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AVALIAÇÃO DOS EFEITOS DE DROGAS ANTI-ADENOSINÉRGICAS E DO DISSELENETO DE DIFENILA NO PREJUÍZO DA MEMÓRIA E ESTRESSE OXIDATIVO RELACIONADOS AO ENVELHECIMENTO

Dissertação de Mestrado

Marlon Régis Leite

Santa Maria, RS, Brasil

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AVALIAÇÃO DOS EFEITOS DE DROGAS ANTI-ADENOSINÉRGICAS E DO DISSELENETO DE DIFENILA NO PREJUÍZO DA MEMÓRIA E ESTRESSE OXIDATIVO RELACIONADOS AO ENVELHECIMENTO

por

Marlon Régis Leite

Dissertação apresentada ao Programa de Pós Graduação em Ciências Biológicas, Área de Concentração em Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Mestre em Bioquímica Toxicológica**

Orientador: Prof º . Dr º. Gilson Zeni

Co-Orientador: Prof º. Dr º. Ricardo Brandão

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Universidade Federal de Santa Maria
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A Comissão Examinadora, abaixo assinada, aprova a Dissertação de Mestrado

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

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Dedico este trabalho a minha mãe, irmãs e ao meu pai e avó, aos quais sou eternamente grato por tudo que fizeram e ainda fazem por mim.

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RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

AVALIAÇÃO DOS EFEITOS DE DROGAS ANTI-ADENOSINÉRGICAS E DO DISSELENETO DE DIFENILA NO PREJUÍZO DA MEMÓRIA E ESTRESSE OXIDATIVO RELACIONADOS AO ENVELHECIMENTO

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Data e Local da Defesa: Santa Maria, 2012

O envelhecimento biológico é o declínio gradual e progressivo das funções físicas que começam com o nascimento e terminam com a morte em todas as espécies animais. Uma das características mais comuns do envelhecimento é a redução da capacidade de aprender e lembrar novas informações. Mudanças relacionadas à idade nos fundamentos neurais dos processos de memória estão intimamente ligadas ao estresse oxidativo. Neste estudo, os efeitos da cafeína e do SCH58261, antagonistas de receptores de adenosina, no prejuízo da memória e estresse oxidativo influenciado pela idade em ratos foram investigados. Ratos jovens (3 meses) e velhos (23 meses) foram tratados diariamente por 10 dias com cafeína (30 mg/kg p.o.) ou SCH58261 (0,5 mg/kg, p.o.). O teste utilizado para avaliar a memória dos animais foi o teste do reconhecimento do objeto novo (**artigo 1)**. Após o teste comportamental, os níveis de acido ascórbico e espécies reativas de oxigênio e nitrogênio, bem como a atividade da Na^+K^+ ATPase foram determinados em amostras de cérebro dos animais. Os resultados mostraram que o déficit de memória induzido pela idade foi revertido pelo tratamento com cafeína ou SCH58261 na memória de longo prazo além de melhorar a memória de curto prazo. Os animais jovens também apresentaram melhora da memória de curto e longo prazo quando tratados com cafeína e SCH58261. Além disso, os níveis aumentados de espécies reativas de oxigênio e nitrogênio no cérebro dos ratos velhos foram revertidos por ambas cafeína e SCH58261. Entretanto os níveis de acido ascórbico, diminuídos no cérebro de ratos velhos, não foram mudados pela cafeína ou SCH58261. A atividade da Na^+K^+ ATPase inibida no cérebro dos animais velhos também foi normalizada pela cafeína e SCH58261. Uma vez que a cafeína melhorou a memória de ratos velhos e levando-se em consideração que muitos compostos orgânicos de selênio apresentam propriedades antioxidantes e neuroprotetoras, também foi investigado se o composto disseleneto de difenila $(PhSe)_2$ seria capaz de melhorar o prejuízo da memória em ratos de meia idade (18 meses) em um modelo de suplementação juntamente com a cafeína. Ratos de meia idade foram tratados pelo período de 30 dias com ambos $(PhSe)_2$ (10 p.p.m. na ração) e cafeína (15 mg/kg, o.p.) ou somente com $(PhSe)_2$ ou cafeína. A memória dos animais foi avaliada no teste do reconhecimento do objeto novo (**manuscrito 1**). Os resultados demonstraram uma melhora na memória de curta duração nos animais tratados somente com $(PhSe)_2$. Entretanto a memória de longa duração foi melhorada apenas nos animais tratados com ambos $(PhSe)_2$ e cafeína. Além disso, os tratamentos melhoraram a atividade locomotora e exploratória. Assim, estes dois estudos sugerem que tanto os antagonistas de receptores adenosinérgicos, quanto o $(PhSe)_2$ são capazes de melhorar o prejuízo da memória influenciado pelo envelhecimento.

Palavras chave: envelhecimento, memória, antioxidante, cafeína, adenosina, selênio

ABSTRACT

Dissertation of Master's Degree
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AVALUATION OF THE EFFCTS OF ANTI-ADENOSINERGIC DRUGS AND DIPHENYL DISELENIDE ON MEMORY IMPAIRMENT AND OXIDATIVE STRESS RELATED TO AGING

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Date and Place of the defense: Santa Maria, 2012

Biological aging is gradual and progressive decline of physical functions that begin in adulthood and ends with death in almost all animals species. One of the most common features of aging is the reduced ability to learn and remember new information. Age-related changes in the neural basis of memory processes are closely linked to oxidative stress. Here, the effects of caffeine caffeine and SCH58261, an A_{2A} receptor antagonist, in the memory impairment and oxidative stress influenced by age in rats were investigated. Young and old rats were treated daily for 10 days with caffeine (30 mg/kg, p.o.) or SCH58261 (0,5 mg/kg, p.o.). The test used to evaluate the memory of animals was the novel object recognition task (**article 1**). After behavioral tasks, ascorbic acid and oxygen and nitrogen reactive species levels as well as Na^+K^+ ATPase activity were determined in rat brain. The results showed that memory deficit influenced by age was reversed by treatment with caffeine or SCH58261 besides improving short-term memory. Young animals also showed improved memory for short and long term when treated with caffeine and SCH58261.Furthermore, increased oxygen and nitrogen reactive species levels in brain of old rats were normalized by both caffeine and SCH58261. However, ascorbic acid levels decreased in brain of old rats were not changed by CAF or SCH58261. The inhibition Na^+K^+ ATPase activity in brain of old rats was also normalized by caffeine and SCH58261. Since caffeine ameliorates memory of aged rats and taking into account that organoselenium compounds have antioxidant and neuroprotective properties, it was also investigated whether diphenyl diselenide $(PhSe)_2$ compound could improve memory impairment in middle-aged rats in a model of supplementation with caffeine. Middle-aged rats were treated for 30 days with both $(PhSe)_2$ (10 p.p.m. in feed) and caffeiene (15 mg/kg, o.p.) or only $(PhSe)_2$ or caffeine. Memory of animals was evaluated in the novel object recognition task (**manuscript 1**). The results showed an improvement in the short-term memory in animals treated only with $(PhSe)_2$. The long-term memory was improved only in animals treated with both $(PhSe)_2$ and caffeine. Moreover, treatments affected locomotor and exploratory activity. Thus, this study suggests that both antagonists of adenosinergic receptor, and $(PhSe)_2$ were effective in ameliorating memory impairment influenced by aging.

Keywords: aging, memory, antioxidant, caffeine, adenosine, selenium

LISTA DE FIGURAS

INTRODUÇÃO

LISTA DE ABREVIATURAS

AGEs - produtos de glicação avançada

DNA - ácido desoxirribonucléico

CREB - elemento ligante de resposta ao AMPc

EROS - espécies reativas de oxigênio

LTM - memória de longo prazo

MAPK - proteína quinase ativada por mitógeno

Na^+K^+ATPase - sódio potássio ATPase

$(PhSe)_2$ - disseleneto de difenila

PKA - proteína quinase

RNA - ácido ribonucléico

RAs - receptores de adenosina

SCH58261- 2-(2-Furanil)-7-(2-feniletil)-7H-pirazolo[4,3-*e*][1,,2,4]triazolo[1,5-*c*]pirimidina-5-amina

SNC - sistema nervoso central

STM - memória de curto prazo

SUMÁRIO

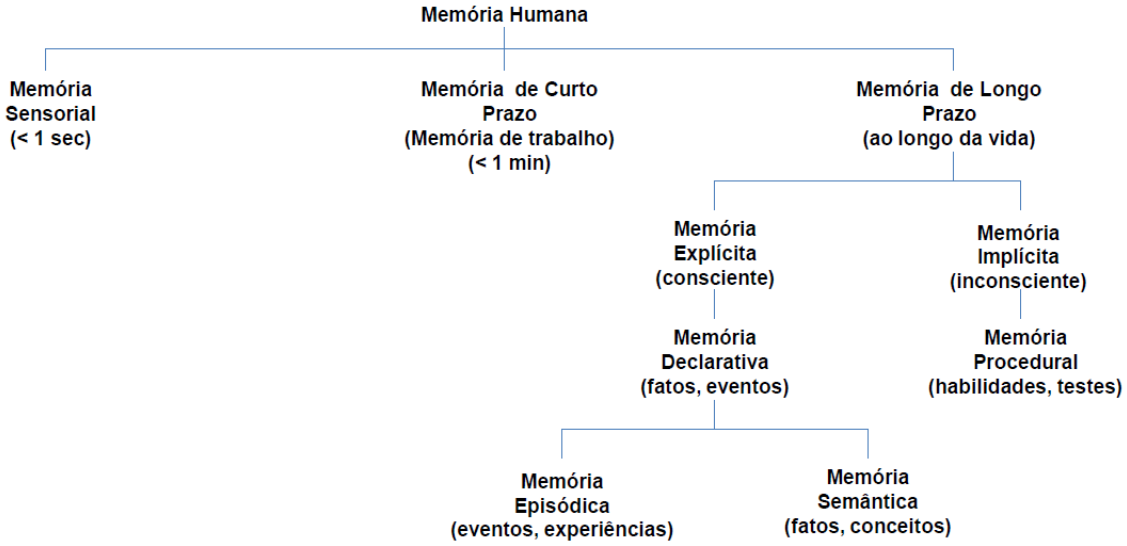
1. INTRODUÇÃO

O funcionamento preciso do sistema nervoso é dependente da exatidão com que as informações são recebidas, processadas e respondidas. Durante o desenvolvimento há o surgimento de um complexo sistema de circuitos neurais que se conectam de maneira precisa para que ocorra o processamento de informações de forma adequada (Heiduschka e Thanos, 2000). É através de uma série de modificações no meio onde as células nervosas estão inseridas bem como nas conexões estabelecidas entre elas, que há a estabilização seletiva das sinapses, gerando uma rede neural funcional. O envelhecimento é associado com o declínio de várias propriedades globais do cérebro, ambas estruturais e funcionais, influenciando de forma significante no desempenho cognitivo (Esiri, 2007).

Entre os processos cognitivos esta a memória, sendo esta a conseqüência usual do aprendizado que reflete as mudanças no sistema nervoso central (SNC), resultado de experiências transitórias (Atkins et. al. 1998). Acredita-se que modificações sinápticas, ambas positivas e negativas, distribuídas através de milhares a milhões de conexões entre os neurônios, são responsáveis por formar substratos físicos e bioquímicos necessários para memória e aprendizado (Squire, 1987; Kendler, 2001;). A deterioração do sistema de circuitos neurais e suas conexões com a idade ocorrem de maneira gradual ocasionando o declínio da memória.

