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Dissertação de Mestrado

INFLUÊNCIA DO EXERCÍCIO FÍSICO SOBRE PARÂMETROS DE COMPORTAMENTO E ESTRESSE OXIDATIVO EM MODELO ANIMAL DE DISCINESIA TARDIA

Angélica Martelli Teixeira

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INFLUÊNCIA DO EXERCÍCIO FÍSICO SOBRE PARÂMETROS DE COMPORTAMENTO E ESTRESSE OXIDATIVO EM MODELO ANIMAL DE DISCINESIA TARDIA

por

Angélica Martelli Teixeira

Dissertação apresentada ao Programa de Pós-Graduação em Farmacologia da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Farmacologia**.

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elaborada por

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como requisito parcial para obtenção do grau de
Mestre em Farmacologia

COMISSÃO EXAMINADORA:

_____
Marilise Escobar Bürger
(Presidente/Orientadora)

_____
Paulo César Ghedini
(UNIMES)

_____
Luiz Fernando Freire Royes
(UFSM)

Santa Maria, 07 de março de 2008.

“Ainda que eu falasse línguas, as dos homens e dos anjos, se eu não tivesse o amor, seria como sino ruidoso ou como címbalo estridente. Ainda que eu tivesse o dom da profecia, o conhecimento de todos os mistérios e de toda a ciência; ainda que eu tivesse toda a fé, a ponto de transportar montanhas, se não tivesse o amor, eu não seria nada.”

(1Cor 13,1-2)

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SUMÁRIO

LISTA DE ABREVIATURAS

CAT – catalase

DA – dopamina

DO _ discinesia orofacial

DT – discinesia tardia

EO – estresse oxidativo

EROs – espécies reativas de oxigênio

GPx – glutationa peroxidase

GSH – glutationa reduzida

H_2O_2 – peróxido de hidrogênio

MAO – enzima monoaminoxidase

MMV – movimentos de mascar no vazio

OH – radical hidroxila

RL – radical livre

SNC – sistema nervoso central

SOD – superóxido dismutase

TBARS – espécies reativas ao ácido tiobarbitúrico

TF – tremor facial

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APRESENTAÇÃO

No item **INTRODUÇÃO**, está descrita uma revisão bibliográfica sobre os temas trabalhados nesta dissertação. Os **RESULTADOS** estão apresentados sob a forma de artigos, os quais se encontram no item **ARTIGOS CIENTÍFICOS**. As seções **MATERIAIS E MÉTODOS, RESULTADOS, DISCUSSÃO DOS RESULTADOS E REFERÊNCIAS BIBLIOGRÁFICAS**, encontram-se nos próprios artigos e representam a íntegra deste estudo.

O item, **DISCUSSÃO E CONCLUSÃO FINAL**, encontrado no final desta dissertação, apresenta interpretações e comentários gerais sobre os artigos científicos contidos neste trabalho.

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

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Farmacologia
Universidade Federal de Santa Maria, RS, Brasil

IFLUÊNCIA DO EXERCÍCIO FÍSICO SOBRE PARÂMETROS DE COMPORTAMENTO E ESTRESSE OXIDATIVO EM MODELO ANIMAL DE DISCINESIA TARDIA

AUTORA: Angélica Martelli Teixeira
ORIENTADORA: Drª Marilise Escobar Burger

LOCAL E DATA DA DEFESA: Santa Maria, março de 2008.

A atividade física praticada de maneira regular promove adaptações benéficas ao organismo, enquanto a inadequação do tempo e intensidade pode exceder a tolerância individual ao exercício gerando estresse oxidativo (EO). Estudos mostram esses efeitos em diversos órgãos como, por exemplo, coração e músculos, mas pouco se conhece sobre sua ação e mecanismos em nível cerebral. Diversas doenças neurológicas e neurodegenerativas estão associadas ao EO e neurotoxicidade. Considerando esses aspectos, o primeiro objetivo desse estudo foi determinar a influência do exercício crônico moderado em modelo de EO induzido por reserpina em ratos. Os animais foram submetidos a sessões diárias de natação com aumento gradual no tempo de treinamento e, após oito semanas, receberam duas doses de solução de reserpina ou controle (1 mg/kg-sc) em dias alternados. Fez-se avaliação comportamental, eutanásia dos animais e retirada da região estriatal do cérebro para determinação enzimática e bioquímica. A reserpina aumentou a freqüência dos movimentos de mascar vazio (MMV) e o tempo de tremor facial (TF); aumentou a atividade da catalase e diminui os níveis de glutationa reduzida (GSH). O exercício preveniu parcialmente o TF e houve recuperação parcial nos níveis de GSH, mas não modificou os efeitos sobre a catalase e MMV. Foi observada uma correlação positiva entre a atividade da catalase e o desenvolvimento de discinesia orofacial (DO), e uma correlação negativa entre GSH e DO. O segundo objetivo desse trabalho foi avaliar os efeitos de uma atividade física intensa sobre este mesmo modelo de EO. Os animais foram submetidos a onze semanas de natação (1 h/dia) com aumento gradual na carga de treinamento até que essa atingisse 7% de seu peso corporal. Realizaram-se as avaliações de comportamento, eutanásia e retirada do estriado para análises. A efetividade do treinamento foi confirmada através dos níveis diminuídos de lactato sérico e do desenvolvimento de hipertrofia cardíaca, observados nos animais exercitados. O exercício intenso reduziu a atividade locomotora e exploratória dos animais, demonstrando desenvolvimento de estresse emocional. Na presença de reserpina, o exercício elevou a peroxidação lipídica (TBARS) e provocou aumento na atividade da catalase, cujos parâmetros apresentaram correlação positiva. Com estes estudos se concluiu que a atividade física crônica de intensidade moderada foi capaz de melhorar as defesas antioxidantes nos distúrbios motores associados ao EO cerebral. Por outro lado, o exercício excessivo provocou alterações emocionais negativas e, quando na presença de um agressor adicional, modificou a

capacidade antioxidante do cérebro, o que poderia agravar casos de doenças neurológicas e/ou neurodegenerativas associadas a processos oxidativos.

Palavras-chave: exercício, discinesia orofacial, reserpina, estresse oxidativo, neurodegeneração.

ABSTRACT

Dissertation of Master's Degree
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Federal University of Santa Maria, RS, Brazil

INFLUENCE OF PHYSICAL EXERCISE ON BEHAVIORAL PARAMETERS AND OXIDATIVE STRESS IN AN ANIMAL MODEL OF TARDIVE DYSKINESIA

AUTHOR: Angélica Martelli Teixeira
ADVISOR: Marilise Escobar Bürger

PLACE AND DATE OF THE DEFENSE: Santa Maria, 2008

Regular practice of physical activity promotes beneficial effects to the body. However, excessive duration and intensity of exercise may surpass individual tolerance to exercise, generating oxidative stress (OS). Studies have shown these effects in various organs, such as the heart and muscles, but little is known about their action and mechanisms in the brain. Various neurological and neurodegenerative diseases are associated with OS and neurotoxicity. Considering these aspects, the first objective of this study was to determine the influence of chronic moderate exercise in an OS model induced by reserpine in rats. The animals were submitted to daily sessions of swimming, with a gradual increase in the length of training. After eight weeks, the animals received two injections of reserpine or control solutions (1 mg/kg-sc), alternately. A behavioral evaluation was performed, after which the rats were euthanized and the striatum was dissected for enzymatic and biochemical assays. Reserpine increased the vacuous chewing movements frequency (VCM) and facial twitching (FT), as well as catalase activity, but decreased reduced glutathione levels (GSH). Exercise partially prevented FT, and partially recovered GSH levels, but did not modify the effects on catalase and VCM. There was a positive correlation between catalase activity and orofacial dyskinesia (OD) and a negative correlation between GSH and OD. The second objective of this study was to evaluate the effects of an intense physical activity in the same model of OS. Rats were submitted to eleven weeks of swimming (1 h/day), where each rat's load was increased according to its body weight until reaching 7% of its weight. Behavioral evaluations were performed before euthanasia and the striatum was then dissected for assays. The effectiveness of the training was confirmed through reduced levels of serum lactate and cardiac hypertrophy, observed in exercised animals. Intense exercise reduced the locomotor index and exploratory activity of the animals, demonstrating the development of emotional stress. In the presence of reserpine, exercise increased lipid peroxidation (TBARS) and caused an increase in catalase activity, which were positively correlated with each other. Based on the results, it was concluded that chronic physical activity of moderate intensity improved the antioxidant defenses in movement disorders associated with cerebral OS. On the other hand, excessive exercise caused negative emotional disorders and, in the presence of another aggressor agent, modified brain antioxidant capacity, which possibly could aggravate cases of neurological and/or neurodegenerative diseases associated with oxidative processes.

Keywords: exercise, orofacial dyskinesia, reserpine, oxidative stress, neurodegeneration.

1. INTRODUÇÃO

1.1 Estresse Oxidativo e Vulnerabilidade Cerebral

Todos os organismos aeróbicos são suscetíveis ao estresse oxidativo (EO) devido ao fato de espécies semi-reduzidas de oxigênio, radical superóxido ($O_2^{-\bullet}$) e peróxido de hidrogênio (H_2O_2) ser produzido na mitocôndria durante a respiração (Chance e cols., 1979). Essas espécies são denominadas radicais livres (RL) e também podem ser geradas no citoplasma ou na membrana celular sendo seu alvo relacionado ao sítio de formação (Anderson, 1996; Yu & Anderson, 1997). O oxigênio é utilizado pela enzima mitocondrial citocromo oxidase em um processo de redução tetravalente que resulta na formação de água (Halliwell & Gutteridge, 1999) e estima-se que em torno de 98% do oxigênio consumido em organismos aeróbios seja reduzido desta forma, sem a geração paralela de RL (Chance e cols., 1979). O cérebro é extremamente sensível ao EO devido sua grande quantidade de ácidos graxos poliinsaturados e baixas defesas antioxidantes, provendo ampla área para atuação da cascata de peroxidação lipídica (Lohr e cols., 2003).

O Homem é constantemente exposto aos RL provenientes do meio ambiente (radiação eletromagnética) e do metabolismo interno celular (aminoácidos excitatórios, neurotransmissores, consumo de O2 mitocondrial, transporte de elétrons pela citocromo P450, atividade da monoamino oxidase - MAO). Os aminoácidos excitatórios (glutamato) e neurotransmissores (dopamina) cujo metabolismo produz RL ou espécies reativas são as principais fontes de EO no cérebro (Gilgun-Sherki e cols., 2001). Em concentrações baixas e moderadas ocorrem os efeitos benéficos dos RL como, por exemplo, na defesa contra agentes infecciosos e na resposta mitogênica sendo utilizados como sinalizadores para a estimulação de antioxidantes e processos de reparo celular (Pani e cols., 2000; Valko e cols., 2007). Por outro lado, quando a produção excede a capacidade natural antioxidante é que ocorre o EO que, dependendo da extensão, pode levar à morte celular por apoptose, pois as espécies reativas de oxigênio formadas (EROs) oxidam componentes celulares vitais como lipídios, proteínas e DNA (Simonian & Coyle, 1996), envolvidos em inúmeras doenças como câncer, inflamação, artrite reumatóide, doenças neurodegenerativas, assim como nos processos de envelhecimento (Goodwin e cols., 1983; Beal, 1995; Perry e cols., 1997; Emerit e cols., 2004; Radak e cols., 2005; Valko e cols., 2007).

