLAROGLU et al, 2005; SENCER et al, 2013.). Deste modo, tais evidências indicam que o Mg também pode exercer um importante papel sobre parâmetros excitotóxicos, os quais também têm sido associados ao desenvolvimento de distúrbios motores (TSAI et al., 1998; ANDREASSEN; JORGENSEN, 2000; KONITSIOTIS et al., 2006).

Analisados em conjunto, os dados observados a partir do presente estudo são consistentes com dados anteriores da literatura, os quais mostraram que o modelo animal de distúrbios do movimento induzidos pela reserpina é capaz de afetar negativamente o *status* oxidativo em áreas do cérebro envolvidas no controle motor (BILSKA et al., 2007; TEIXEIRA et al, 2009; 2011; BUSANELLO et al., 2011; BARCELOS et al, 2011; RECKZIEGEL et al.,

2011; PATIL; KASTUREB, 2012). Por conseguinte, as ações preventivas e de reversão dos distúrbios do movimento observados nos animais tratados com Mg, levam-nos a propor que tal suplementação é capaz de exercer uma ação adjuvante ao tratamento farmacológico convencional em situações clínicas, nas quais as alterações motoras estão associadas aos processos oxidativos e/ou excitotóxicos. Deste modo, o Mg pode configurar uma terapia suplementar, retardando ou prevenindo os efeitos adversos motores associados à terapia antipsicótica.

7 CONCLUSÕES

Através dos resultados obtidos com o presente estudo, conclui-se:

1. A suplementação de Mg antes da administração da reserpina foi suficiente para prevenir os distúrbios do movimento, observados pela menor frequência dos MMV e menor tempo de catalepsia;
2. A suplementação de Mg antes da administração de reserpina foi capaz de prevenir a geração de ER e a oxidação de proteínas, tanto no córtex, como na *substantia nigra*, regiões envolvidas no controle motor, prevenindo também a LP nos eritrócitos;
3. A suplementação de Mg após a administração com reserpina minimizou a consequente elevação da frequência dos movimentos orais e o tempo de catalepsia;
4. A suplementação de Mg após administração de reserpina foi capaz de reduzir a geração de ER e os níveis de carbonilação proteica no córtex e no corpo estriado, sendo também capaz de reduzir o nível de LP nos eritrócitos, os quais foram aumentados pela administração de reserpina.

PERSPECTIVAS

Com base nos resultados apresentados no presente estudo, faz-se necessária a sua continuidade, a qual tem a seguinte proposta:

- ✓ Avaliar o efeito da suplementação de Mg na prevenção e reversão de distúrbios do movimento em modelo animal sub-crônico e crônico induzido por Haloperidol;
- ✓ Avaliar o efeito preventivo e protetor contra geração de espécies reativas nas áreas dopaminérgicas responsáveis pelo controle do movimento;
- ✓ Avaliar a expressão de receptores e dos transportadores de dopamina e glutamato, através de Western-blotting;
- ✓ Avaliar os níveis destes neurotransmissores através de microdiálise e HPLC;
- ✓ Investigar possível influência do Mg sobre as cascatas pró-inflamatória e apoptótica (caspases e interleucinas).

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