1.1. Memória

A memória consiste em um conjunto de procedimentos que permite manipular e compreender o mundo, levando em conta o contexto atual e as experiências individuais. Esses procedimentos envolvem mecanismos de aquisição, retenção e evocação. A primeira tentativa abrangente de dividir a memória humana em diferentes sistemas foi proposta por Atkinson e Shiffrin (1968) e passou a ser conhecida como o "Modelo de Memória Modal." O Modelo Modal postulou três grandes grupos de sistemas de memória: a memória sensorial, memória de curto prazo (STM), e memória de longo prazo (LTM), sendo estes responsáveis pela realização das operações de aquisição, retenção e evocação (Esquema 1).



Esquema 1: Modelo de memória modal postulado por Atkinson e Shiffrin

Memória sensorial

A memória sensorial funciona como um depósito de capacidade ilimitada, armazenando informações do ambiente através de entradas sensoriais de todos os órgãos do sentido. Estas informações sensoriais são armazenadas nos 'buffers' sensoriais que podem armazenar grande quantidade de informação. Ela consiste em uma memória de muito curto prazo, com duração de aproximadamente 0,1 a 0,5 segundos (Sperling, 1960; Haber e Standing, 1969).

Como a duração de armazenamento é curta, uma decisão deve ser tomada depressa sobre se a informação será transferida para o próximo sistema de memória para ser analisada ou será esquecida. Uma vez decidido qual informação será armazenada, esta informação codificada será transferida do armazenamento sensorial para a memória de curto prazo.

Memória de curto prazo

A memória de curto prazo, ou memória de trabalho, pode ser determinada como a capacidade de recordar e processar a informação ao mesmo tempo. Ela possui duas importantes características. Primeiro, ela contém um número limitado de elementos que podem ser retidos. Um estudo de George Miller (1956) sobre as limitações da memória de curto prazo demonstrou que uma pessoa pode reter 7±2 itens. A segunda característica é que a memória de curto prazo pode reter informações durante 15 a 30 segundos. Sendo esta duração de informação pequena, esta informação pode ser copiada ou pode ser transferida para a memória de longo prazo antes do termino deste período. Depois deste tempo a informação será perdida.

O conhecimento, ou a experiência do tipo de conteúdo, favorecem a passagem da informação da memória de curto prazo até a de longo prazo favorecendo a retenção prolongada de informações. A memória de curto prazo determina se a informação é útil para o organismo e deve ser armazenada. Uma vez que existem informações semelhantes nos arquivos de memória de longo prazo esta informação será descartada (Brown, 1964).

Memória de longo prazo

A memória de longo prazo tem o processo de formação de aquisição e consolidação, e pode durar minuto, horas, meses ou décadas. Ela permite a evocação de informações depois de décadas que esta foi armazenada, e os limites da sua capacidade são desconhecidos.

A memória de longo prazo é dividida em dois tipos principais: memória declarativa (ou explicita) e memória procedural (ou implícita) (Ullman, 2004).

Memória declarativa

A memória declarativa é a memória de fatos e acontecimentos, e refere-se a memórias que podem ser recordadas conscientemente. É chamada muitas vezes de memória explicita, uma vez que é a informação que é explicitamente armazenada e recuperada, embora seja mais propriamente um subconjunto da memória explicita A memória declarativa ainda pode ser subdividida em memória episódica e memória semântica (Eichenbaum, 1997).

A memória episódica representa nossa memória de experiências e acontecimentos específicos no tempo em forma de série, a partir do qual podemos reconstruir os fatos reais que ocorrem em um determinado ponto em nossas vidas. E a memória de eventos autobiográficos (tempos, lugares, emoções associadas e outros conhecimentos contextuais) que podem ser explicitamente declarados. A carga emocional e todo o contexto em torno de um evento geralmente é parte da memória, não apenas os fatos nus do evento em si (Tulving, 1984).

A memória semântica, por outro lado, é um registro mais estruturado dos fatos, significados, conceitos e conhecimento sobre o mundo externo que adquirimos. Refere-se ao conhecimento factual geral, compartilhada com os outros e independente da experiência pessoal e do contexto espacial / temporal em que foi adquirido. Por conseguinte, inclui coisas como os tipos de alimentos, capitais, costumes sociais, as funções dos objetos, o vocabulário, a compreensão da matemática, etc. Muito da memória semântica é abstrata e relacional e está associada com o significado dos símbolos verbais (Saumier and Chertkow, 2002).

Memória procedural

A memória procedural (ou de procedimentos) é a memória inconsciente de competências e de como fazer as coisas, nomeadamente a utilização de objetos ou

movimentos do corpo, tais como tocar um piano ou andar de bicicleta. Ela é composta por comportamentos sensório-motores automáticos, que estão tão profundamente internalizados que não se tem mais consciência deles, e uma vez aprendida, estas "memórias corporais" nos permitem realizar ações motoras comuns automaticamente. Refere-se à memória procedural muitas vezes como a memória implícita, porque ajuda experiências anteriores na execução de tarefas sem o conhecimento explicito e consciente dessas experiências anteriores, embora seja mais propriamente um subconjunto da memória implícita (Bullemer et al, 1989).

1.2. Envelhecimento do cérebro e memória

O envelhecimento tem sido longamente associado com decorrentes prejuízos no sistema nervoso central, sendo este o sistema biológico mais comprometido com o processo de envelhecimento. As alterações nos processos bioquímicos do cérebro relacionadas ao envelhecimento têm como conseqüência a ocorrência de mudanças tanto a nível macroscópico quanto microscópico. Tais modificações influenciam significativamente no desempenho da memória em uma ampla variedade de tarefas e situações (Esiri, 2007).

Mudanças macroscópicas e microscópicas do cérebro com a idade

Macroscópicamente, diversos estudos *pos mortem* no cérebro humano relatam que em adultos com idade entre 20 a 60 anos, ocorre uma pequena perda do peso do cérebro de cerca de 0,1%/ano, sendo esta perda ainda mais acentuada depois dos 60 anos (Miller et al.,1977; Dekaban et al., 1978; Ho et al., 1980; Blinkov et al., 1968).

Em 1970, quando foi introduzida a técnica de processamento de imagens não invasivas do cérebro, estabeleceu-se que o volume do cérebro realmente diminuia com a idade (Coffey et al., 1992; Jerningan et al. 1990; Pfefferbaum et al. 1994). Esta diminuição é relativamente difusa e uniforme na substância branca cerebral, mas mostra as mesmas diferenças regionais na substância cinzenta, com o córtex frontal e parietal mais afetados que o córtex temporal e occipital, além do estriado também ser afetado (Trollor e Valenzuela, 2001; Resnick et al., 2003; Scahill et al., 2003; Raz, 2004;) bem como o hipocampo (Ball et al. 1977). Além disso, como conseqüência da redução do volume do cérebro, o sistema ventricular expande-se para preencher o espaço vago gerado por esta redução.

Cerca de duas décadas antes do advento de técnicas que permitiram o monitoramento do cérebro com maior precisão, Brody (1955) sugeriu que as reduções relacionadas com a idade no peso do cérebro eram devidas, em parte, a um declínio no número de neurônios corticais, refletindo assim em alterações de caráter microscópico. Investigações posteriores

corroboraram com esta idéia, relatando um declínio de 10 a 60% na densidade de neurônios corticais entre a infância tardia e a velhice (Coleman e Flood, 1987). Entretanto, algumas populações foram indentificadas por não apresentar perda de neurônios com a idade (Esiri, 1994). Além disso, estimar com exatidão a magnitude da perda neural em humanos por ação do envelhecimento normal é complicado, pois o cérebro de individuos idosos com idade superior a 80 anos são afetados por alterações patológicas como formação de placas amilóides e emaranhados neurofibrilares, dois marcadores, quando presentes em número considerável, de doença de Alzheimer. Além disso, também são afetados por doença cérebrovascular (Hunter et al., 2012)

Embora os estudos a cerca do número de neurônios apresentem resultados controversos, há um acordo maior com relação ao tamanho dos neurônios. Aparentemente, há uma modesta diminuição dos neurônios com a idade, particularmente no córtex cerebral (Meier-Ruge et al., 1980; Buell et al., 1981; Anderson et al., 1983; Haugh, 1985; Terry et al., 1987). Acredita-se que o tamanho do neurônio reflete a extensão das arborizações dentríticas e axonais da célula. Assim, estudos de sinapses, localizados principalmente , mas não exclusivamente, nas espinhas dentriticas do córtex cerebral demonstraram reduções globais com a idade, embora os padrões de ramificação dos dentritos sugira que pode haver aumentos compensátórios em alguns dentritos para compensar a perda de outros (Buell et al., 1981). Também é observado aumento do número e tamanho dos astrócitos e micróglias. Microglias são geralmente cosideradas como patogênicas quando ativadas, mas podem também ser neuroprotetoras em algumas circunstâncias (Esiri, 2007).

Envelhecimento do cérebro e estresse oxidativo

Um fator chave no envelhecimento cerebral, é a grande demanda que os neurônios têm pelo metabolismo oxidativo na geração de energia. A atividade mitocondrial necessária para o metabolismo oxidativo com a conseqüente produção de energia, envolve a geração inevitável de espécies reativas de oxigênio (EROS). O ânion superóxido é produzido e leva a geração de peróxido de hidrogênio, que por meio da reação de Fenton forma o radical hidroxil (Zecca et al., 2004). O excesso destas moléculas leva as mitocondrias a sofrer dano oxidativo, o que pode torna-las menos eficientes na geração de energia e suscetiveis a gerar mais ânions superóxido, desencadeando o processo de estresse oxidativo. A diminuição da eficiência das mitocondrias com o envelhecimento (Xiong et al., 2002), e seus efeitos em cadeia no aumento do estresse oxidativo nos neurônios, está intimamente ligado a mudanças na homeostase do cálcio (Thibault et al., 2001).

Cada uma das principais classes de moléculas celulares, incluindo proteínas, ácidos nucléicos e lipídios é oxidativamente modificada durante o envelhecimento do cérebro por intermédio de EROS. Modificações de proteínas incluem a formação de carbonil (Dubey et al. 2006; Butterfield et al., 1997; Cakatay et al., 2001); modificação covalente de residuos de cisteína, lisina e histidina pelo produto da peroxidação lipídica 4-hidroxinonenal (Papaionnou et al., 2001; Pedersen et al., 1998; 2000) nitração da tirosina (Sloane et al., 1999) e glicação (Munch et al., 1997). O DNA e RNA estão sujeitos a modificação oxidativa, sendo a formação de 8-hidroxi-2-deoxiguanosina um exemplo proeminente (Shimura et al., 2001). Danos ao DNA conduzem a expressão reduzida de genes ou a geração de proteínas anormais que devem ser eliminadas por processos tais como a degradação proteossomal. A formação de isoformas com conformação estrutural diferente da proteína original, tem sido considerada como a causa de degenerações no cérebro (Goyns, 2002). Um estudo do DNA obtido do cortéx frontal humano de individuos na faixa etária de 26 a 106 anos, descobriu que muitos gens, cerca de 4% dos 11000 genes estudados, apresentaram expressão reduzida após a idade de 40 anos. Estes genes estavam envolvidos na função sináptica e plasticidade celular (Lu et al., 2004). O cérebro possui um alto conteúdo de ácidos graxos insaturados. As ligações duplas nos lipídios de membrana são oxidados resultando na produção de uma variedade de peróxidos lipídicos e aldeídos (Mattson, 1998).

Produtos da oxidação de lipídios podem reagir com outras moléculas adicionais. A lipofuscina é um composto classicamente associado ao envelhecimento, tendo um aumento com a idade em muitos tecidos (Kato et al., 1998). Considera-se que sua formação ocorre pela reação de produtos de peroxidação lipídica com proteínas. Observa-se um aumento no conteúdo de lipofuscina nos neurônios. Seu acúmulo, como um produto da degradação lisossomal, esta relacionado a autofagia (Mann et al., 1978).