1.2 Defesas Antioxidantes

O delicado balanço entre benefícios e malefícios dos RL é de extrema importância para os seres vivos, sendo alcançado através do mecanismo de "regulação redox", mantendo a homeostase celular. Este estado de equilíbrio é determinado pela razão entre produção e remoção de RL através de antioxidantes endógenos e/ou exógenos (Dröge, 2002). Os mecanismos de defesa antioxidante incluem a remoção de espécies reativas de oxigênio/nitrogênio e seus precursores, inibição da formação de EROs, ligação aos íons metálicos necessários à catálise da geração das EROs e regulação das defesas antioxidantes endógenas. O efeito eficaz dos antioxidantes depende do tipo de radical gerado, do local de formação e da severidade do dano causado (Halliwell, 1994; 1997).

O sistema de defesa antioxidante está dividido em enzimático e não enzimático. O primeiro inclui as enzimas superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx). A SOD catalisa a formação de H_2O_2 a partir do radical superóxido, enquanto a catalase age na eliminação do H_2O_2 promovendo sua catálise até água. A GPx converte a glutationa reduzida (GSH) à glutationa oxidada (GSSG), removendo H_2O_2 e formando água (revisado por Lohr e cols., 2003). O sistema não enzimático inclui compostos sintetizados pelos seres vivos como bilirrubina, ceruloplasmina, hormônios sexuais, melatonina, coenzima Q, ácido úrico, e outros compostos presentes na dieta como ácido ascórbico (vitamina C), α-tocoferol (vitamina E), β-caroteno (precursor da vitamina A) e grupos fenóis de plantas (flavonóides) (Schneider & Oliveira, 2004).

1.3 Doenças Neurodegenerativas e Estresse Oxidativo

O cérebro é exposto durante toda a vida ao EO e vários distúrbios do sistema nervoso central (SNC) estão associados à geração de RL e danos oxidativos, como causa primária ou como conseqüência da própria doença. (Gilgun-Sherki e cols., 2001). Descrevemos abaixo algumas destas doenças, com especial enfoque sobre a discinesia tardia:

1.3.1 Doença de Alzheimer

Caracterizada por degeneração neuronal e deterioração cognitiva especialmente em pacientes idosos (Flynn & Runho, 1999), cuja fisiopatologia tem sido relacionada ao EO através de diversas constatações como: aumento da peroxidação lipídica em áreas específicas de cérebro (Lovell e cols., 1995); aumento na atividade da catalase, SOD, GPx e glutationa redutase na região do hipocampo e amígdala (Zemlan e cols., 1989; Pappella e cols., 1992). Além disso, Pappolla et al. (1998) evidenciaram que a proteína β-amilóide (principal constituinte da placa senil) é neurotóxica e que tal toxicidade é mediada por RL.

1.3.2 Doença de Parkinson

Doença progressiva caracterizada por tremor, rigidez muscular, anormalidades posturais e bradicinesia. Dados de estudos cerebrais *postmortem* indicam que o estresse oxidativo tem fundamental importância nos neurônios dopaminérgicos da região nigro-estriatal (Fahn & Cohen, 1992). Espécies reativas de oxigênio podem ser produzidas durante o metabolismo normal de dopamina, cujos produtos de oxidação se polimerizam para formar neuromelanina, que pode também ser tóxica (Offen e cols., 1999). Além disso, existem várias evidências sobre a toxicidade de dopamina em culturas de células, provocando uma morte celular programada (Ziv e cols., 1994; Offen e cols., 1995).

1.3.3 Doença de Huntington

É uma doença progressiva, com perda maciça de neurônios estriatais (Bartzokis e cols., 1999), com elevados níveis de ferro (Chen e cols., 1993). Estudos dão suporte à teoria de uma disfunção metabólica associada ao EO na doença de Huntington (Gu e cols., 1996; Browne e cols., 1997), assim como o envolvimento de uma ativação glutamatérgica excessiva, conduzindo a produção de EROs (Olney & Gubareff, 1978).

1.3.4 Esclerose lateral amiotrófica

Caracterizada por uma degeneração seletiva e progressiva dos neurônios motores inferiores da medula espinhal e dos neurônios motores superiores do córtex cerebral (Gilgun-Sherki e cols., 2001). Detectou-se o conteúdo de proteína carbonilada (indicador de oxidação de proteínas) elevado em 85% dos pacientes com a doença, indicando o envolvimento de EO em

todos os tipos de esclerose lateral amiotrófica (Coyle & Puttfarcken, 1993). Em pacientes com doença esporádica foi observado um aumento acentuado de substâncias reativas ao ácido tiobarbitúrico (TBARS) no plasma, as quais são produtos de peroxidação lipídica (Oteiza e cols., 1997).

1.3.5 Esquizofrenia e Discinesia Tardia (DT)

A presença de níveis elevados de EROs é descrita tanto para a esquizofrenia como para a DT induzida por antipsicóticos (Lohr e cols., 1990). A esquizofrenia é uma desordem psiquiátrica que acomete aproximadamente 1% da população, e o envolvimento do EO na fisiopatologia da doença é indicado pela presença de produtos de peroxidação lipídica no plasma e fluido cérebro-espinhal de pacientes, e em níveis alterados de antioxidantes enzimáticos e não-enzimáticos (Reynolds, 1992; Mahadik & Scheffer, 1996). A discinesia tardia (DT) é uma síndrome extrapiramidal que pode desenvolver-se após uso prolongado de fármacos antipsicóticos clássicos, manifestando-se através de movimentos involuntários anormais e repetidos da região orofacial e pescoço, principalmente (American Psychiatric Association, 1994). Atualmente, a síndrome é considerada um dos problemas psiquiátricos mais graves, desenvolvendo-se em cerca de 20 a 25% dos pacientes que fazem uso de neurolépticos (Andreassen & Jorgensen, 2000). Antipsicóticos clássicos utilizados no tratamento da esquizofrenia podem aumentar a neurotransmissão glutamatérgica estriatal através do bloqueio de receptores pré-sinápticos de dopamina (DA), promovendo EO e degeneração neuronal. Essa degeneração pode resultar em lesões irreversíveis dos neurônios nigro-estriatais com conseqüente morte celular, o que explicaria a irreversibilidade da DT (Elkashef & Wyatt, 1999; Sachdev e cols., 1999). Além disso, regiões do cérebro como substância negra e gânglios basais contêm diferentes metais de transição e altas concentrações de DA, aumentando a vulnerabilidade ao EO e o envolvimento dessas regiões na patogênese de desordens degenerativas e do movimento (Hanig & Aprison, 1967; Larson e cols., 1979).

Como se pode evidenciar existe uma variedade de doenças relacionadas ao EO e nossas defesas podem não ser completamente efetivas, considerando também o aumento da exposição ambiental aos agentes pró-oxidantes. Deste modo, torna-se importante o estudo de novas substâncias e/ou alternativas terapêuticas, já que o EO tem sido relacionado com a patogênese de diferentes doenças neurológicas e neurodegenerativas, além do envolvimento em outras doenças como as cardiovasculares, câncer, diabetes, isquemia/reperfusão e envelhecimento (Dhalla e cols., 2000; Sayre e cols., 2001; Jenner 2003; Dalle-Donne e cols., 2006).

1.4 Atividade Física

1.4.1 Efeitos benéficos do exercício

A prática de atividade física tem sido cada vez mais reconhecida e recomendada por médicos e estudiosos da área da Saúde, com evidente importância para a manutenção de uma boa qualidade de vida. O mecanismo bioquímico pelo qual o exercício beneficia a saúde e bem estar, incluindo a incidência de certas doenças e depressão ainda não é bem conhecido (Holloszy, 1993; Sarna e cols., 1993; Blair e cols., 1995). Alguns estudos demonstram que a atividade física crônica pode aumentar a produção de EROs especialmente no fígado e músculos (Ji & Fu, 1992; Atalay e cols., 1996). Por outro lado, um treinamento regular é capaz de reduzir esta produção frente a um exercício intenso, pois vários agentes de defesa contra o estresse oxidativo (enzimas antioxidantes, vitamina C e E, GSH, etc.) estão presentes nas células e podem ser reforçados durante o exercício crônico (Niess e cols., 1996; Leeuwenburgh e cols., 1997).

Os efeitos da relação exercício físico/estresse oxidativo sobre músculos e órgãos como coração principalmente, são bem descritos. Foi demonstrada uma estreita conexão entre as funções cardiovascular e cognitiva, sugerindo efeitos do exercício sobre a função cerebral (Hicks & Birren, 1970). Ao manter a integridade cerebrovascular (McFarland, 1963) o exercício aumenta os capilares (Black e cols., 1987) e as conexões dendríticas (Pysh & Weiss, 1979), melhorando as funções cerebrais como na prevenção e/ou melhora do déficit cognitivo da velhice (Chodzko-Zajko & Moore, 1994). Os efeitos benéficos do exercício são observados em modelos animais através do aumento da plasticidade e neurogênese, resultando na proteção das funções cerebrais, melhora do aprendizado e redução do EO cerebral (Fordyce & Wehner, 1993; Van

Praag e cols., 1999; Radak e cols., 2001a; Cotman & Berchtold, 2002; Molteni e cols., 2004). Na doença de Parkinson até um nível moderado, o exercício melhora de forma significativa a atividade motora, a cognição, o estado de humor e as atividades diárias dos pacientes (Hurwitz, 1989; Sunvisson e cols., 1997; Baatile e cols., 2000; Miyai e cols., 2000). Independente do grau da referida doença, Kuroda e cols. (1990,1992) constataram uma redução na taxa de mortalidade em pacientes submetidos ao exercício físico regular. Além disso, a atividade física está associada a um menor risco para o desenvolvimento da doença de Alzheimer e demência de qualquer grau (Broe e cols., 1990; Shimamura e cols., 1998; Laurin e cols., 2001), benefícios também comprovados em portadores de esquizofrenia (Marder e cols., 2004; Connolly e Kelly, 2005).

Tem sido demonstrado que os efeitos benéficos da atividade física regular (crônica) dependem de processos adaptativos iniciais, pois o exercício agudo estimula o estado redox cerebral (Radal e cols., 2001a,b; 2002, 2003, 2004). De acordo com o exposto, os agentes oxidantes produzidos pelo exercício podem agir como sinalizadores para a ativação de defesas antioxidantes, enquadrando-se no conceito de “hormesis”, onde se discute que uma dose baixa de determinada substância causaria uma adaptação celular benéfica enquanto uma dose elevada seria prejudicial (Radak e cols, 2005; Ji e cols., 2006).

1.4.2 Efeitos prejudiciais do exercício

De acordo com a teoria da “hormesis”, Gomez-Cabrera e cols. (2006) demonstraram que o exercício provoca uma importante regulação nas enzimas antioxidantes, e que uma produção elevada de oxidantes pode causar dano às células. Peijie e cols. (2003) observaram em estudo com animais, que o exercício extremo (*overtraining*) pode comprometer o sistema imunológico celular e humoral, provocando alterações sobre a secreção hormonal e neurotransmissores de estresse. Neste estudo, foi observado um aumento no conteúdo de beta-endorfina, dinorfina A, arginina, vasopressina e ocitocina no hipotálamo e uma diminuição na hipófise, sugerindo um aumento na produção de neuropeptídeos cerebrais relacionados ao estresse.

Tem sido descrito que o estresse emocional pode ativar tanto o sistema nervoso simpático quanto o eixo hipotálamo-pituitário-adrenal (Ganong, 2001), aumentando a liberação de noradrenalina, dopamina, epinefrina, fator liberador de corticotrofina e glicocorticóides em certas áreas do cérebro e na circulação periférica (Kandel e cols., 2000). Além disso, o estresse fisiológico e patológico pode aumentar a concentração de glicocorticóides no plasma e diminuir

sua capacidade de ligação aos receptores (Xu & Tan, 1990; Chen e cols., 1991). Howells e cols. (2005) sugeriram através de observações comportamentais que o exercício voluntário oferece neuroproteção, mas que agentes estressores são capazes de cancelar este benefício. Demonstrou-se que o exercício exaustivo está relacionado a um aumento de RL em diferentes tecidos biológicos (Reide e cols., 1992; Sastre e cols., 1992; Bejma e cols., 2000), podendo gerar diferentes sintomas centrais como depressão, dificuldade de concentração, dores de cabeça e perda de peso (Fry e cols., 1991).

1.5 Modelo Animal de Estresse Oxidativo Induzido por Reserpina

A reserpina vem sendo utilizada por diferentes laboratórios para induzir discinesia orofacial (um modelo animal de discinesia tardia), relacionada à formação de RL e EO (Raghavendra e cols., 2001; Dutra e cols., 2002; Abílio e cols., 2003; Carvalho e cols., 2003; Burger e cols., 2003, 2004, 2005), e também como modelo experimental para a doença de Parkinson (Colpaert, 1987; Menzaghi e cols., 1997; Dawson e cols., 2000). Está estabelecido que a reserpina seja capaz de prevenir a estocagem de DA nas vesículas neuronais interferindo com o transporte vesicular de monoaminas (bloqueio do transportador). Como resultado, ocorre aceleração do catabolismo de DA citosólica através da enzima MAO, seguido da formação simultânea de metabólitos acídicos e peróxido de hidrogênio, associados ao EO (Abílio e cols., 2002; Burger e cols., 2003; Naidu e cols., 2004; Bilzka & Dubiel, 2007). Independente da ação da MAO e do transportador pode ocorrer uma auto-oxidação da dopamina formando aminocromo, um importante precursor de RL. Este metabólito poderá ter diferentes destinos como à formação de subprodutos (neuromelanina, leucoaminocromo), descritos como fontes geradoras de espécies reativas endógenas envolvidas em processos degenerativos (Figura 1).

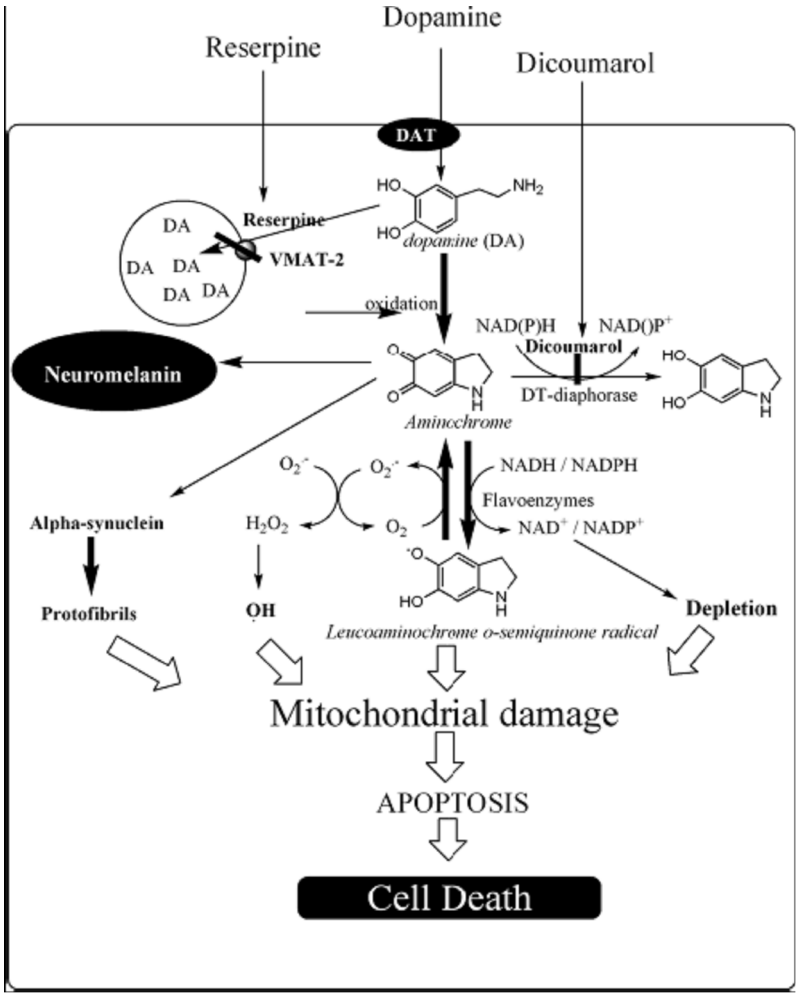

Figura 1. Proposta do mecanismo de ação da reserpina e auto-oxidação da dopamina (Fuentes e cols., 2007).

2. OBJETIVOS

2.1 Objetivo Geral

Considerando o envolvimento do estresse oxidativo no desenvolvimento de patologias neurodegenerativas, este estudo propõe avaliar a influência do exercício físico em diferentes intensidades sobre um modelo animal de estresse oxidativo, relacionado ao desenvolvimento de discinesia orofacial.

2.2 Objetivos Específicos

- Avaliar o desenvolvimento de estresse oxidativo estriatal através da observação de discinesia orofacial (tempo de tremor facial- TF e freqüência de movimentos de mascar vazio-MMV) induzida por reserpina em animais submetidos ou não a sessões de exercício físico moderado e intenso;

- Avaliação comportamental de animais submetidos ao exercício físico intenso através da observação do índice locomotor (movimentação espontânea e freqüência de erguer as patas dianteiras-“rearing”) em campo aberto (open-field);

- Avaliações bioquímica e enzimática de estresse oxidativo (TBARS, GSH, catalase) em estriado (região cerebral envolvida em distúrbios de movimento) de animais submetidos ao exercício físico moderado e intenso.

3. ARTIGOS CIENTÍFICOS

O **artigo 1** está disposto na forma que foi publicado na edição do periódico **Pharmacology, Biochemistry and Behavior.** O **artigo 2** está disposto na forma submetida para publicação.

3.1 – EFEITO DO EXERCÍCIO CRÔNICO MODERADO SOBRE O MODELO DE ESTRESSE OXIDATIVO INDUZIDO POR RESERPINA EM RATOS

Artigo 1

INFLUENCE OF CHRONIC EXERCISE ON RESERPINE-INDUCED OXIDATIVE STRESS IN RATS: BEHAVIORAL AND ANTIOXIDANT EVALUATIONS

TEIXEIRA, A.M., TREVIZOL, F., COLPO, G., GARCIA, S.C., CHARÃO, M., PEREIRA, R.P., FACHINETTO, R., ROCHA, J.B.T., BURGER, M.E.

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Influence of chronic exercise on reserpine-induced oxidative stress in rats: Behavioral and antioxidant evaluations

Angélica M. Teixeira [a], Fabíola Trevizol [a], Gabriela Colpo [a], Solange C. Garcia [c], Mariele Charão [c], Romaiana P. Pereira [b], Roselei Fachinetto [b], João B.T. Rocha [b], Marilise E. Bürger [a,*]

[a] *Departamento de Fisiologia e Farmacologia, Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria, RS, Brazil*
[b] *Departamento de Química, Universidade Federal de Santa Maria, RS, Brazil*
[c] *Departamento de Toxicologia, Universidade Federal de Santa Maria, RS, Brazil*

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Abstract

Several neurological diseases are related to oxidative stress (OS) and neurotoxicity. Considering that physical exercise may exert beneficial effects on antioxidant defenses, our objective was to evaluate the influence of a swimming exercise on an OS animal model (reserpine-induced orofacial dyskinesia). In this model, the increased dopamine metabolism can generate OS and neuronal degeneration, causing involuntary movements. The increase in vacuous chewing movements and facial twitching caused by reserpine (1 mg/kg sc) was partially prevented by exercise. An increase in catalase activity and a decrease in GSH levels were observed in the striatum. Physical training did not change the effects of reserpine on catalase, however it partially recovered GSH. Exercise *per se* caused a significant GSH decrease. There was a positive correlation between catalase and OD ($r=0.41$; $r=0.47$, $P<0.05$) and a negative correlation between GSH and OD ($r=0.61$; $r=0.71$, $P<0.05$). These results reveal the benefit of exercise in attenuating the motor disorder related to OS.

Keywords: Exercise; Orofacial dyskinesia; Reserpine; Oxidative stress; Excitotoxicity

1. Introduction

Physical activity is recognized as an important component of a healthy life style and is recommended by clinicians and scientists (Donaldson, 2000). The favorable effects of exercise on the cardiovascular system and on cognition suggest its influence on brain function (Cotman and Engesser-Cesar, 2002; Fordyce and Wehner, 1993; Hicks and Birren, 1970). Alterations elicited by exercise are associated with improvements in a variety of age-related diseases. However, the mechanisms are not yet well understood (Blair et al., 1995; Gündüz et al., 2004; Holloszy, 1993). Furthermore, habitual exercise has been related to OS resistance and the inhibition of carcinogenesis at the initiation stage (Nakatani et al., 2005).

Exercise is also associated with an increase of oxygen uptake (Sen, 2001), of which as much as 2% may be converted to reactive oxygen species (ROS) (Inoue et al., 2003; Wickens, 2001). However, this increased uptake of oxygen that occurs in the body is not observed in the brain and it has been reported that chronic exercise caused an increase in the brain's antioxidant defenses (Liu et al., 2000). The few studies available on the effect of exercise on oxidative damage or the antioxidant status in brain show conflicting results (Asha and Kiran, 2004; Radak et al., 1995; Somani et al., 1995). Suzuki et al. (1983) reported that voluntary exercise increased the lipid peroxidation in the brain of rats. On the other hand, regular exercise attenuated an age-associated decline in memory and reduced the accumulation of proteins affected by oxidative damage in the brain (Radak et al., 2001a,b). In line with this, regular physical exercise has been related to an increase in the number of new

* Corresponding author. Centro de Ciências da Saúde, Programa de Pós-Graduação em Farmacologia, 97105-900, Santa Maria, RS, Brazil. Tel.: +55 55 3220 8342; fax: +55 55 3220 8241.
E-mail address: mariliseeb@yahoo.com.br (M.E. Bürger).