Além disso, lipídeos oxidados ou açucares redutores também podem formar produtos de glicação avançada (AGEs) a partir da interação com proteínas, aminofosfolipídios ou ácidos nucléicos (Monnier, 2003). Tem sido relatado o acúmulo de AGEs com a idade em muitos tecidos, incluindo o cérebro (Kimura et al., 1996; 1998). Os efeitos patológicos dos AGEs está relacionado a capacidade destes compostos de modificar as propriedades químicas e funcionais das mais diversas estruturas biológicas. Por meio da geração de radicais livres, de ligações cruzadas com proteínas ou de interações com receptores celulares, os AGEs promovem, respectivamente, estresse oxidativo, alterações morfofuncionais e aumento da expressão de mediadores inflamatórios (Jakus et al., 2004; Ahmed, 2005; Bierhaus et al., 1998).

Por fim, as defesas antioxidantes no cérebro, responsáveis por combater o dano oxidativo, são relativamente baixas (Halliwell, 1996); tendo seus efeitos modificados por alterações em suas vias de sinalização ou menor produção com o envelhecimento (Mattson et al., 2004).

1.3. Antagonistas de receptores de adenosina e prejuízo da memória ocasionado pelo envelhecimento

A adenosina atua como um modulador citoprotetor em resposta ao estresse de órgãos ou tecidos (Fredholm et al. 2001; Haskó et al. 2008; Jacobson e Gao, 2006), ativando quatro subtipos de receptores acoplados a proteína G (RAs): A_1, A_{2A}, A_{2B}, e A_3 (Fredholm e Jacobson, 2009). Para cada um dos quatro subtipos de RA, agonistas e antagonistas seletivos foram estudados e usados para desenvolver novos conceitos de drogas terapêuticas.

Por outro lado, a adenosina, através de RA A_1, inibe a plasticidade sináptica de longo prazo, tais como a potenciação de longa duração (de Mendonça e Ribeiro, 1994) e a depressão de longa duração (LTD) (de Mendonça et al., 1997), fenômenos caracterizados por aumento ou redução na eficácia da comunicação sináptica, respectivamente, sendo os principais correlatos moleculares dos processos de aprendizado e memória (Bliss e Collingridge, 1993).

A cafeína, uma metilxantina, desencadeia seus efeitos no SNC principalmente por sua ação antagonista de RA A_1 e A_{2A} (Fredholm e Jacobson 2009). Os efeitos cognitivos da cafeína são principalmente devido à sua capacidade de antagonizar RA A_1 no hipocampo e no córtex, áreas do cérebro principalmente envolvidas na cognição (Fredholm et al. 1999). As ações positivas da cafeína sobre o processamento da informação e o desempenho também podem ser atribuídas a melhorias nas rotinas de comportamento, aumento da excitação e acoplamento sensório-motor. Realmente, a habilidade da cafeína para prevenir o prejuízo da memória tem sido relatada (Mendonça e Cunha, 2010; Nehling et al., 2010; Ribeiro e Sebastião, 2010).

A redução da ativação de RA A_{2A} também pode ser relevante para melhorias cognitivas, uma vez que camundongos knock-out para RA A_{2A} melhoraram a memória de reconhecimento espacial (Wang et al., 2006). Aparentemente, a superexpressão de RA A_{2A} leva a déficits de memória (Giménez-Llort et al. De 2007). Além disso, Prediger et al. (2005) demonstraram que o antagonista RA A_{2A} SCH58261 foi capaz de prevenir o prejuízo da memória induzido pela proteína β amilóide. Isto é reforçado pelo fato da densidade de RA

A_{2A} ter um aumento significativo no córtex (Cunha et al. 1995) e hipocampo (Diógenes et al. 2007) de ratos velhos.

1.4. Selênio e memória

O selênio (Se) é um elemento traço essencial de importância biológica para saúde humana, particularmente em relação à resposta imune e prevenção ao câncer (Margaret, 2000). Como um constituinte de selenoproteínas, apresenta função estrutural e enzimática estando presente na forma de um resíduo de selenocisteína no sitio ativo de enzimas como glutationa peroxidase (GPx) (Forstrom et al., 1978), tioredoxina redutase (Holmgren, 1985) e 5´-deiodinase (Behne e Kyriakopoulos, 1990). Além disso, o Se desempenha um importante papel em diversas vias metabólicas, incluindo o metabolismo dos hormônios tireoidianos, a função imunológica, o crescimento celular e principalmente o sistema de defesa antioxidante (Stazi e Trinti, 2008). Níveis insuficientes de Se no cérebro tem efeitos potencialmente negativos sobre o seu funcionamento, podendo agravar a perda neuronal e disfunções subseqüentes aos estímulos endógenos ou exógenos, trauma e outras condições neurodegenerativas (Schweizer et al., 2004a) além de alterar a taxa de *turnover* de neurotransmissores (Castano et al., 1997).

Compostos orgânicos de selênio mostraram potencial farmacológico em roedores como efeitos antidepressivo (Savegnago et al., 2007; Brüning et al., 2011), anticonvulsivante (Prigol et al., 2009) bem como o de melhorar a cognição (Pinton et al., 2010; 2011).

O composto disseleneto de difenila $(PhSe)_2$, por exemplo, possui capacidade de transpor a barreira hematoencefálica (Prigol et al., 2010). Corroborando com este fato, demonstrou-se que o mesmo exibe atividade antioxidante *in vivo* (Burger et al., 2004) em modelos animais de neurodegeneração.

Com relação à cognição, o $(PhSe)_2$, administrado de forma aguda, melhorou o desempenho de camundongos no teste de reconhecimento do objeto (Rosa et al., 2003). Também foi demonstrado que tanto ratos quanto camundongos tratados com $(PhSe)_2$ apresentaram uma melhora cognitiva no teste do labirinto aquático de Morris (Stangherlin et al., 2008; Souza et al.,2010). Além disso, da Rocha et al. (2012) mostrou os efeitos do $(PhSe)_2$ na melhora da memória em um modelo de déficit cognitivo em ratas ovariectomizadas.

Uma vez que o envelhecimento é acompanhado pelo declínio cognitivo em um segmento importante da população e é o fator de risco primário para a doença de Alzheimer e outras

doenças neurodegenerativas, o desenvolvimento de novas estratégias que possibilitem a redução ou eliminação de declínios nas habilidades de memória que ocasionam a diminuição da qualidade de vida das pessoas torna-se necessário.

2. OBJETIVOS

2.1. Objetivo geral

Investigar os efeitos de antagonistas de RA e do $(PhSe)_2$ no déficit de memória e estresse oxidativo em ratos envelhecidos.

2.2. Objetivos específicos

Considerando os aspectos mencionados, os objetivos específicos deste estudo compreendem:

- Verificar os efeitos da administração aguda dos antagonistas de RA SCH58261 e cafeína no déficit de memória e dano oxidativo em ratos velhos.
- Avaliar os efeitos da administração crônica da cafeína associada à suplementação com disseleneto de difenila sobre o déficit de memória em ratos de meia idade.

3. ARTIGO E MANUSCRITO

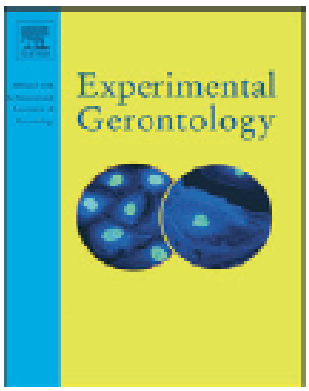
Os resultados que fazem parte dessa dissertação estão apresentados na forma de um artigo científico e de um manuscrito. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas do artigo e do manuscrito estão dispostos de acordo com a recomendação dos periódicos científicos aos quais estes foram enviados.

3.1 Artigo 1

Efeito protetor da cafeína e de um antagonista seletivo de receptor A_{2A} no prejuízo da memória e estresse oxidativo de ratos velhos

PROTECTIVE EFFECT OF CAFFEINE AND A SELECTIVE A_{2A} RECEPTOR ANTAGONIST ON IMPAIRMENT OF MEMORY AND OXIDATIVE STRESS OF AGED RATS

Marlon Régis Leite, Ethel A. Wilhelm, Cristiano R. Jesse, Ricardo Brandão, Cristina Wayne Nogueira*



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Protective effect of caffeine and a selective A_{2A} receptor antagonist on impairment of memory and oxidative stress of aged rats

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ABSTRACT

In this study, the effects of caffeine (CAF) and SCH58261, a selective A_{2A} receptor antagonist, on memory impairment and oxidative stress generated by aging in rats were investigated. Young and aged rats were treated daily per 10 days with CAF (30 mg/kg p.o.) or SCH58261 (0.5 mg/kg, p.o.) or vehicle (1 ml/kg p.o.). Rats were trained and tested in a novel object recognition task. After the behavioral test, ascorbic acid and oxygen and nitrogen reactive species levels as well as Na^+K^+ ATPase activity were determined in rat brain. The results demonstrated that the age-related memory deficit was reversed by treatment with CAF or SCH58261. Treatment with CAF or SCH58261 significantly normalized oxygen and nitrogen reactive species levels increased in brains of aged rats. Na^+K^+ ATPase activity inhibited in brains of aged rats was also normalized by CAF or SCH58261 treatment. A decrease in basal ascorbic acid levels in brains of aged rats was not changed by CAF or SCH58261. These results demonstrated that CAF and SCH58261, modulators of adenosinergic receptors, were able to reverse age-associated memory impairment and to partially reduce oxidative stress.

1. Introduction

The cognitive function decline is closely related with brain changes generated by advancing age. Results of a previous study demonstrated that cognitive impairment may be due to the excessive formation of reactive species (RS) (Lu et al., 2006). The central nervous system (CNS) is particularly more vulnerable to oxidative damage because of its high oxygen consumption, high tissue concentration of iron (Dröge and Schipper, 2007), high lipid contents and the low activity of its antioxidant defenses (Halliwell, 1996). Moreover, Jayakumar et al. (2007) have reported a decrease in enzymatic and non-enzymatic antioxidant defenses in rat brain during aging.

Several drugs, caffeine (CAF) for example, have been exploited to combat RS and the age-related changes. CAF and its metabolites have been shown to present antioxidant effects (Gómez-Ruiz et al., 2007). Central effects of CAF are mediated by binding in a non-selective way to adenosine receptors in the brain. Four adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3) have been cloned and characterized from several mammalian species including humans and rats, and they all belong to the G-protein-coupled receptor family (Fredholm et al., 2005). The effects of CAF on CNS appear to be mediated primarily by its antagonistic actions at the A_1 and A_{2A} subtypes of adenosine receptors (Fredholm et al., 1999, 2001, 2005).

The ability of CAF to prevent memory impairment has been reported (Mendonça and Cunha, 2010; Nehling et al., 2010; Ribeiro and Sebastião, 2010). Costa et al. (2008) demonstrated that treatment with CAF during 12 months prevents memory impairment in aging mice. Positive actions of CAF on information processing and performance might also be attributed to improvement of behavioral routines, arousal enhancement and sensorimotor gating, and these actions may be not solely related to A_1 receptor function (Ribeiro and Sebastião, 2010). However, some beneficial effects triggered by CAF were related to the preferential blockade of adenosine A_{2A} receptors (Fredholm et al., 2005). Moreover, it has been generally accepted that caffeine and/or selective A_{2A} receptor antagonists prevent memory impairment associated with different insults affecting the brain such as Alzheimer's disease (Arendash et al., 2006; Dall'Igna et al., 2007; Cunha, 2008; Canas et al., 2009a, 2009b; Arendash et al., 2009), diabetes (Duarte et al., 2009), convulsions (Cognato et al., 2010), sleep deprivation (Alhaider et al., 2010) or attention-deficit hyperactivity disorder (Pires et al., 2009, 2010).

Not only CAF but also selective antagonists of receptors A_{2A}, such as SCH58261, can be used to ameliorate the memory impairment. Prediger et al. (2005) have reported that A_{2A} receptor antagonists prevent memory impairment in animal models of aging. SCH58261 and CAF have the ability of preventing β-amyloid-induced cognitive

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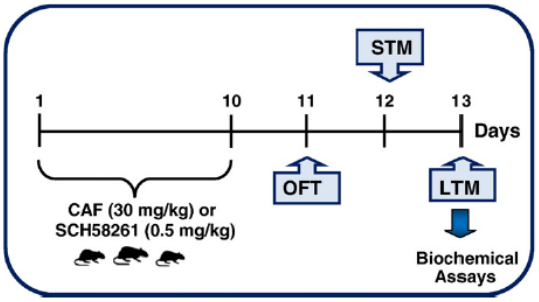
deficit (Arendash et al., 2006, 2009; Dall'Igna et al., 2007; Cunha et al., 2008; Canas et al., 2009a, 2009b).

Diverse behavioral tasks are used for evaluating alterations occasioned in memory caused by the influence of aging, neurodegenerative diseases and other factors. The object recognition memory test, developed by Ennaceur and Delacour (1988), is a non-spatial and non-aversive memory test which has been increasingly used as a important experimental tool in assessing drug effects on memory and investigating the neural mechanisms underlying learning and memory (Norman and Eacott, 2004). Thus, the present study was designed to determine whether treatment with CAF and a selective A_{2A} receptor antagonist, SCH58261, reverses the memory impairment and oxidative stress in aged rats.



Scheme 1. Exposure design.

2. Materials and methods

2.1. Animals

Male young (3 month-old, weighing 170–250 g) and aged (23 month-old, weighing 550–700 g) rats were kept in groups during aging and these animals were not exposed to any environmental enrichment. Wistar rats were obtained from a local breeding colony. Animals were kept in an air conditioned room (22 ± 2 °C), on a 12 h light/dark cycle. Commercial diet (Guaiba, RS, Brazil) and tap water were supplied *ad libitum*. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil.

2.2. Drugs

2-(2-Furanyl)-7-(2-phenylethyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine (SCH58261, Sigma-Aldrich, Dorset, UK) was dissolved in dimethyl sulfoxide (DMSO) (5 mg/ml) which was further diluted in phosphate buffered saline (PBS) to give a final concentration of 15% DMSO (Fischer Scientific, Loughborough, UK) in the drug injection solution. CAF was purchased from Sigma-Aldrich (Dorset, UK) and dissolved in warmed PBS. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.3. Exposure

Seven to ten animals per group were usually tested in the experiments. Both young and aged rats were treated orally (p.o.; by gavage) with CAF (30 mg/kg body weight) or A_{2A} receptor antagonist, SCH58261 (0.5 mg/kg body weight), daily per 10 days (around 10 a.m.). The period of treatment was chosen because a period of circa 1–2 weeks is required to offset the biochemical and behavioral consequences. The doses were calculated as free base form. The dose of 30 mg/kg, corresponding to the equivalent of 4–6 cups of coffee in humans, causes the maximal behavior effects in rodents (Fredholm et al., 1999). The dose of SCH58261 was chosen on the basis of previous study indicating neuroprotection (Chen et al., 2001). The control groups of each age received only vehicle (1 ml/kg p.o., daily per 10 days) (Scheme 1).

2.4. Behavioral tests

2.4.1. Novel object recognition memory

Twenty-four hours after the open field test (OFT) (Scheme 1), animals were trained and tested in a novel object recognition task as previously described (De Lima et al., 2005). Training in the object recognition task took place in the same arena used for the OFT, except that the arena floor was covered with sawdust during the recognition memory task training and test trials. The open field exploration was thus used as a context habituation trial for the recognition memory task. The object recognition test required that the rats recalled which of two plastic objects they had been previously familiarized with the environment where the test was performed. Twenty-four hours after arena exploration, training was conducted by placing individual rats into the field, in which two identical objects (objects A1 and A2; duple Lego toys) were positioned in two adjacent corners, 9 cm from the walls. Animals were left to explore the objects until they had accumulated 30 s of total object exploration time or for a maximum of 20 min. In a short-term memory (STM) test given 1.5 h after training, the rats explored the open field for 5 min in the presence of one familiar (A) and one novel (B) object. All objects presented similar textures, colors and sizes, but distinctive shapes. A recognition index calculated for each animal was expressed by the ratio $TB/(TA+TB)$ [TA = time spent exploring the familiar object A; TB = time spent exploring the novel object B]. Between trials the objects were washed with 10% ethanol solution. In a long-term memory (LTM) test given 24 h after training, the same rats explored the field for 5 min in the presence of familiar object A and a novel object C. Recognition memory was evaluated as for the short-term memory test. Time intervals for testing STM and LTM were chosen on the basis of previous studies characterizing the neurochemical pathways mediating the formation of STM and LTM for object recognition (De Lima et al., 2005) and other tasks (Quevedo et al., 2004) in rats. The exploration of objects by the animal is traditionally defined as approaching the object headfirst within a short distance. In this study, exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the object was not considered exploration. A longer exploration of the new object represents that the animal remembers.

2.4.2. Open field test (OFT)

In order to control for possible sensorimotor effects induced by CAF and SCH58261, behavior during exploration of an open field was evaluated 24 h after the last injection (Scheme 1). The open field was a 40 × 45 cm arena surrounded by 50 cm high walls, made of plywood with a frontal glass wall. The floor of the arena was divided into 9 (3 × 3) equal squares by black lines. Animals were placed in the rear left corner and left to explore the field freely for 5 min. Line crossings and rearings were counted (Walsh and Cummins, 1976). Young and aged animals used in the first experiment (evaluation of possible age-related impairment in object recognition memory) were also left to explore the open field for 5 min in order to allow animals to familiarize with the arena used for object recognition training.

2.5. Biochemical determinations

After the behavioral test (Scheme 1), all rats were euthanized after light isoflurane anesthesia. The brains of animals were removed, dissected and kept on ice until the time of assay. The samples of whole brains were homogenized in 50 mM Tris–HCl, pH 7.4 (1/5, w/v), centrifuged at 4000 × *g* for 10 min. The low-speed supernatants (S_1) were separated and used for biochemical assays.

2.5.1. Ascorbic acid levels

Ascorbic acid (vitamin C), a non-enzymatic antioxidant defense that is involved in protecting against the injurious effects of RS, was measured in the brain of young and aged rats. Ascorbic acid level determination was performed as described by Jacques-Silva et al. (2001) with some modifications. Briefly, S_1 was precipitated in 10% trichloroacetic acid solution. An aliquot of the supernatant (300 µl) at a final volume of 575 µl of the solution was incubated at 38 °C for 3 h then 500 µl H_2SO_4 65% (v/v) was added to the medium. The reaction product was determined using a color reagent containing 4.5 mg/ml dinitrophenyl hydrazine and $CuSO_4$ (0.075 mg/ml) at 520 nm. The content of ascorbic acid is related to tissue amount (µmol ascorbic acid/g tissue).

2.5.2. Oxygen and nitrogen reactive species levels

In order to determine the presence of oxidative imbalance caused by age factor, oxygen and nitrogen reactive species levels in brain from young and aged rats were measured. S_1 was incubated with 10 µl of 1 mM 2′,7′ dichlorofluorescein diacetate (DCHF-DA). The oxygen and nitrogen reactive species levels were determined by a spectrofluorimetric method, using DCHF-DA assay. The oxidation of DCHF-DA to fluorescent dichlorofluorescein is measured for the detection of intracellular oxygen and nitrogen reactive species. The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation) 15 min after the addition of DCHF-DA to the medium. The results were expressed as arbitrary units (AU).

2.5.3. Na^+K^+ ATPase activity

Na^+K^+ ATPase, a well-known SH-containing enzyme found in the brain, is highly sensitive to pro-oxidant elements (Carfagna et al., 1996). The reaction mixture for Na^+K^+ ATPase activity assay contained 50 µl of S_1, 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl and 50 mM Tris/HCl, pH 7.4, in a final volume of 500 µl. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Control samples were carried out under the same conditions with the addition of 0.1 mM ouabain. The samples were incubated at 37 °C for 30 min, the incubation was stopped by adding trichloroacetic acid solution (10% TCA). Na^+K^+ ATPase activity was calculated by the difference found between two assays (with and without ouabain). Released inorganic phosphate (Pi) was measured by the method of Fiske and Subbarow (1925) (nmol Pi/mg protein/min).

2.6. Protein quantification

Protein concentration was measured by the method of Bradford (1976), using bovine serum albumin as the standard.

2.7. Statistical analysis

Data are expressed as means ± S.E.M. The statistical significance was assessed by analysis of variance (ANOVA). One-way ANOVA was used to assess the age effect (Fig. 1). Two-way ANOVA was used to assess the effect of age (young and aged) and treatment (CAF and SCH58261) (Figs. 2–6). Post hoc Duncan's test was carried out when appropriated. A value of $p<0.05$ was considered to be significant.

3. Results

3.1. Novel object recognition memory

In the STM retention test, there was no significant difference between groups (One-way ANOVA of young×aged, $p>0.05$). In the LTM retention test, one-way ANOVA revealed a significant decrease in recognition index of aged rats ($p<0.05$) (Fig. 1).

A significant main effect of CAF [$F(1,32)=11.35$, $p<0.001$] was found on object recognition memory in STM retention in young and aged rats (Two-way ANOVA). Post hoc comparisons showed an increase in STM retention in young and aged rats treated with CAF when compared to the respective controls (Fig. 2A).

Two-way ANOVA of STM retention data showed a significant main effect of SCH58261 treatment [$F(1,32)=12.08$, $p<0.001$]. Post hoc comparisons revealed an increase in STM retention in young and aged rats treated with SCH58261 when compared to the respective controls (Fig. 2A).

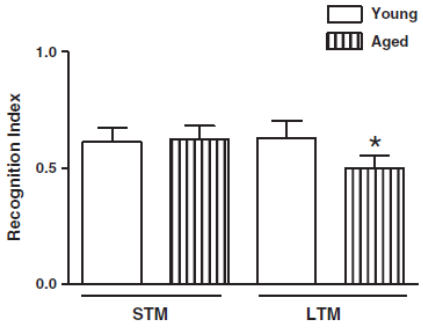


Fig. 1. Novel object recognition memory in young adult (3 months of age) and aged (23 months of age) male rats. Data are median (interquartile ranges) recognition indexes in short-term (STM) and long-term (LTM) retention test trials. $N=7–10$ animals per group. * $p<0.05$ compared to the LTM young group.

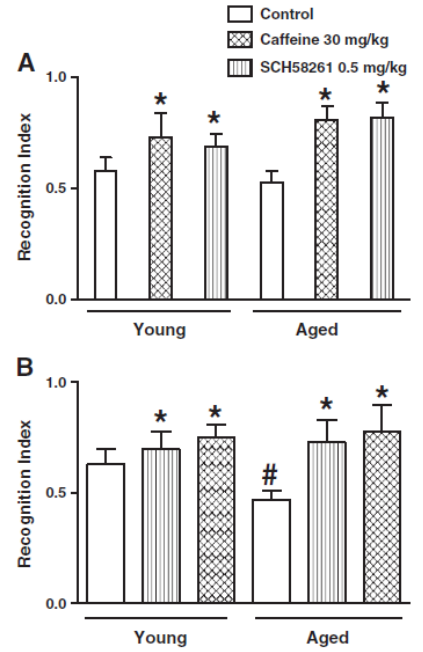


Fig. 2. Novel object recognition memory in young and aged rats treated with a daily administration of vehicle, caffeine (30 mg/kg, p.o.) or SCH58261 (0.5 mg/kg, p.o.) for 10 days. Animals were given a training trial in the object recognition memory task 48 h after the last injection. Data are median (interquartile ranges) recognition indexes in short-term (STM) (A) and long-term (LTM) (B) retention test trials. $N=7–10$ animals per group. * $p<0.05$ compared to the respective control group.