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hippocampal cells (Van Praag et al., 1999a,b), an increase in brain plasticity (Cotman and Berchtold, 2002), an increase in the production of neurotrophic factors (Neeper et al., 1996; Ogonovszky et al., 2005), and an increase in learning and memory (Fisher et al., 2000; Ogonovszky et al., 2005; Radak et al., 2001a, 2006; Van Praag et al., 1999a).

Of particular importance, the brain is more susceptible to oxidative damage when compared to other organs or systems (Halliwell and Gutteridge, 1999), mainly because it contains high levels of membrane lipids, excitotoxic amino acids, low levels of antioxidant defenses and autoxidizable neurotransmitters. For instance, dopamine (DA) reacts with molecular oxygen to form dopamine-quinones which can deplete glutathione, generating ROS during this process (Graham, 1978). When the production of ROS exceeds the ability of the antioxidant system to eliminate them, oxidative damage results (Jenkins and Goldfarb, 1993). Considering the OS, reserpine is a monoamine depletor that exerts a blockade on the vesicular monoamine transporter (VMAT) for neuronal transmission or storage, promoting dopamine-autoxidation and oxidative catabolism by monoamine oxidase (MAO) (Fuentes et al., 2007). This accelerated mechanism leads to the formation of dopamine-quinones and hydrogen peroxide, related to the OS process (Abílio et al., 2002, 2003a; Bilzka and Dubiel, 2007; Burger et al., 2003; Calvente et al., 2002; Naidu et al., 2004; Spina and Cohen, 1988, 1989). In particular, areas of the brain, such as the basal ganglia, are rich in monoamines and therefore more vulnerable to free radical damage that may result in OS (Lohr et al., 2003). The neuronal damage of the basal ganglia is associated with damage of voluntary movements (Dawson et al., 2000; Graybiel et al., 1995) and related to different diseases such as Huntington, ballism, Parkinson and tardive dyskinesia (Albin et al., 1989; Andreassen and Jorgensen, 2000; Bartzokis et al., 1999; Fahn and Cohen, 1992; Gilgun-Sherki et al., 2001; Lohr et al., 1990).

Various laboratories have demonstrated the development of a movement disorder upon the administration of reserpine in rats/mice (Abilio et al., 2002, 2003b, 2004; Bergamo et al., 1997; Burger et al., 2003, 2004, 2005b; Calvente et al., 2002; Carvalho et al., 2003; Castro et al., 2006; Colpaert, 1987; Dawson et al., 2000; Dutra et al., 2002; Menzaghi et al., 1997; Naidu et al., 2004; Neisewander et al., 1994; Peixoto et al., 2005; Queiroz and Frussa-Filho, 1999; Raghavendra et al., 2001; Silverdale et al., 2001; Sussman et al., 1997; Vital et al., 1997). This animal model, known as orofacial dyskinesia, has been related to OS in the basal ganglia (striatum) and is attenuated and prevented by antioxidant substances such as melatonin, ebselen and quercetin (Abílio et al., 2002, 2003a,b; Burger et al., 2003, 2004, 2005b; Faria et al., 2005; Naidu et al., 2004; Raghavendra et al., 2001).

As the brain is particularly vulnerable to free radical damage and the enhancement of OS has been associated with various neurodegenerative diseases, we investigated the effects of an exercise program in an animal model of OS and its relationship with the antioxidant defenses. Smith and Zigmond (2003) considered that exercise afforded protection against a variety of diseases including Parkinson and dopaminergic degeneration. In the same way, Howells et al. (2005) demonstrated that voluntary exercise afforded neuroprotection in a Parkinson's disease rat model. With this in mind, our objective is to evaluate whether chronic physical exercise is capable of attenuating or preventing neuronal damage related to reserpine-induced OS.

2. Method

2.1. Drugs

Reserpine (methyl reserpate 3,4, 5-trimethoxybenzoic acid ester—Sigma Chemical) was dissolved in glacial acetic acid and then diluted to a final concentration of 0.5% acetic acid with distilled water. The vehicle consisted of a 0.5% acetic acid solution. These solutions were injected subcutaneously (sc) in a volume of 1.0 ml/kg body weight.

2.2. Animals

Male Wistar rats weighing 270–320 g (about 3-month of age) were used. Groups of six 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 12 h-light/dark cycle with lights on at 7:00 a.m. The animals were maintained and used in accordance to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil. The rats were randomly assigned into four groups: sedentary-control (SC), sedentary-reserpine (SR), exercise-control (EC), exercise-reserpine (ER).

2.3. Training and experimental procedure

All rats from the exercise groups were subjected to swimming in a plastic container (depth 45 cm) and continuously supervised, with the water temperature set to 28 °C±1 °C, 1 h/day, 5 times per week during 8 weeks. The swimming duration increased about 15 min every day until it reached 60 min per day in the first week, which was maintained until the sixth week; in the seventh and eighth week they swam 90 min/day. The control rats (sedentary) were transported to the experimental room and placed in the swimming pool for a short time (3 min), to force them to get wet, however, they were not in contact with water for the same time that the swimmers were. One day after the last training, all the animals were treated with vehicle or reserpine solution. The drugs were then subcutaneously administered, for 3 days every other day, as follows:

SC sedentary rats injected with 0.5% acetic acid solution (vehicle for reserpine);
SR sedentary rats injected with 1 mg/kg reserpine solution;
EC exercised rats injected with 0.5% acetic acid solution;
ER exercised rats injected with 1 mg/kg reserpine solution.

On the fourth day, 24 h after the second reserpine or vehicle injection, all the rats were observed for the quantification of

orofacial dyskinesia. The animals were decapitated 24 h after behavioral measurements.

The animal's body weights were monitored once a week during the experiment.

2.4. Behavioral testing

With the objective of analyzing the development of reserpine-induced orofacial dyskinesia, the rats were submitted to behavioral observation as follows: Rats were placed individually in cages (20×20×19 cm) containing mirrors under the floor and behind the back wall of the cage to allow behavioral quantification when the animal was faced away from the observer. To quantify the occurrence of oral dyskinesia, the incidence of vacuous chewing movements (VCM) and the duration of facial twitching (FT) were recorded during 10 min. Observers were blind to the drug treatment. In a preliminary study (using 5 control and 10 rats treated with reserpine) of interrater reliability, we found that the use of this method of observation and definition for the parameters evaluated usually resulted in >90 and 91% agreement between 4 different observers for vacuous chewing and duration of facial twitching, respectively. All the calculated P values were significant for $P<0.05$.

2.5. Biochemical assays

The brains were removed immediately after decapitation, put on ice and cut coronally at the caudal border of the olfactory tubercle. The striatum was dissected from the anterior part and separated into two parts. The right striatum was homogenized in 10 volumes (w/v) of 0.1 M Tris–HCl, pH 7.4, centrifuged for 10 min at 3000 ×g, and used for catalase determination by spectrophotometry (Aebi et al., 1995).

The left striatum was homogenized in 50 volumes (w/v) of 0.5 N perchloric acid and centrifuged under the same conditions previously cited. The supernatant was used to measure reduced glutathione (GSH). A volume of 130 µl of supernatant was mixed with 500 µl of Tris–HCl 0.5 M. The derivatization of the samples was carried out with 350 µl DTNB (5.5′-dithiobis(2-nitro-benzoic acid) for high performance liquid chromatography (Schott et al., in press).

2.6. Statistical analysis

Data were analyzed by two-way ANOVA (2 (sedentary/exercise)×2 (control/reserpine) followed, when appropriate, by univariate analysis and Duncan's multiple range test.

3. Results

Eight weeks of swimming did not cause a difference in body weight between exercised and control animals, which is in accordance with other research using swimming as an exercise model (Gündüz et al., 2004; Radak et al., 2001a).

Two-way ANOVA of vacuous chewing frequency revealed a significant main effect of reserpine [$F(1, 20)=136.6$, $P<0.001$]. Univariate ANOVA followed by Duncan's multiple range test revealed that reserpine (SR) and exercise+reserpine (ER)-treated groups displayed an increase in vacuous chewing frequency when compared to control (SC) and exercise-treated (EC) groups (Fig. 1A).

Analyses of the duration of facial twitching yielded a significant main effect of reserpine [$F(1,20)=57.6$, $P<0.001$],

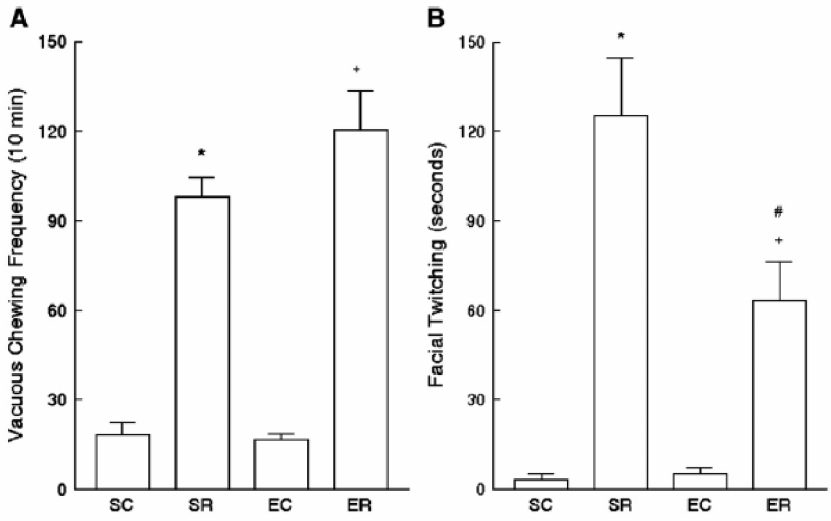

Fig. 1. Effects of administration of reserpine (1.0 mg/kg sc every other day, for 3 days) (SR) or vehicle (SC) on sedentary rats and rats submitted to exercise training (ER) or (EC) for vacuous chewing frequency (A) and duration of twitching of the facial musculature (in seconds) (B). Data (mean±SEM) were analyzed by one-way analysis of variance followed by Duncan's test. * indicates a significant difference from control group (SC) for $P<0.001$, $^{+}$ indicates a significant difference from exercised rats (EC) for $P<0.05$, and $^{\#}$ indicates a significant difference from reserpine-treated animals (SR) for $P<0.05$ (Duncan's multiple range test).

Table 1
Catalase activity (mU/g tissue) and GSH levels (mM/g tissue) in the striatum of rats as the result of chronic exercise training

	SC	SR	EC	ER
	$n=6$	$n=6$	$n=6$	$n=6$
Catalase activity	1.95±0.16	2.51±0.16*	1.81±0.1	2.21±0.13
GSH levels	3.17±0.12	1.26±0.1**	2.18±0.1**	2.07±0.13^{+}

Values are means±SEM, *$P<0.05$, **$P<0.001$, differences from sedentary-control group (SC); $^{+}P<0.001$, difference from sedentary-reserpine group (SR) (Duncan's multiple range test).

exercise [$F(1, 20)=6.4$, $P<0.05$] and a significant reserpine×exercise interaction [$F(1, 20)=7.2$, $P<0.05$]. Univariate ANOVA followed by Duncan's multiple range test revealed that reserpine considerably increased the duration of facial twitching and that the exercise training partially reversed the effect of reserpine. In fact, the duration of facial twitching for the exercise+reserpine-treated animals (ER) was significantly lower than that of rats treated with reserpine (SR) and significantly higher than that of control (SC) or exercise-treated (EC) animals (Fig. 1B).