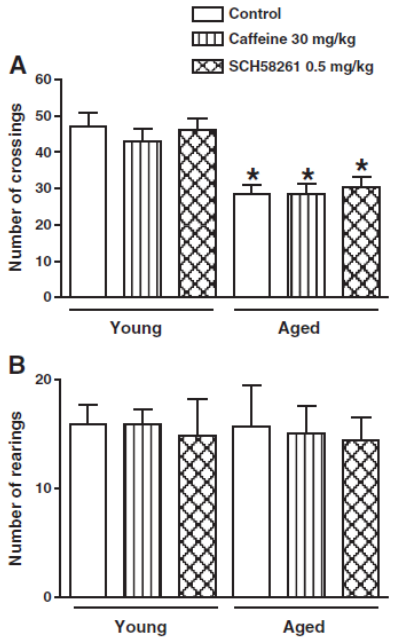


Fig. 3. Open field behavior in young and aged rats treated with a daily systemic injection of vehicle, caffeine (30 mg/kg) or SCH58261 (0.5 mg/kg) for 10 days. Animals were left to explore the arena for 5 min 24 h after the last injection. Data are mean + SEM number of crossings (A) and number of rearings (B). $N=7–10$ animals per group. $^{*}P<0.05$ compared to the young control group.

Two-way ANOVA of LTM retention data revealed a CAF×age interaction [$F(1,32)=1.86$, $p<0.04$] (Fig. 2B). Treatment with CAF [$F(1,32)=5.44$, $p<0.01$] significantly increase LTM retention in aged and young rats when compared to the respective controls. Two-way ANOVA of LTM retention data revealed a SCH58261 × age interaction [$F(1,32)=6.12$, $p=0.01$]. Post hoc comparisons showed a significant increase in LTM retention in aged and young rats treated with SCH58261 [$F(1,32)=19.99$, $p<0.001$] (Fig. 2B).

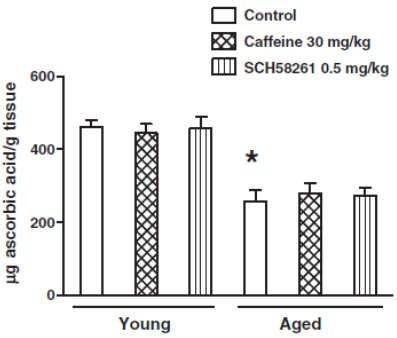


Fig. 4. Effect of CAF and SCH58261 on ascorbic acid levels in young adult and aged male rats. Results are expressed as mean ± S.E.M. of seven to ten animals per group. $^{*}p<0.05$ compared to the young control group (two-way ANOVA/Duncan).

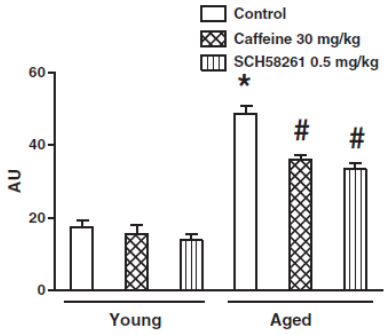


Fig. 5. Effect of CAF and SCH58261 on RS levels in young adult and aged male rats. Results are expressed as mean ± S.E.M. of seven to ten animals per group. $^{*}p<0.05$ compared to the young control group (two-way ANOVA/Duncan). $^{\#}p<0.05$ compared to the control group of each age (two-way ANOVA/Duncan).

3.2. Open field test

Two-way ANOVA (CAF×age) performed on the number of crossings showed a significant main effect of age [$F(1,32)=24.14$, $p<0.001$]. Post hoc comparisons demonstrated that the decrease in the number of squares crossed by aged rats was not protected by CAF [$F(1,32)=0.42$, $p<0.52$] (Fig. 3).

Two-way ANOVA (SCH58261×age) of the number of crossings demonstrated a significant main effect of age [$F(1,32)=32.04$, $p<0.001$]. Post hoc comparisons revealed that SCH58261 was not effective in protecting against the decrease in the number of squares crossed caused by age [$F(1,32)=0.57$, $p<0.45$)].

Neither age nor treatment affected the number of rearings ($p>0.05$) (Fig. 3B).

3.3. Ascorbic acid levels

A significant main effect of age [$F(1,32)=35.65$, $p<0.001$] on ascorbic acid levels was found (Two-way ANOVA). Treatment with CAF [$F(1,32)=0.08$, $p=0.88$] or SCH58261 [$F(1,32)=0.12$, $p=0.91$] did not change the decrease in basal ascorbic acid levels in brain of aged rats. Ascorbic acid levels were not altered in all groups of young rats (Fig. 4).

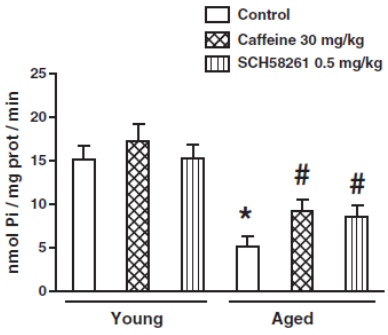


Fig. 6. Effect of CAF and SCH58261 on Na^{+} K^{+} ATPase activity in young adult and aged male rats. Results are expressed as mean ± S.E.M. of seven to ten animals per group. $^{*}p<0.05$ compared to the young control group (two-way ANOVA/Duncan). $^{\#}p<0.05$ compared to the control group of each age (two-way ANOVA/Duncan).

3.4. Oxygen and nitrogen reactive species levels

Two-way ANOVA (age×CAF) on oxygen and nitrogen reactive species levels revealed a significant main effect of age [$F(1,32)=13.56$, $p<0.001$]. Treatment with CAF [$F(1,32)=12.01$, $p<0.001$] significantly reduced oxygen and nitrogen reactive species levels increased in brains of aged rats (Fig. 5).

Two-way ANOVA (age×SCH58261) on oxygen and nitrogen reactive species levels demonstrated a significant interaction [$F(1,32)=5.66$, $p<0.02$]. Treatment with SCH58261 [$F(1,32)=11.23$, $p<0.001$] reduced oxygen and nitrogen reactive species levels increased in brains of aged rats [$F(1,32)=17.66$, $p<0.001$] (Fig. 5). The levels of oxygen and nitrogen reactive species were not altered in young rats from all groups.

3.5. Na^+K^+ ATPase activity

Two-way ANOVA (age×CAF) of Na^+K^+ ATPase activity revealed a treatment×age interaction [$F(1,32)=4.55$, $p<0.01$]. Treatment with CAF [$F(1,32)=15.02$, $p<0.001$] significantly increased Na^+K^+ ATPase activity inhibited in brains of aged rats [$F(1,32)=21.34$, $p<0.001$] (Fig. 6).

Two-way ANOVA (age×SCH58261) of Na^+K^+ ATPase activity showed a significant interaction [$F(1,32)=6.10$, $p<0.01$]. SCH58261 [$F(1,32)=13.89$, $p<0.001$] was effective in increasing Na^+K^+ ATPase activity inhibited in brains of aged rats [$F(1,32)=17.89$, $p<0.001$]. Na^+K^+ ATPase activity was not altered in all groups of young rats (Fig. 6).

4. Discussion

In this study we provide evidence for an age-related deficit in novel object recognition memory in rats. CAF or SCH58261, antagonists of adenosine receptors, reverses memory impairment in aged rats. This study also demonstrated the influence of age on locomotor activity. Moreover, CAF or SCH58261 ameliorated the index of recognition in young animals.

The antioxidant action of CAF and SCH58261 could be related to the reverse in memory impairment since they restored oxygen and nitrogen reactive species levels and Na^+K^+ ATPase activity altered in aged rats.

Like humans, aged rodents exhibit age-related decline in cognitive function (Erickson and Barnes, 2003). The novel object recognition memory task evaluates natural behavior of rodents, such as approaching and exploring novel objects rather than familiar ones. Thus this task deals with the natural motivation of the animals to explore novelty, an innate instinct that animals use to recognize their environment (Heldt et al., 2007). The novel object recognition memory test has been widely used in the investigation of the neurobiological mechanisms of learning and memory. Whereas most studies investigating learning and memory in rodents use spatial and/or emotionally motivated behavioral tasks, the object recognition task provides a tool for assessing non-spatial, non-aversive memory sensitive to genetic and pharmacological manipulations as well as aging process (De Lima et al., 2005; Schröder et al., 2003).

The present study investigated the hypothesis that treatment with CAF or SCH58261 attenuates age-related memory deficits using the novel object recognition memory task. It was clearly demonstrated an age related deficit in novel object recognition memory in the LTM retention test. Accordingly, De Lima et al. (2005) have reported an age related deficit in novel object recognition memory measured 24 h after training in rats and the absence of effect in the STM retention test. The memory deficit was reversed by treatment with CAF or SCH58261 in aged animals. In addition, CAF or SCH58261 improved the novel object recognition memory in young rats. Taken into consideration these results, it is tempting to speculate the beneficial role of A_{2A} and A_1 receptor antagonists as CAF or A_{2A} receptor antagonist selective, SCH58261, on cognitive deficit caused by aging. There is also considerable evidence that CAF presents cognition enhancing properties (Gevaerd et al., 2001; Costenla et al., 2010; Mendonça and Cunha, 2010; Ribeiro and Sebastião, 2010) and its use has been proposed as a potential treatment to counteract age-related cognitive decline (Riedel and Jolles, 1996; Cunha and Agostinho, 2010). A modulatory role of adenosine in learning and memory, including processes such as long-term potentiation (LTP) (de Mendonça and Ribeiro, 1994) and long-term depression (LTD) (Mendonça et al., 1997) that are considered to be basic mechanisms involved in memory processes, has been proposed. Prediger et al. (2005) have reported that adenosine modulates the short-term social memory in rats by acting on both A_1 and A_{2A} receptors, with adenosine receptor agonists and antagonists, respectively, disrupting and enhancing the social memory.

It is important to point out that there is a main difference in this study compared with others investigating the effects of CAF and A_{2A} receptor antagonists on memory performance (Angelucci et al., 1999; Corodimas et al., 2000; Costa et al., 2008; Takahashi et al., 2008). In the present study, CAF and SCH58261, an A_{2A} receptor antagonist, ameliorated memory performance both in control subjects (adult rats) and in test (aged) rats. Even though, there seems to be a general consensus in the literature that chronic CAF consumption is not a memory enhancer but rather a memory stabilizer (Cunha and Agostinho, 2010). An explanation for this difference is that the effects of CAF depend on the tested dose, the schedule of administration (acute *versus* chronic), the timing of administration (before training, affecting memory acquisition, or after training, affecting memory consolidation or retrieval) and on the mode of administration (locally in defined brain structures or peripherally affecting different brain structures) (Takahashi et al., 2008; Cunha, 2008).

Clearly, the different impact of CAF and A_{2A} receptor antagonists on memory performance is related to changes in adenosine receptor expression, density on aging (Cunha et al., 1995; Alfaro et al., 2004; Canas et al., 2009a, 2009b) and functional properties of adenosine A_{2A} receptors (Lopes et al., 1999; Corsi et al., 1999, 2000; Rebola et al., 2003; Rodrigues et al., 2008).

Treatment with CAF or SCH58261 was not able to reverse a decrease in the number of crossings in aged animals. Accordingly, Pardon et al. (2000) tested the behavioral parameters of different age of rats (5, 11, 17 and 23 months) in the OFT and argued that locomotor activity and exploring behavior decrease with the age. However, there was no effect of age in the number of rearings, in the present study.

Additionally, the current study showed that CAF or SCH58261 treatment partially decreased RS levels increased in aged animals. Although the major factors involved in age-related decline remain to be specified, oxidative stress generated by RS has been mainly implicated in cognitive impairment and neuropathological disorders in both old experimental animals and aged humans (Liu et al., 2003). It is possible that due to its antioxidant action, CAF reduced the levels of oxygen and nitrogen reactive species in the brain of aged rats, resulting in the improvement of the performance of aged animals in the novel object recognition memory task.

In addition, both adenosine receptors, A_1/A_{2A}, have the ability to control neuronal damage after different insults: A_1 receptors constitute a hurl that increases the threshold for brain damage, whereas the blockade of adenosine A_{2A} receptors affords neuroprotection against chronic noxious brain insults (Cunha, 2005). A_{2A} receptors affect the impact and formation of free radicals in neuronal preparations (Masino et al., 1999; Rego et al., 1999; Agostinho et al., 2000; Behan and Stone, 2002; Almeida et al., 2003). In addition, there has been demonstrated the impact of A_{2A} receptors on NMDA receptors (Hussey et al., 2007; Rebola et al., 2008) and on mitochondrial function (Silva et al., 2007), i.e., the main triggers of oxidative stress in brain tissue. Thus, the antagonistic effects of both CAF and SCH58261 could also be involved in reducing oxygen and nitrogen reactive species levels in aged rats, but more studies are needed to support this assertion.

Alterations on non-enzymatic and enzymatic antioxidant systems are determined as parameters of oxidative stress (Jayakumar et al., 2007). The results presented in this study clearly showed that aging depressed ascorbic acid levels in the brains of old rats. Antioxidant function in the brain declines during aging (Siqueira et al., 2005). Shahidi et al. (2008) concluded that short- and long-term supplementation with ascorbic acid has facilitatory effects on acquisition and retrieval processes of passive avoidance learning and memory in rats. Moreover, an observational study in elderly individuals indicated that ascorbic acid may have beneficial effect on the development of Alzheimer dementia (Landmark, 2006). Plasma ascorbic acid levels were lower in subjects with dementia compared to controls (Charlton et al., 2004), and it was suggested that ascorbic acid might protect against cognitive impairment (Paleologos et al., 1998). In this context, we suggest that the depressed ascorbic acid levels are associated with the cognitive impairment in aged rats.

The results on Na^+ K^+ ATPase activity are in accordance with Kaur et al. (2001) who demonstrated the age-related decline in Na^+ K^+ ATPase activity. Thus, it is possible that oxidative stress (decrease in ascorbic acid and increase of oxygen and nitrogen reactive species levels) and inhibition of Na^+ K^+ ATPase activity have some influence in the cognitive deficit in aged rats. As previously reported, the decreased activity of Na^+K^+ATPase is the consequence of either aging or peroxidative process. Such effect might depend on the modification of the lipidic composition and decreased membrane fluidity, which occurs during aging (Viani et al., 1991). In this study, treatment with CAF or SCH58261 was effective in normalizing Na^+ K^+ ATPase activity inhibited in aged rats. In this context, Bavaresco et al. (2003) have reported that vitamins E and C prevent the inhibition of Na^+K^+ ATPase activity. The inhibition of Na^+K^+ ATPase activity has been related to memory consolidation of step-down inhibitory avoidance in the hippocampus (Wyse et al., 2004). Thus, the increase in Na^+K^+ ATPase activity by CAF or SCH58261 may be involved in the improvement of memory in aged rats.

In the CNS, the increase of the neuronal excitability would consume more cell energy. It has been shown that approximately one half or more of total ATP generated in brain at resting state is consumed by Na^+K^+ATPase to maintain proper transmembrane ionic gradients (Erecinska and Silver, 1989). Establishing and maintaining high K^+ and low Na^+ in the cytoplasm are required for normal resting membrane potentials and various cellular activities. Taken these into consideration, the decrease in Na^+K^+ATPase activity consequently, reducing the Na^+ and K^+ electrochemical gradient, and disturbed the ionic homeostasis and the physiological functions of neurons, could be related to the impairment of spatial recognition memory in aged rats.

It has been well documented the importance of Na^+K^+ATPase activity in the spatial recognition memory (Hu et al., 2010), in learning (Brunelli et al., 1997) and in activity-dependent synaptic plasticity, such as long-term potentiation (LTP) (Glushchenko and Izvarina, 1997). In this context, Na^+K^+ATPase inhibition by ouabain impairs learning and memory in Morris water maze (Zhan et al., 2004) and step-through passive avoidance tasks (Gibbs and Ng, 1977; Ng and Gibbs, 1991; Sato et al., 2004), further supporting the main role of this enzyme on learning and memory.

In summary, the present study demonstrated that CAF and SCH58261, modulators of adenosinergic receptors, were able to reverse age-associated memory impairment and to partially reduce oxidative stress, suggesting its use in the treatment of cognitive decline associated with aging. However, more studies are needed to unravel the exact mechanisms by which CAF and SCH58261 improve memory.

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3.2 Manuscrito 1

Manuscrito em fase de preparação

Cafeína e Disseleneto de Difenila Melhoram o Prejuízo da Memória de Longo Prazo em Ratos de Meia Idade

CAFFEINE AND DIPHENYL DISELENIDE IMPROVE THE IMPAIRMENT OF LONG-TERM MEMORY IN MIDDLE-AGED RATS

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Caffeine and Diphenyl Diselenide Improve the Impairment of Long-term Memory in Middle-aged Rats

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Abstract

The aim of the present study was to evaluate the effects of diphenyl diselenide $(PhSe)_2$ supplemented diet (10 ppm) associated to the administration of caffeine (15 mg/kg) for 30 days on the novel object recognition memory in middle-aged rats. The present findings showed that $(PhSe)_2$ supplemented diet induced an improvement in short-term memory of middle-aged rats in the object recognition task. By contrast, the same effect was not observed with respect to long-term memory. Moreover, $(PhSe)_2$ supplemented diet associated with caffeine administration reversed the impairment of long-term memory in middle-aged rats. The animals treated only with caffeine showed no improvement in short and long-term memory. In addition, treatments with $(PhSe)_2$ and $(PhSe)_2$ plus caffeine increased the number of crossings in the open field in middle-aged rats. The number of rearings were also affected by treatments, caffeine and $(PhSe)_2$ and caffeine increased the exploratory activity of middle-aged rats. These results suggest that $(PhSe)_2$ and caffeine reverse memory deficit caused by aging.

Key-words: caffeine, organoselenium, memory, object recognition, middle-aged

1. Introduction

The global phenomenon of increase life expectancy in recent decades has reflected directly in increased incidence of age-related diseases. Particularly, it is observed a deficiency on cognitive functions in elderly individual, causing a decrease in a variety of brain functions including processing speed, inductive reasoning, and spatial learning and memory (Hedden and Gabrieli 2004). Thus, the age-dependent loss of cognitive functions has stimulated the development of strategies to contain this decline.

Caffeine is certainly the most widely consumed psychoactive substance in the world. It is estimated that more than 50% of the world's adult population consume caffeine daily (Fredholm et al. 1999). The wide consumption of caffeine associated with common beverages, such as teas and coffee, together with the impact of xanthines on biomedical research, prompted many studies that have focused on specific caffeine effects (Daly 2007; Ferré 2008). The caffeine crosses the blood–brain barrier and triggers its effects in the central nervous system (CNS) by antagonizing primarily adenosine receptors A_1 and A_{2A} (Fredholm et al., 2005). It has been reported that caffeine induces several cellular and pharmacological responses, such as the CNS and motor activity stimulation (Fredholm et al., 1999), anxiety and sleep disturbance (Nardi et al., 2009; Paterson et al., 2009), antioxidant activity (Noschang et al., 2009; Shi et al., 1991) among others.

The beneficial effects of caffeine on cognition have been shown in humans. In fact, epidemiological studies in elderly population demonstrated that habitual caffeine consumption lower cognitive decline in men (van Gelder et al., 2007) and women (Santos et al., 2010). In addition, several animal studies have reported the effectiveness of caffeine in enhancing cognition (Villa-luna et al., 2012; Duarte et al. 2012; Leite et al., 2011).

Selenium has an important biological role in mammalian species. Enzymes such as gluthatione peroxidase (Forstrom et al., 1978), thioredoxin reductase (Holmgren, 1985) and

5′-deiodinase (Behne and Kyriakopoulos, 1990) have a selenocysteine residue in their active sites. Selenium present in these residues has redox activity which is essential for the catalytic activity of these enzymes (Stangherlin et al., 2008).

Persuasive evidence has been found to indicate that organoselenium compounds are promising pharmacological agents (Nogueira and Rocha 2011). Particularly, the compound diphenyl diselenide $(PhSe)_2$ has been proven to be neuroprotector (Posser et al., 2008), anti-hyperlipidemic (da Rocha et al., 2011), hepatoprotector (Borges et al., 2008) and antioxidant (Luchese et al., 2007; Prigol et al., 2009) in different experimental models. Regarding memory, $(PhSe)_2$ enhances cognition of mice in a model of cognitive impairment induced by scopolamine (Souza et al. 2010) and in the novel object recognition test (Rosa et al., 2003) and improves the performance of rats in the water-maze test (Stangherlin et al., 2008).

Based on the above considerations the aim of the present study was to evaluate the effects of $(PhSe)_2$ supplemented diet associated to the administration of caffeine on memory deficit related to aging in rats.

2.1 Materials and methods

2.1 Animals

Male adult (3 month-old, weighing 170–250 g) and middle-aged (18 month-old, weighing 500–600 g) Wistar rats were obtained from a local breeding colony. Animals were kept in an air conditioned room (22 ± 2 °C) with free access to water and food, under a 12 h light/dark cycle. All manipulations were carried out between 08:00 a.m. and 04:00 p.m. All experiments were performed on separate groups of animals. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources (license number 040/2012), the Federal University of Santa Maria, Brazil.

2.2 Drugs

Diphenyl diselenide $(PhSe)_2$ was prepared in our laboratory according to the method described by Paulmier (1986) and the chemical purity (99.9%) was determined by GC/MS. Analysis of ^{1}H and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. Caffeine was purchased from Sigma-Aldrich (Dorset, UK).

2.3 Dietary supplementation

Animals were fed daily with 50 g/animal standard diet chow or standard chow supplemented with $(PhSe)_2$. The standard diet was pulverized with ethyl alcohol, whereas the supplemented diet was pulverized with $(PhSe)_2$ dissolved in ethyl alcohol (1 mg/10 ml). The standard and supplemented diets were kept at room temperature for 3 h to evaporate the alcohol and then kept at 4^0 C by not more than 1 week (Bem et. al., 2009).

2.4 Experimental procedure

The rats were divided into five groups of seven animals each, these being:

• Adult: rats received standard diet chow and saline;

• Middle-aged:

Control: rats received standard diet chow and saline;

$(PhSe)_2$: rats received 10 ppm of $(PhSe)_2$ supplemented diet for 30 days;

Caffeine: rats received 15 mg/kg of caffeine (dissolved in saline 0,9 %; p.o., by gavage) for 30 days;

$(PhSe)_2$ + caffeine: rats received both $(PhSe)_2$ + caffeine

The dosage and regimen of caffeine and $(PhSe)_2$ were chosen based on previous studies (Fredholme et al. 1999; De Bem et al., 2009).