Two-way ANOVA of catalase activity is shown in Table 1. Two-way ANOVA revealed a significant main effect of reserpine on catalase activity [$F(1, 20)=12.8$, $P<0.05$]. Univariate ANOVA followed by Duncan's multiple range test revealed that reserpine significantly increased catalase activity (SR) and that chronic exercise training did not reverse the effect of reserpine (ER).

Two-way ANOVA for GSH (Table 1) revealed a significant reserpine effect [$F(1, 20)=109.22$, $P<0.001$] as well as exercise×reserpine interaction [$F(1, 20)=86.74$, $P<0.001$]. Post-hoc analysis revealed that rats treated with reserpine (SR), exercise (EC) and exercise+reserpine (ER) presented a decrease in GSH levels when compared to controls (SC). The animals that received the exercise-reserpine (ER) co-treatment showed a partial recovery of GSH levels, when compared to the group treated with reserpine (SR).

Statistical analyses revealed a significant positive correlation between vacuous chewing frequency ($r=0.41$, $P<0.05$, Fig. 2) and facial twitching ($r=0.47$, $P<0.05$, Fig. 2) with striatal catalase activity of rats. To the contrary, regression analyses between vacuous chewing frequency ($r=0.61$, $P=0.001$ Fig. 3) and facial twitching ($r=0.71$, $P<0.001$, Fig. 3) with GSH levels in the striatum of rats revealed a significant negative correlation.

4. Discussion

The results of the present study clearly indicate that moderate chronic physical exercise is capable of exerting a protective role against reserpine-induced orofacial dyskinesia, shown through increased vacuous chewing movements and facial twitching. Exercise training partially reversed the increase in FT duration and, interestingly, did not change VCM frequency. This result is in accordance with other findings from our laboratory, where ebselen (an antioxidant agent) reversed the reserpine-induced increase in FT but did not modify VCM (Burger et al., 2003).

Of particular interest for the animal model chosen here, different laboratories have associated OS with neurodegeneration and movement disorders (Cadet et al., 1986, Cadet and Kahler, 1994; Naidu et al., 2003; Post et al., 1998; Sagara, 1998;), and have searched for antioxidant substances (Abílio et al., 2002, 2003a,b; Burger et al., 2003, 2005a; Dabiri et al., 1994; Egan et al., 1992; Faria et al., 2005; Naidu et al., 2003; Raghavendra et al., 2001; Singh et al., 2003). In fact, Sussman et al. (1997) showed that reserpine administration causes a decrease in striatal dopamine levels and an increase in the

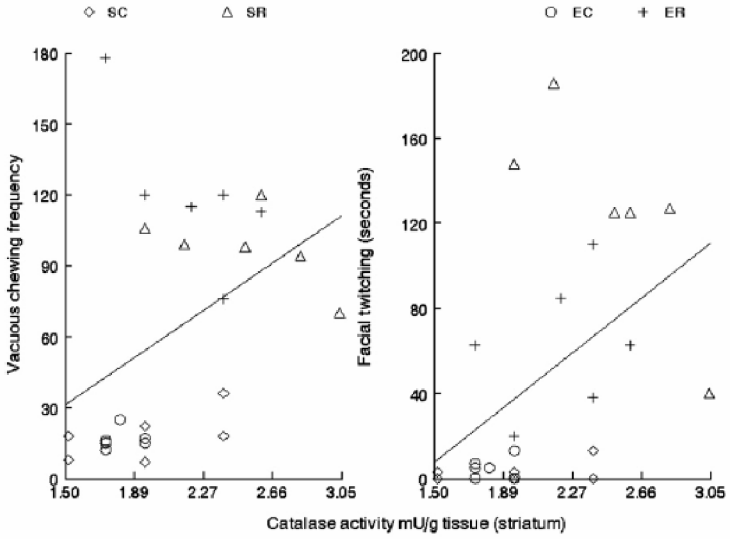

Fig. 2. Linear regression analysis between vacuous chewing frequency, facial twitching and catalase activity in the striatum of rats treated with reserpine (1.0 mg/kg sc every other day, for 3 days) following 8 weeks of chronic exercise training. (Statistical analysis revealed the following P significance levels for the r values: 0.41 and 0.47 respectively).

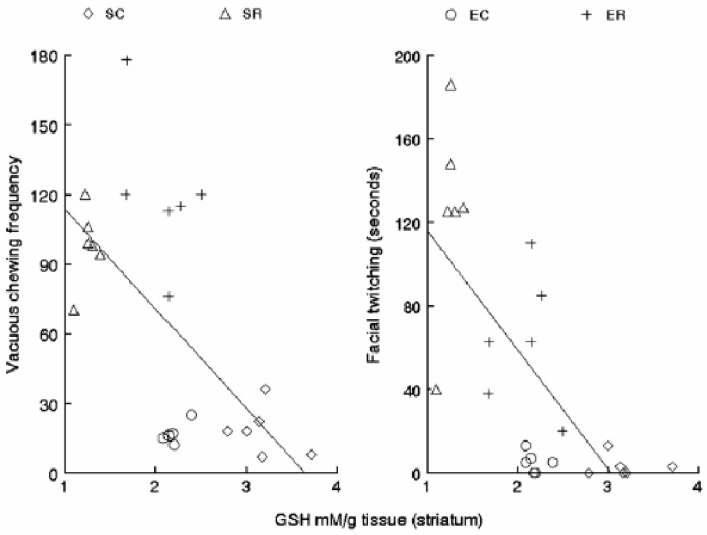

Fig. 3. Linear regression analysis between vacuous chewing frequency, facial twitching and GSH levels in the striatum of rats treated with reserpine (1.0 mg/kg sc every other day, for 3 days) following 8 weeks of chronic exercise training. (Statistical analysis revealed the following *P* significance levels for the *r* values: 0.61 and 0.71 respectively).

metabolite of dopamine ratios (DOPAC/dopamine and HVA/dopamine) in rats. Recently, we showed a negative relationship between the glutamate transporter and the manifestation of orofacial dyskinesia in rats exposed to reserpine or haloperidol (Burger et al., 2005b), contributing to the relation between OS and excitotoxicity. Coyle and Puttfarcken (1993) considered that these events may very well act together, since they are closely related. In line with this, the basal ganglia, involved in motor function, is particularly more vulnerable to free radical damage, since this brain region is rich in transition metals and contains large amounts of catecholamines such as dopamine. The antioxidant enzymes SOD, CAT and GSH-Px and the ratio of GSH to oxidized glutathione (GSSG) are critical for protection against oxyradical toxicity. Glutathione in its reduced state plays an important role in cellular protection against damage from free radicals and oxyradicals (Werner and Cohen, 1993), and its deficiency leads to mitochondrial damage in the brain (Jain et al., 1991).

Physical exercise early in life may be protective against the development of Parkinson's disease (PD) (Brasted et al., 1999; Sasco et al., 1992) and it ameliorates motor symptoms and neurochemical deficits in rodent models of induced striatal damage (Tillerson et al., 2003). In addition, symptoms of senile dementia might be improved by exercise (Sutoo and Akiyama, 2003) and may also protect against a variety of neurodegenerative conditions (Döbrössy and Dunnett, 2003; Smith and Zigmond, 2003).

Considering neurological diseases and OS, we found it interesting to examine whether moderate chronic exercise training changed the effect of repeated reserpine treatment in striatal catalase activity and GSH levels.

The results presented here demonstrate that reserpine administration increased catalase activity in the striatum. Recently, Abilio et al. (2004) and Faria et al. (2005) demonstrated the critical role of this antioxidant enzyme in the development of oral dyskinesia and OS. In line with this, it has been reported that catalase activity is low in the brain (Gaunt and De Duve, 1991), however, chronic physical exercise did not modify its activity. Of particular importance for the increase in free radicals induced by reserpine, the increased catalase activity found here may be a compensatory response or a signaling mechanism of an oxidative damage (Gomez-Cabrera et al., 2006).

We also demonstrated that reserpine reduced striatal GSH levels and this result occurred in parallel to an increase in orofacial dyskinesia. This negative correlation reinforces the role of free radicals in this putative animal model. In this study, the effect of reserpine on GSH levels was partially prevented by moderate physical training, although exercise *per se* reduced these levels and deserves further investigations. Abilio et al. (2003b) demonstrated a relationship between the development of reserpine-induced orofacial dyskinesia and an increase of the striatal GSSG/GSH ratio, where both effects were attenuated by vitamin E. These results show clearly that physical training is capable of exert beneficial effects on this important brain defense system. Different from our results, Somani et al. (1995) and Liu et al. (2000) did not observe the influence of exercise training on GSH levels in the striatum of rats, although the experimental procedures used were different. In reserpine-treated mice, a considerable rise was reported in the striatal GSSG level (Spina and Cohen, 1989) as well as in striatum and prefrontal cortex of rats (Bilska and Dubiel, 2007).

Swimming was chosen as the model of exercise over the treadmill since it is a natural behavior of rodents (Kramer et al., 1993; Venditti and Di Meo, 1996). It is less stressful and can prevent foot injury, which may generate ROS unrelated to exercise (Venditti and Di Meo, 1996). In this sense, the exercise

program and water temperature employed here were not stress factors, as demonstrated by the reduced facial twitching. In fact, the influence of stress has been demonstrated to elevate orofacial movements (Andreassen et al., 1996; Egan et al., 1996; Glenthoj et al., 1990, 1993; Glenthoj and Hemmingsen, 1991; Levy et al., 1987; Waddington, 1990). In addition, different animal models have associated oxidative damage to stress, including cold stress (Kaushik and Kaur, 2003; Liu et al., 1996; Madrigal et al., 2001; Sahin and Gümüslü, 2004a,b; Voronych and Iemel'ianenko 1994), corroborating with our results.

In conclusion, in this study, through the measurement of orofacial dyskinesia we have demonstrated for the first time that chronic moderate physical exercise reduces reserpine-induced OS and attenuates the reserpine-induced decrease in striatal GSH levels. These results establish the beneficial effect of exercise on special clinical disorders associated with movement such as Huntington and Parkinson diseases, tardive dyskinesia, ballism and other neurological diseases.

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3.2 – EFEITO DO EXERCÍCIO INTENSO SOBRE UM MODELO ANIMAL DE ESTRESSE OXIDATIVO

Artigo 2

BRAIN RESPONSE TO HEAVY EXERCISE IN AN ANIMAL MODEL OF OXIDATIVE STRESS

TEIXEIRA, A., SANTOS, A., BONIATTI, T., REICKZIEGEL, P., MÜLLER, L., PEREIRA, R., ROOS, D., ROCHA, J.B.T., BURGER, M.E.