2.5 Behavioral tests

2.5.1. Open field test (OFT)

The open field test was performed 24 h after the last treatment day (Scheme 1) in order to discard possible sensorimotor effects induced by caffeine and $(PhSe)_2$. The open field was a 40×45 cm arena surrounded by 50 cm high walls, made of plywood with a frontal glass wall. The floor of the arena was divided into 9 (3×3) equal squares by black lines. Animals were placed in the rear left corner and left to explore the field freely for 5 min. Line crossings and rearings were counted (Walsh and Cummins, 1976). Adult and middle-aged animals used in the first experiment (evaluation of possible age related impairment in object recognition memory) were also left to explore the open field for 5 min in order to allow animals to familiarize with the arena used for object recognition training.

2.5.2. Novel object recognition memory

Twenty-four hours after the OFT (Scheme 1), the animals were trained and tested in a novel object recognition task as previously described (De Lima et al., 2005). Training in the object recognition task took place in the same arena used for the OFT, except that the arena floor was covered with sawdust during the recognition memory task training and test trials. The open field exploration was thus used as a context habituation trial for the recognition memory task. The object recognition test required that the rats recalled which of two plastic objects they had been previously familiarized with the environment where the test was performed. Twenty-four hours after arena exploration, training was conducted by placing individual rats into the field, in which two identical objects (objects A1 and A2; duple Lego toys) were positioned in two adjacent corners, 9 cm from the walls. Animals were left to explore the objects until they had accumulated 30 s of total object exploration time or for a maximum of 20 min. In a short-term memory (STM) test given 1.5 h after training, the rats explored the open field for 5 min in the presence of one familiar (A) and one novel (B) object. All objects presented similar textures, colors and sizes, but distinctive shapes.

A recognition index calculated for each animal was expressed by the ratio TB/(TA+TB) [TA=time spent exploring the familiar object A; TB=time spent exploring the novel object B].

Between trials the objects were washed with 10% ethanol solution. In a long-term memory (LTM) test given 24 h after training, the same rats explored the field for 5 min in the presence of familiar object A and a novel object C. Recognition memory was evaluated as for the short-term memory test. Time intervals for testing STM and LTM were chosen on the basis of previous studies characterizing the neurochemical pathways mediating the formation of STM and LTM for object recognition (De Lima et al., 2005) and other tasks (Quevedo et al., 2004) in rats. The exploration of objects by the animal is traditionally defined as approaching the object headfirst within a short distance. In this study, exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the object was not considered exploration. A longer exploration of the new object represents that the animal remembers.

2.6. Statistical analysis

Data are expressed as means ± S.E.M. The statistical significance was assessed by analysis of variance (ANOVA). The Student's t-test was used to assess the age effect (adult x middle-aged). Two-way ANOVA was used to assess the effect of treatment (caffeine and $(PhSe)_2$). Post hoc Duncan's test was carried out when appropriated. A value of $p<0.05$ was considered to be significant.

2. Results

3.1. Open field test

The Student's t-test revealed a significant decrease in the number of crossings [$F(1,24)=11,81$, $p<0.05$] (Fig. 3A) and in the number of rearings [$F(1,24)=19,86$, $p<0.05$] (Fig. 3B) in middle-aged rats.

A significant increase in the number of crossings in middle-aged rats treated with $(PhSe)_2$ and caffeine x $(PhSe)_2$ was demonstrated. The two-way ANOVA of number of crossings data revealed significant differences for $(PhSe)_2$ treatment [$F(1,24)=9.33$, $p<0.05$] and caffeine x $(PhSe)_2$ interaction [$F(1,24)=5.32$, $p<0.05$] (Fig. 4A).

The two-way ANOVA of number of rearings indicated significant differences for caffeine treatment [$F(1,24)=4.64$, $p<0.05$] and caffeine x $(PhSe)_2$ interaction [$F(1,24)=6.54$, $p<0.05$] (Fig.4B).

3.2. Novel object recognition memory

In both STM and LTM retention tests, the Student's t-test analysis revealed a significant decrease in the recognition index of middle-aged rats ($p<0.05$) when compared to adult rats (Fig. 1A and B). The two-way ANOVA analysis showed a significant main effect of $(PhSe)_2$ [$F(1,24)=9.12$, $p<0.05$] on object recognition memory in STM retention in middle-aged rats. Post hoc comparisons demonstrated an increase in STM retention in middle-aged rats treated with $(PhSe)_2$ when compared to control rats (Fig. 2A).

Figure 2B shows a significant increase in LTM retention in middle-aged rats treated with both caffeine x $(PhSe)_2$ when compared to the control group (Fig. 2B). The two-way ANOVA of LTM retention data revealed a significant caffeine x $(PhSe)_2$ interaction [$F(1,24)=9.86$, $p<0.05$].

4. Discussion

In recent decades, the increasing average life-span has been accompanied by the high incidence of age- related cognitive disorders, stimulating the interest in the search for agents

that have the ability to prevent or retard such disorders. Previous studies have shown the protective potential of $(PhSe)_2$ (da Rocha et al., 2012; Dias et al., 2012; Rosa et al. 2003) or caffeine (Costa et al., 2008; Cunha et al. 2010, Botton et al., 2010) against cognitive disorders in animal models.

The current study demonstrated that supplementation with $(PhSe)_2$ reversed the cognitive deficit caused by aging in the STM in middle-aged rats evaluated in the novel object recognition task. By contrast, $(PhSe)_2$ supplemented diet was not effective in reversing the deficit of LTM in middle-aged rats. It is well reported a decrease in the antioxidant defense mechanisms with a consequent increase of the oxidative damage in the aging brain, which contributes to cognitive dysfunctions (Berr et al. 1998; Floyd, 1999; Floyd et al., 2002). Therefore, the use of antioxidants could be an interesting alternative for reducing oxidative damage associated with aging and consequently the cognitive impairment (Hou et al., 2012). The neuroprotective effect of $(PhSe)_2$ in the rat brain has been related to its antioxidant property (Rossato et al., 2002;Warren, 2002). Therefore, a possible hypothesis is that the antioxidant property of $(PhSe)_2$ is in some way related to the enhancement of STM in middle-aged rats. Another plausible explanation for the $(PhSe)_2$ action on memory is its ability to stimulate adenylyl cyclase (Nogueira et al., 2001) and/or mitogen-activated protein (MAP) kinase (Stapleton et al., 1997), since both are involved in cAMP/protein kinase A (PKA)/CREB signaling pathway. This pathway is crucial to memory formation in the hippocampus and other brain areas (Atkins et al., 1998; Bach et al., 1999; Izquierdo et al., 2000; Walz et al., 2000).

The findings of this study clearly demonstrated that chronic treatment of middle-aged rats only with caffeine was not effective in improving STM and LTM. On the other hand, we have previously reported that caffeine at a dose of 30 mg/kg, dose corresponding to the equivalent of 4–6 cups of coffee in humans, causing the maximal behavior effects in rodents

(Fredholm et al., 1999), reversed the long-term memory impairment caused by aging in rats (Leite et al., 2011). It is important to highlight that discrepancies found between these studies could be attributed to different doses of caffeine, the protocol of exposure and the age of rats. In fact, in our first study (Leite et al., 2011) caffeine was administered to old rats (23 month-old) at a dose of 30 mg/kg for 10 days, while in the present study middle-aged (18 month-old) rats received 15 mg/kg of caffeine for 30 days. Indeed, chronic treatment with caffeine showed inconsistent results regarding the prevention of cognitive decline during aging, as shown in previous studies (Costa et al., 2008; Arendash et al. 2009).

In addition, the results of the present study demonstrated that $(PhSe)_2$ supplemented diet associated with caffeine administration reversed the impairment of LTM in middle-aged rats. The main molecular target of the psychostimulant effects of caffeine is the non-selective antagonism of A_1 and A_{2A} adenosine receptor (Fredholm, 1980). However, evidence has been found to suggest that effects on excitation and neuroprotection of caffeine appears to be preferred due to the blockage of A_{2A} adenosine receptor (Huang et al., 2005; Higgins et al, 2007; Silva et al., 2007). Accordingly, the selective A_{2A} receptor antagonist SCH58261 reversed memory impairment in old rats (Leite et al., 2011). Furthermore, Wang et al. (2006) demonstrated that knock-out mice for A_{2A} adenosine receptor improved spatial recognition memory. These findings are reinforced by the fact that overexpression of A_{2A} adesonine receptor lead to memory deficits (Giménez-Llort et al., 2007). Since the density of A_{2A} adenosine receptor has a significant increase in the cortex (Cunha et al. 1995) and hippocampus (Diogenes et al. 2007) of old rats; A_{2A} blockade by caffeine along with the effects already reported by $(PhSe)_2$ (Stangherlin et al., 2008; Souza et al., 2010) seems to have been sufficient to reverse the impairment of LTM in middle-aged rats, showing what appears to be an additive effect of these two compounds.

The open-field is generally used to evaluate animal behavior based on natural conflict between exploration and aversion against open areas in a novel environment. The total number of grid squares traversed in the field normally serves as an index of locomotion activity, whereas the sum of rearings and grid squares traversed reflects exploratory activity (Schmitt and Hiemke, 1998). The locomotor activity and exploratory behavior in the OFT decrease with aging in rodents (Pardon et al. 2000). In this study, the administration of caffeine and $(PhSe)_2$ supplemented diet to middle-aged rats ameliorated locomotion and exploratory activities impaired by age. The stimulant actions of caffeine on locomotion are attributed to the antagonism of A_1 and A_{2A} adenosine receptors in the striatum (Ferré, 2008). With respect to $(PhSe)_2$, this is the first report that showed that this organoselenium compound ameliorated locomotor activity in aged rats. Although $(PhSe)_2$ more caffeine treatments increased locomotion, the reversal of impaired memory shown by middle-aged rats treated with $(PhSe)_2$ or $(PhSe)_2$ more caffeine does not seem to be related to locomotion because animals treated with caffeine had an increase in exploratory activity and no improvement in memory.

In conclusion, this study demonstrated that $(PhSe)_2$ associated to caffeine ameliorated LTM in middle-aged rats. $(PhSe)_2$ supplemented in the diet improved STM in middle-age rats. Chronic administration of caffeine did not improve memory impaired by age. $(PhSe)_2$ associated to caffeine treatment was effective in increasing locomotor and exploratory activities in middle-aged rats. These results suggest that $(PhSe)_2$ associated to caffeine could be a promising alternative to prevent the memory impairment caused by aging. More experiments are needed for precisely delineating the mechanism by which caffeine and $(PhSe)_2$ supplementation improved locomotor and exploratory activities and memory in middle-aged rats.