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ABSTRACT

Regular physical activity exerts beneficial effects for mental and physical health, but little is known about intense chronic exercise, which may cause an increase in brain monoamines and oxidative stress, mainly in the striatal region. Both intense exercise and reserpine can cause oxidative stress, mainly in regions such as the striatum. The neurotoxicity developed by reserpina may be accessed through behavioral and biochemical evaluations. Considering that moderate physical exercise is beneficial for antioxidant defenses, the objective of this study was to evaluate the influence of an intense exercise program in an animal model of oxidative stress. Male rats were submitted to swimming training (1 hour/day, during eleven weeks), where each rat's load was increased according to its body weight until reaching 7% of its weight in the last week of exercise. After the last training session, all the animals were treated with vehicle or repeated reserpine solution (1mg/kg-sc). After behavioral evaluations, all rats were sacrificed and the striatum was dissected for enzymatic and biochemical assays. The effectiveness of the physical training was assessed by reduced lactate levels and cardiac hypertrophy. The intense exercise *per se* reduced locomotor index, demonstrating emotional stress development. Exercise and reserpine together increased lipid peroxidation and striatal catalase activity (positively correlated with each other). These results suggest that heavy exercise can promote oxidative stress and emotional changes and when acting together with an oxidant agent (reserpine) its harmful effects are not overcome by the brain.

Keywords: intense exercise, heavy exercise, orofacial dyskinesia, reserpine, oxidative stress, neurotoxicity.

1. Introduction

Regular physical exercise exerts many benefits for mental and physical health, reducing disease incidence, improving cognitive processes and increasing resistance to brain injury [28,51,58]. Accumulated evidence has shown that regular physical activity may increase antioxidant defenses against brain oxidative damage [60,75] promoting antiapoptotic effects [81]. In this sense, physical activity can offer protection against seizures [68] and neurodegenerative diseases [32,73,77], as well as alleviating symptoms of anxiety and depression [45].

Of particular importance, the brain is more susceptible to oxidative stress (OS) when compared to other organs or systems, because it contains high levels of membrane lipids, excitotoxic amino acids, low levels of antioxidant defenses and mainly autoxidizable neurotransmitters [31]. Free radicals are formed in the central nervous system (CNS) as part of normal metabolic processes [30], but when the production of reactive oxygen species (ROS) exceeds the ability of the antioxidant system to eliminate them, oxidative damage occurs [34]. This subject is extremely important since the brain is exposed throughout life to OS and there is evidence of the involvement of free radicals, which arise from oxidative damage, in the pathophysiology of several diseases of the brain and nervous system [26]. Among these diseases are included Alzheimer's [53], Parkinson's [50,84], Huntington's [11], schizophrenia [43] and tardive dyskinesia [82].

There is growing evidence that the continuous presence of low ROS concentrations may induce the expression of antioxidant enzymes, DNA repair molecules and protein degrading enzymes, resulting in decreased OS [59]. This phenomenon has been related to the hormesis concept (a low dose of a substance is stimulatory and a high dose is inhibitory) and has been extended to the ROS generating effects of exercise [36,59,83]. In this sense, free radicals may be beneficial since they act as signals to enhance antioxidant defenses. Antioxidant enzymes, which constitute a defense mechanism against free radicals, may also be affected by exercise [17]. Gomez-Cabrera et al. [27] indicated that when oxidants are produced at moderate

levels, they act as signals to adapt cells to exercise, but when overproduced they may cause cellular damage.

Susuki et al. [78] observed an increase in brain lipid peroxidation caused by voluntary exercise, while Radak et al. [58] showed an attenuation of the age-associated decline in memory by regular swimming, and unmodified levels of lipid peroxidation. These conflicting data have shown that exercise can alter the brain response in the generation and inhibition of oxidative damage. Considering different forms and intensity of physical activity, some research groups have demonstrated a relationship between exhaustive exercise and the increased generation of ROS in different biological tissues [8,62,66]. Excessive physical activity may lead to overtraining and generate psychological symptoms that resemble clinical depression [5,12]. A concept of overtraining is that it generally occurs after a long term period of strenuous exercise or when the duration of exercise sessions increases abruptly [57]. Recently it was reported that a stress-related condition may alter physiological and immunological functions associated with biochemical abnormalities [6]. Besides depression, this effect can generate alterations in emotionality leading to other symptoms such as impaired concentration, anorexia, headache, low appetite and loss of body mass and/or motor coordination [24].

Considering OS, reserpina is a monoamine depletor which acts preventing the storage of DA in neuronal vesicles by interfering with the vesicular monoamine transporter (VMAT), resulting in oxidative catabolism of cytosolic DA (by MAO). This accelerated metabolism leads to the formation of metabolites and hydrogen peroxide, associated with the OS process in dopaminergic neurons [1,9,13,48]. Besides the MAO action, the autoxidation of DA is also precursor of *O*-quinone aminochrome, with different pathways in DA neurons: a polymerization to neuromelanin and a one-electron reduction to the leukoaminochrome *O*-semiquinone radical, which is thought to be one of the major sources of endogenous reactive species involved in the degenerative processes [25,54,55,67]. Particularly, the brain basal ganglia is rich in monoamines and therefore more vulnerable to free radical damage leading to OS [42]. Experimentally,

reserpine has been used by various laboratories to study movement disorder related to OS and neurodegenerative disease [2,9,15,21,48,56].

Recently, the beneficial effects of moderate chronic exercise were showed in an animal model of reserpine-induced OS [79], however the effects of an intense exercise program on oxidative damage and brain function continue to be unknown. The aim of the present study was to evaluate the influence of intense (heavy) physical activity on brain susceptibility to OS, either alone or in association with reserpina, an OS-inductor (behavior, enzymatic and biochemical evaluation).

2. Method

2.1. Drugs

Reserpine (methyl reserpate 3,4,5-trimethoxybenzoic acid ester- Sigma Chemical) was dissolved in glacial acetic acid and then diluted to a final concentration of 0.5% acetic acid with distilled water. The vehicle consisted of a 0.5% acetic acid solution. These solutions were injected subcutaneously (sc), at a volume of 1.0 mL/kg body weight.

2.2. Animals

Male Wistar rats weighing 270-360g (about 3-month of age) were used. Groups of five animals were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (23ºC ± 1°C) and on a 12h-light/dark cycle with lights on at 7:00 a.m. The animals were maintained and used in accordance to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil. The rats were randomly assigned to four groups: sedentary-control (SC), sedentary-reserpine (SR), exercise-control (EC), exercise-reserpine (ER).

2.3. Training protocol and experimental procedure

All exercised rats were subjected to swimming exercise in a plastic container (depth 45 cm) under continuous supervision, with the water temperature set to 34ºC ±

1ºC, 1 h/day, 5 times per week during 11 weeks. The animals had a 1 week adaptation period in which swimming time was increased every day so that, by the second week, they could swim 1 h/day. Exercise intensity was then increased every week, until the animals' load reached 7% of their body weight (swimming with a weight fixed around their abdomens) [16].

One day after the last training session, all the animals received an injection of vehicle (0.5% acetic solution) or reserpine solution (1mg/kg body weight in 0.5% of acid acetic solution), subcutaneously, for 3 days every other day, totaling two injections, in accordance with the following protocol:

SC: sedentary rats - Vehicle

SR: sedentary rats -Reserpine solution;

EC: exercised rats - Vehicle

ER: exercised rats - Reserpine solution.

The animals' weight was recorded weekly during the experiment.

2.4. Behavioral testing

On the fourth day (24h after the second reserpine or vehicle injection), all the rats were observed for the quantification of orofacial dyskinesia (OD): The animals were placed individually in cages (20x20x19cm) containing mirrors under the floor to allow behavioral quantification when the animal was faced away from the observer. To quantify the occurrence of oral dyskinesia, the incidence of vacuous chewing movements (VCM) frequency and the duration of facial twitching (FT) was recorded during 5 min after a period of 2 min adaptation (hand operated counters were employed). Observers were blind to the drug treatments.

Open field test: The rats were placed individually in an open-field arena, for measurement of locomotion (number of floor units entered) and rearing (number of times that the animal stood on hind legs) frequencies for 5 min.

All behavioral experiments were conducted between 09:00 and 11:00 a.m.

2.5. Tissue preparation and oxidative stress parameters

After behavioral evaluation the rats were decapitated, the brains were removed and put on ice, cut coronally at the caudal border of the olfactory tubercle. The striatum was dissected from the anterior part, weighed and homogenized for analysis.

For catalase activity determination, brain homogenates were centrifuged at 3000 x g for 10 min and the supernatants were used for enzyme quantification. Catalase was quantified by measuring the disappearance of H_2O_2 at 240 nm. One unit of catalase was defined as the number of mol of H_2O_2 degraded per minute [4].

Lipid peroxidation was determined by measuring accumulation of thiobarbituric acid reactive substances (TBARS) as described by Ohkawa et al. [52].

2.6. Statistical analysis

Data of orofacial movements (VCM and FT), TBARS and catalase activity were analyzed by a two-way ANOVA (2 (sedentary/exercise) X 2 (control/reserpine)) followed by Duncan's multiple range test, when appropriated. Behavioral data obtained in open-field (locomotion and rearing) were analyzed by Kruskal-Wallis analysis of variance followed by the two-tailed Mann-Whitney *U*-test [69].

3- Results

Verification of training program: heart weight (mg), final body weight (g), heart weight/body weight ratios (heart hypertrophy index) and lactate levels are shown in Table 1. The exercised group showed heart hypertrophy [$F(1,16)=9.75$, $P<0.05$] and reduced blood lactate levels [$F(1,16)=17.51$, $P<0.01$], indicating the effectiveness of the exercise. Body weight was not modified by physical training.

Open-field test: The effects of the intense exercise program on locomotor index are represented by the number of crossing and rearing in the open-field test (Fig.1). Kruskal-Wallis analyses of variance revealed significant differences in the two behavioral parameters observed ($H=16.5$, $P<0.001$ and $H=16.0$, $P<0.001$, locomotion and rearing respectively). Mann-Whitney *U*-test revealed that exercised rats (EC) displayed a reduced number of crossings and rearings, when compared to the sedentary group

(SC). Reserpine treatment caused a marked reduction in crossing and rearing in both sedentary (SR) and exercised (ER) rats (Figure 1a and 1b).

Orofacial movements: The effects of reserpine administration on orofacial movements are shown in Fig.2. For VCM and FT, two way ANOVA showed only a significant main effect of reserpine [$F(1,16)=116.7$; $P<0.001$] and [$F(1,16)=147.77$; $P<0.001$], respectively. Univariate analysis, followed by Duncan's multiple range test, revealed that sedentary and exercised rats treated with reserpine (SR and ER) displayed an increase in VCM and FT when compared to control groups (SC and EC). In fact, intense exercise did not modify the effect of reserpine administration (Figure 2a and 2b).

Biochemical analysis:

Two way ANOVA revealed a significant main effect of heavy exercise on catalase activity [$F(1,16)=8.21$, $P<0.05$]. Univariate ANOVA followed by Duncan's multiple range test revealed that co-treatment of reserpine and exercise (ER) significantly increased catalase activity when compared to the rat exercise-control group (EC) (Table 2)

Two way ANOVA of striatal TBARS levels revealed a significant main effect of heavy exercise [$F(1,16)=45.98$; $P<0.001$], reserpine treatment [$F(1,16)=40.76$; $P<0.001$] and interaction of exercise and reserpine [$F(2,16)=19.16$; $P<0.001$). Post hoc comparisons by Duncan's multiple range test indicated a significant difference in TBARS levels in the exercise-reserpine treated rats (ER) when compared to the other groups (SR and EC) (Table 2).

Regression analysis revealed a significant positive correlation between vacuous chewing frequency ($r=0.56$, $P<0.05$, Fig. 3a), catalase activity ($r=0.52$, $P<0.05$, Fig. 3b) and TBARS in striatum of rats.

4- Discussion

Physical exercise, as a stimulus, is dependent on the time of day, frequency (how many times a day, or a week) and content (aerobic, weight bearing and so on), and these variations have an influence on brain functions [40] leading to various disturbances, including emotional stress, which can be measured by behavioral, biochemical and genetic modifications [64]. Disturbances of emotionality were considered in our study through behavioral evaluation in an open-field arena, which has been used by other laboratories to study emotional and anxiolytic effects [19,37,80]. The results presented here showed that animals submitted to intense exercise program were more emotional (a less exploratory behavior) than the other groups, indicating that the exercise intensity employed was strong enough to provoke the development of emotional stress. Interestingly, we did not observe variation in the body weight of the exercised animals at the end of the experiment, which may indicate a physiological adaptation to this kind of exercise.

According to Kiraly & Kiraly [39], exercise is a major protective factor against neurodegeneration of various etiologies. Regular physical training of moderate intensity can have beneficial effects against senile dementia, Parkinson's and Alzheimer's disease [10,65,73,77] as well as in status epilepticus [20,68] and can induce resistance to OS [7,35]. In accordance with these findings, recently the beneficial effects of chronic physical exercise (moderate intensity) were shown in an OS animal model, suggesting beneficial effects in neurological diseases associated with movements [79]. After behavioral observations, Howells et al. [32] confirmed that voluntary exercise can exert neuroprotective effects, but also that mild stressors cancel this afforded neuroprotection. According to Kandel et al. [38], any kind of stress can increase the release of norepinephrine, DA, glutamate, epinephrine and corticotrophin releasing factor in certain areas of the brain and peripheral circulation. More specifically, DA and glutamate have the capacity to be neurotoxic during prolonged stress as they act synergistically to promote neuronal loss [71,72].

Freed and Yamamoto [23] considered dopaminergic neurons to play important roles during motor activation. In line with this, an increase in dopamine metabolism was observed in several brain regions of rats during physical activity [46]. Theoretically, exhaustive exercise may increase the brain's synthesis of DA, whose metabolites, formed by autoxidation or MAO action, could form ROS [77]. Fortifying this hypothesis, some studies have indicated an increase in lipid peroxidation in different brain regions after acute exercise [33,74].

Recently, Nybo and Secher [49] discussed the effects of strenuous exercise and their relation with homeostatic disturbances, showing important consequences on the cardiorespiratory, locomotive and mainly central nervous systems. Therefore, in view of the sometimes conflicting data found in the literature, our group was motivated to study the influence of intense exercise and its relationship to OS in the CNS.

Of particular interest for the animal model chosen here, different laboratories have associated OS with neurodegeneration and movement disorders [14,47,63], while searching for antioxidant therapeutic agents [2,13,22,61,70]. In fact, Sussman et al. [76] showed that reserpine administration causes long-term spontaneous orofacial dyskinesia (OD) in rats by decreasing striatal DA levels and increasing DA metabolite ratios (DOPAC/DA and HVA/DA).

In a current study, moderate chronic exercise displayed a beneficial effect by partially preventing the increase in FT [79]. In this experiment, we observed that the reserpine treatment (used as an OS inductor) increased VCM and FT and that intense physical activity did not alter these parameters. The exercise model employed here was not able to change either catalase activity or TBARS striatal levels, but when associated with the OS inductor (reserpine), increased these parameters. The harmful effect of the association was confirmed through the positive correlation between OD development (VCM) and lipid peroxidation (TBARS determination). Similarly, the positive correlation between lipid peroxidation (TBARS) and catalase activity observed in our study may be a compensatory response or a signaling mechanism of oxidative damage, since this enzyme exerts a critical role in the development of oral dyskinesia and OS [3,22]. In fact, different researchers have submitted rats to moderate chronic exercise and observed an increase of enzymatic activity, including catalase activity, in cerebral and other tissues,

relating this effect to an increase of antioxidant defenses [18,29,41,75]. In our study, the rats that were submitted chronically to an intense exercise program and co-treated with reserpine, presented an increase of striatal catalase activity, suggesting OS development. In line with our findings, Margonis et al. [44] explained a similar increase of catalase activity by a compensatory mechanism to scavenge hydrogen peroxide in times of greater demand (catalase increased only when overtraining and peak oxidative stress levels occurred).

In conclusion, the results found here reinforce the relationship between the generation of oxidative stress and a compensatory response toward aggressor agents, which was more evident for the association of chronic intense exercise and reserpine treatment. Further studies are necessary considering the activity of other enzymes and endogenous antioxidants, as well as the relationship between physical training, the release of dopamine and its effects on emotional stress.

Legends

Figure 1 – Effects of reserpine administration (1.0 mg/kg sc every other day, for 3 days) or vehicle on sedentary rats (SR and SC, respectively) and rats submitted to intense exercise (ER) or (EC) on open-field rearing frequency (a) and locomotion frequency (b). Data are reported as mean ± S.E.M. Analysis of variance followed by Mann-Whitney Test. *Indicates a significant difference from control group (SC) for $P<0.05$, $^{+}$indicates a significant difference from exercised rats (EC) for $P<0.05$.

Figure 2 - Effects of reserpine administration (1.0 mg/kg sc every other day, for 3 days) (SR) or vehicle (SC) on sedentary rats and rats submitted to intense exercise (ER) or (EC) on vacuous chewing frequency (a) and facial twitching duration (b). Data (mean±SEM) were analyzed by two-way analysis of variance followed by Duncan's test. *Indicates a significant difference from control group (SC), $^{+}$indicates a significant difference from exercised rats (EC) for $P<0.001$.

Figure 3 - Linear regression analysis between vacuous chewing frequency, catalase activity and lipid peroxidation (TBARS) in striatum of rats treated with reserpine (1.0 mg/kg sc) following 11 weeks of intense exercise (Statistical analysis revealed the following *P* significance levels for the *r* values: 0.56 and 0.52 respectively).

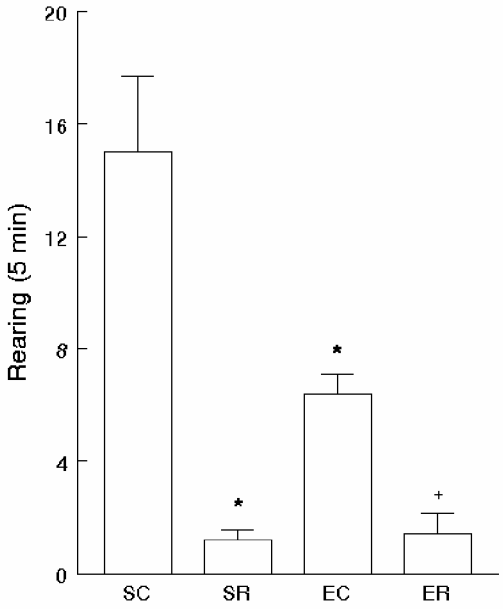

Fig. 1a

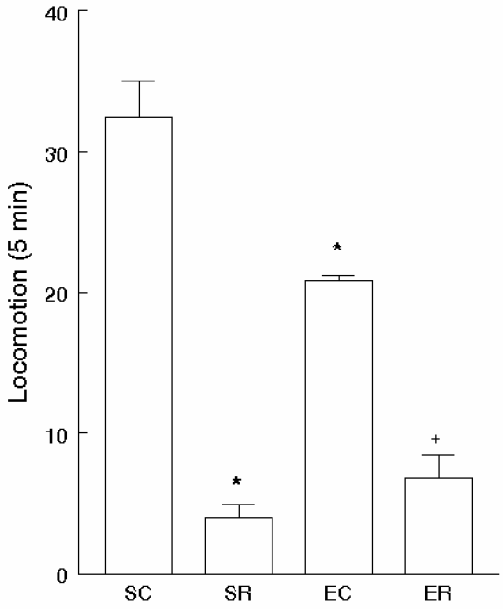

Fig. 1b

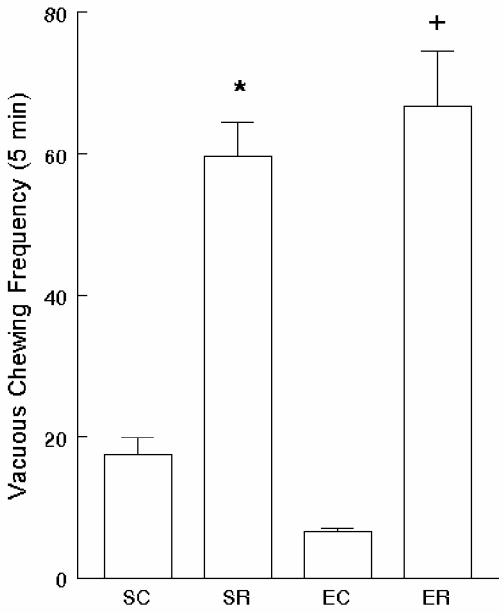

Fig. 2a

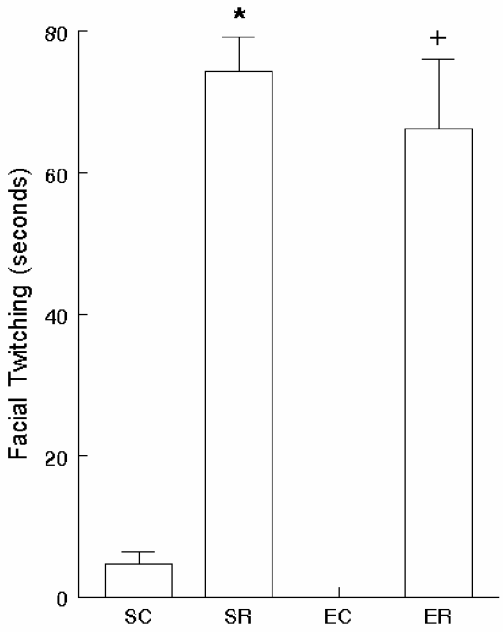

Fig. 2b

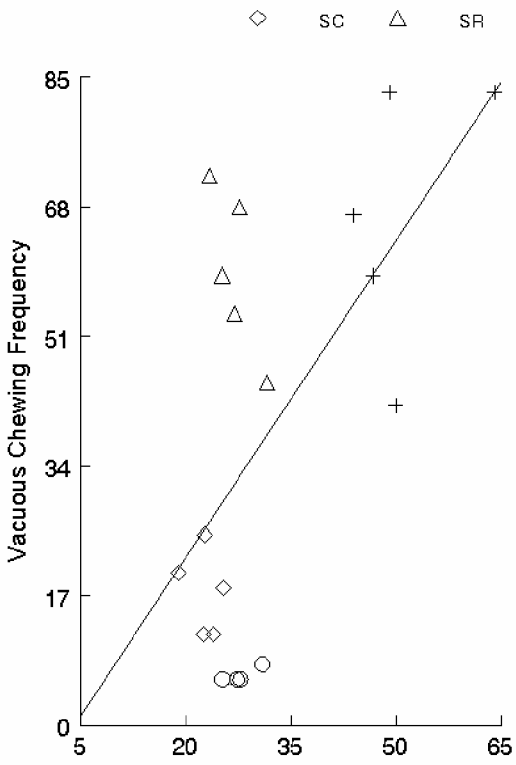

Figure 3a

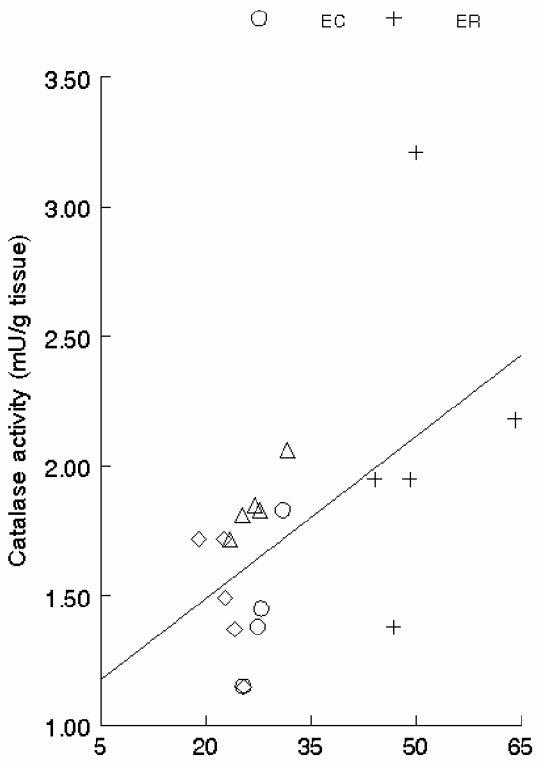

Figure 3b

TBARS striatum (nmol MDA / g tissue)

Table 1: Verification of training: Mean values of heart weight, final body weight, heart weight/body weight ratios and blood lactate levels.

Group	Heart Wt. (mg)	Body Wt. (g)	***Ratio (mg/g)***	***Lactate (mmol/L)***
S	997.80±60.8	353.80±25.6	2.83±0.11	5.05±0.17
E	1317.60±55.4*	385.80±12.1	3.42±0.15 **	3.94±0.2*

Data (mean ± S.E.M.) were analyzed by one-way analysis of variance followed by Duncan's test.

* ($P<0.01$) and **($P<0.05$) difference from sedentary-control group (SC)

Table 2- Catalase activity (mU/g tissue) and TBARS (nmol MDA/g tissue) in rat striatum after intense exercise.

	SC n=5	SR n=5	EC n=5	ER n=5
Catalase activity	1.49±0.1	1.85±0.1	1.45±0.1	2.13±0.3^{+}
TBARS	22.75±1.0	27.04±1.3	27.88±1.1	50.86±3.0$^{++\#}$

Values are means ± S.E.M., $^{+}p<0.05$, $^{++}p<0.001$ difference from exercised-control group (EC); $^{\#}p<0.001$ difference from sedentary-reserpine (SR) (Duncan's multiple range test).

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4. DISCUSSÃO E CONCLUSÃO FINAL

Modelos animais de discinesia orofacial (DO) têm sido empregados no estudo de novos compostos que possam aliviar sintomas e/ou prevenir o desenvolvimento da discinesia tardia (DT) humana, e também no conhecimento da fisiopatologia da síndrome e de outras doenças neurodegenerativas associadas ao EO (Abílio e cols., 2002, 2003; Burger e cols. 2003, 2005; Faria e cols., 2005). A reserpina, como um indutor de DO em animais, é capaz de desenvolver EO através de múltiplos mecanismos, o que resulta na elevação da formação de metabólitos associados à geração de radicais livres e conseqüentemente ao processo oxidativo (Sussman e cols., 1997; Paris e cols., 2001; Dutra e cols., 2002; Bilska & Dubiel, 2007).

No **Artigo 1,** demonstramos os efeitos benéficos do exercício físico regular de intensidade moderada sobre este modelo animal de estresse oxidativo induzido por reserpina, observado através do desenvolvimento de DO.

No **Artigo 2**, associamos duas fontes deletérias: o exercício físico intenso, desenvolvendo um estresse emocional e a administração de reserpina, desenvolvendo o estresse oxidativo. A atividade física intensa modificou parâmetros de comportamento locomotor (atividade exploratória), sugerindo o desenvolvimento de estresse emocional. Tem sido descrito que alterações emocionais tornam os animais mais tímidos quando expostos a um ambiente diferente, fazendo com que o medo se sobreponha ao seu comportamento exploratório natural (Tharp & Carson, 1975). Recentemente, Howells e cols. (2005) demonstraram através de um modelo animal para a doença de Parkinson, que o estresse emocional reduz o efeito neuroprotetor do exercício, e é capaz de alterar a secreção de diferentes neurotransmissores e hormônios em determinadas regiões do cérebro e na circulação periférica (Kandel e cols., 2000). De acordo com Smith e cols. (2002) estas alterações, principalmente as provocadas por DA e glutamato, podem exercer efeitos benéficos sobre regiões cerebrais como o estriado. No entanto, quando este estresse se torna crônico, pode atuar sinergicamente na promoção da perda neuronal através da neurotoxicidade.

A geração de RL através dos processos fisiológicos ou exógenos (incluindo a reserpina empregada nos experimentos, **Artigos 1 e 2**), pode desencadear cascatas de peroxidação lipídica e processos oxidativos que nem sempre o organismo consegue controlar. O sistema enzimático faz parte do mecanismo de defesa dos seres vivos contra os danos causados por RL, agindo através da eliminação dessas espécies reativas ou convertendo-as em substâncias menos tóxicas.

Os resultados obtidos no **Artigo 1** demonstram que a administração de reserpina foi capaz de aumentar a atividade da catalase estriatal, relacionada diretamente ao desenvolvimento de DO, e o exercício moderado não modificou a atividade desta enzima. Com relação ao exercício intenso **(Artigo 2),** foi observado um aumento na atividade da catalase somente com o grupo de animais que recebeu administração de reserpina, mostrando uma resposta sinérgica entre os dois agentes agressores: o estresse emocional do exercício intenso e o estresse oxidativo da reserpina, demonstrado através dos níveis elevados de peroxidação lipídica. A importância crítica da catalase no desenvolvimento de EO já foi anteriormente descrita por diferentes pesquisadores (Abílio e cols. 2004; Faria e cols. 2005), assim como sua resposta compensatória ou mecanismo de sinalização de dano oxidativo (Gómez-Cabrera e cols., 2006).

Neste trabalho, observamos que a administração de reserpina reduziu os níveis de GSH no estriado, demonstrando uma correlação negativa com o desenvolvimento de movimentos orofaciais. O exercício físico moderado *per se* também reduziu os níveis estriatais de GSH, entretanto, quando associado à administração de reserpina foi capaz de reverter parcialmente este parâmetro, acompanhado da reversão parcial da discinesia orofacial (**Artigo 1**). Existem evidências de que um descontrole na produção de RL pode contribuir para o desenvolvimento de DT humana e outras desordens do movimento como distonias e parkinsonismo (Cadet & Kahler, 1994). Este efeito deletério pode estar relacionado, em parte, a uma diminuição dos mecanismos antioxidantes endógenos, entre eles, a diminuição dos níveis de GSH (Shivakumar & Ravindranath, 1993).

Tem sido evidenciado que o exercício regular está associado à redução do risco de uma diversidade de doenças incluindo cardiovasculares, câncer de mama e cólon, obesidade, diabetes tipo II e depressão (Polidori e cols., 2000; Dishman e cols, 2006). Estudos prospectivos têm

encontrado uma menor probabilidade para o desenvolvimento de Alzheimer e outras formas de demência em indivíduos mais ativos fisicamente (Laurin e cols., 2001; Podewils e cols., 2005; Larson e cols. 2006). Além disto, a atividade física mostrou melhora da memória recente (Hurwitz, 1989) e da coordenação motora e tremores relacionados à doença de Parkinson (Palmer e cols., 1986).

Mesmo com o reconhecimento dos efeitos vantajosos do exercício físico sobre funções fisiológicas e neurológicas, esta atividade também está associada ao aumento de consumo de oxigênio molecular, elevando a geração de RL (Heunks e cols., 1999; Viña e cols., 2000). Este evento se reflete na elevação dos níveis de substâncias reativas ao ácido tiobarbitúrico (TBARS) e glutationa oxidada (GSSG) (Laaksonen e cols., 1999) e pode ser responsável por danos oxidativos (Alessio & Goldfarb, 1988; Sen, 1995). Alessio e cols. (2000) demonstraram que o exercício exaustivo provocou EO observado através da elevação de hidroperóxidos lipídicos, carbonilação de proteínas e da capacidade de absorbância do radical oxigênio na circulação sangüínea. Neste sentido, os resultados apresentados no **Artigo 2** mostram uma elevada produção de TBARS estriatal, como conseqüência do exercício intenso e do tratamento com reserpina, assim como uma correlação positiva entre os níveis de TBARS e movimentos orofaciais (movimentos de mascar vazios-MMV), confirmando os efeitos deletérios do exercício excessivo sobre este modelo animal de estresse oxidativo aqui proposto.

Analisados em conjunto, os resultados encontrados nos **Artigos 1** e **2** fortalecem a relação entre EO e neurodegeneração e fortalecem o emprego de reserpina como um modelo experimental de EO cerebral. Além disto, o avanço dos estudos e o conseqüente conhecimento da fisiopatologia das doenças neurodegenerativas conduzem à busca de mecanismos que possam tornar estes processos lentos, ou mesmo, evitá-los. A atividade física quando bem empregada, pode contribuir com o tratamento farmacológico clássico e servir como uma terapêutica alternativa, acompanhada por profissionais qualificados e integrados, formando uma equipe de saúde completa, preocupada não só com a saúde dos indivíduos, mas com a qualidade de vida dos mesmos.

5. PERSPECTIVAS

Com base nos resultados obtidos nesta dissertação, tem-se por objetivos posteriores:

1. Investigar diferentes modalidades e intensidades de exercício físico, em busca da minimização de estresse emocional dos animais, observando também as defesas antioxidantes sobre diferentes órgãos e tecidos;

2. Estudar e comparar as respostas de outros indutores de estresse oxidativo no SNC, em comparação ao modelo induzido com reserpina;

3. Avaliar o papel da dopamina estriatal, através de modelos animais de distúrbios do movimento provocados por estresse oxidativo, e seus possíveis benefícios com a atividade física estudada no item 1;

4. Associar ao modelo de exercício (1) outras fontes de proteção contra os danos oxidativos como, por exemplo, plantas medicinais com potencial antioxidante;

5. Avaliar os efeitos desta atividade física (1) e /ou plantas (4) sobre parâmetros de estresse oxidativo em diferentes regiões do cérebro e outros tecidos, em diferentes fases do desenvolvimento de animais.

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