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Legends and figures

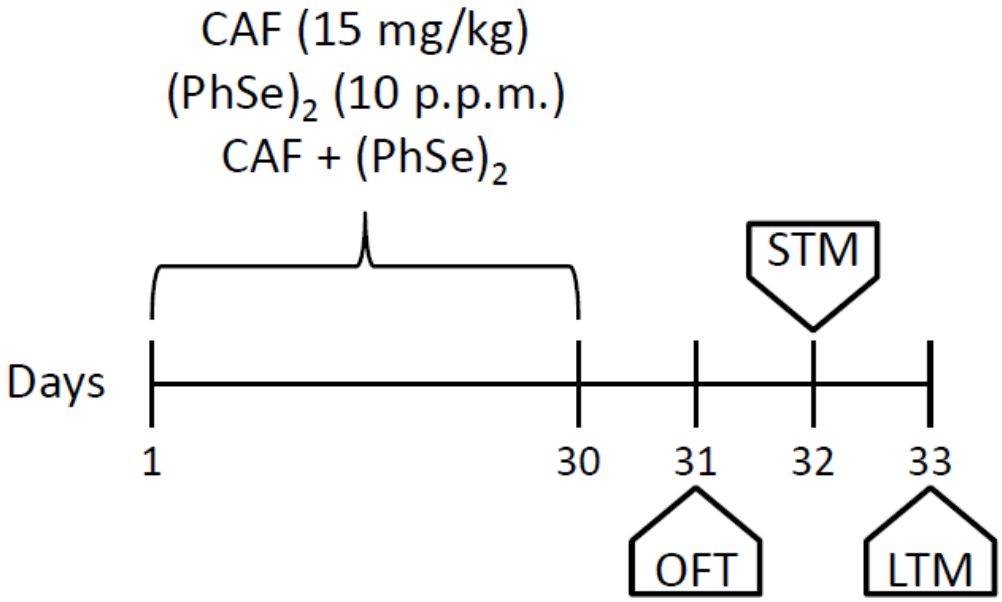
Scheme 1. Experimental design

Figure 1. Novel object recognition memory in adult (3 month-old) and middle- aged (18 month-old) male rats. Data are the mean (s) +SEM of recognition indexes in short-term (STM) (A) and long-term (LTM) (B) retention test trials. N=7 animals per group. $^{\#}p<0.05$ compared to the adult group.

Figure 2. Effect of a daily administration of $(PhSe)_2$ (10 ppm supplemented diet), caffeine (15 mg/kg, p.o.) or both for 30 days in middle-aged (18 month-old) male rats in the novel object recognition memory test. Animals were given a training trial in the object recognition memory task 48 h after the last injection. Data are the mean (s) +SEM of recognition indexes in short-term (STM) (A) and long-term (LTM) (B) retention test trials. N=7 animals per group. * $p<0.05$ compared to the control group.

Figure 3. The open-field test in adult (3 month-old) and middle-aged (18 month-old) male rats. Animals were left to explore the arena for 5 min 24 h after the end of treatment. Data are the mean (s) +SEM number of crossings (A) and number of rearings (B). $^{\#}p<0.05$ compared to the adult control group.

Figure 4. Effect of a daily administration of $(PhSe)_2$ (10 ppm supplemented diet), caffeine (15 mg/kg, p.o.) or both for 30 days in middle-aged (18 month-old) male rats in the open field test. Animals were left to explore the arena for 5 min 24 h after the end of treatment. Data are the mean (s) +SEM of the number of crossings (A) and the number of rearings (B). N=7–10 animals per group. *$P<0.05$ compared to the control group.



Scheme 1

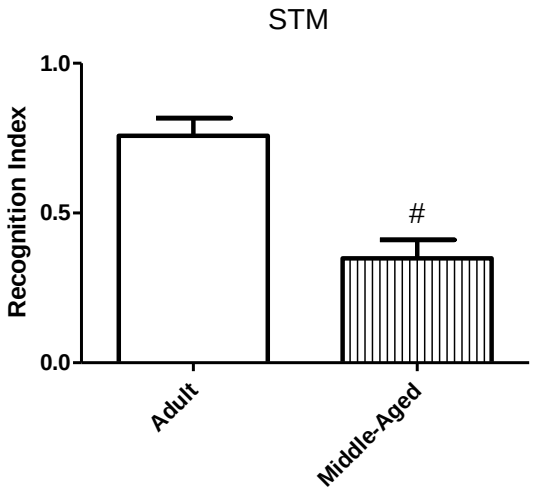


Fig. 1A

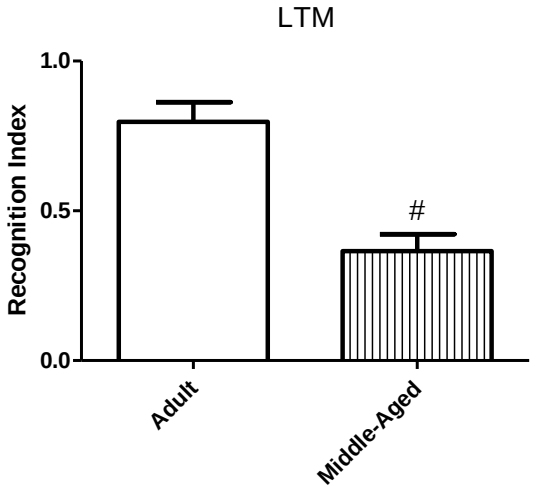

LTM
Recognition Index
1.0
0.5
0.0
#
Adult
Middle-Aged

Fig. 1B

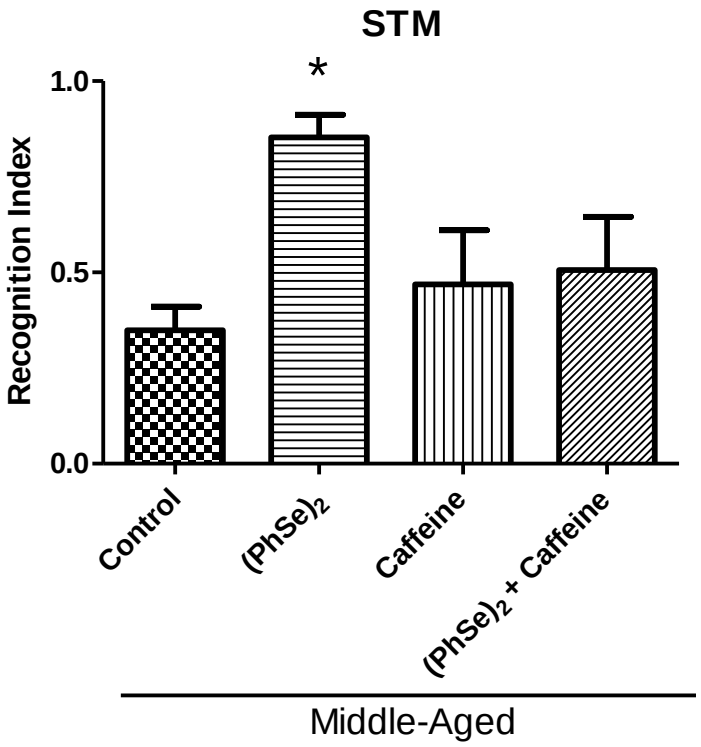

STM
Recognition Index
1.0
0.5
0.0
*
Control
(PhSe)2
Caffeine
(PhSe)2 + Caffeine
Middle-Aged

Fig. 2A

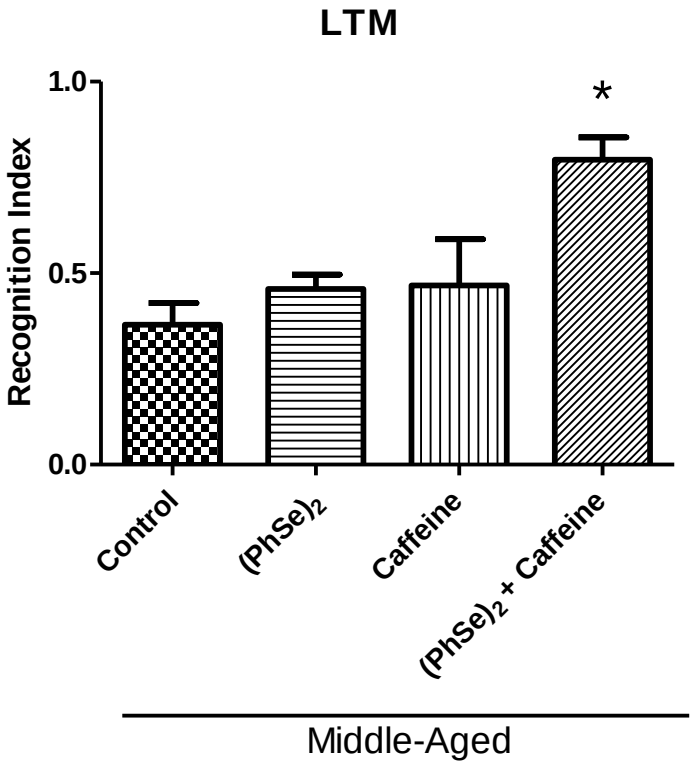

LTM
Recognition Index
1.0
0.5
0.0
*
Control
(PhSe)2
Caffeine
(PhSe)2 + Caffeine
Middle-Aged

Fig. 2B

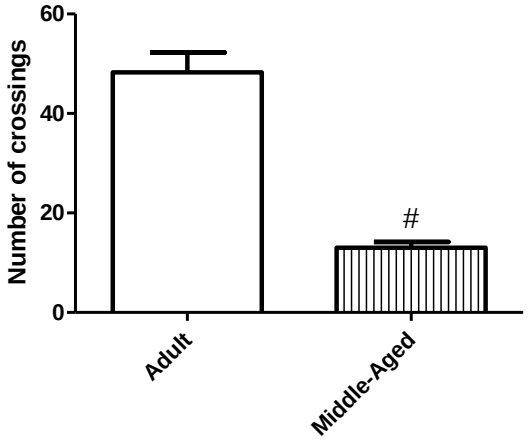

Number of crossings
60
40
20
0
#
Adult
Middle-Aged

Fig. 3A

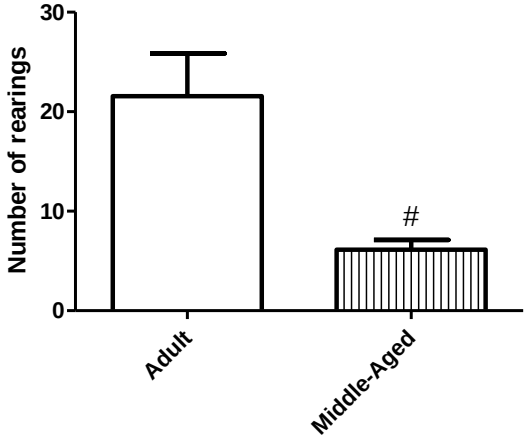

30
20
10
0
Number of rearings
#
Adult
Middle-Aged

Fig. 3B

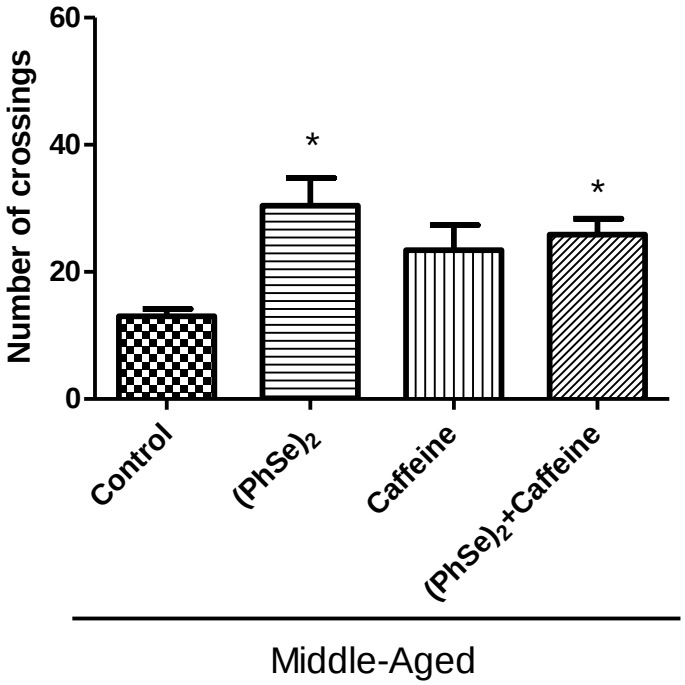

60
40
20
0
Number of crossings
*
*
Control
(PhSe)2
Caffeine
(PhSe)2+Caffeine
Middle-Aged

Fig. 4A

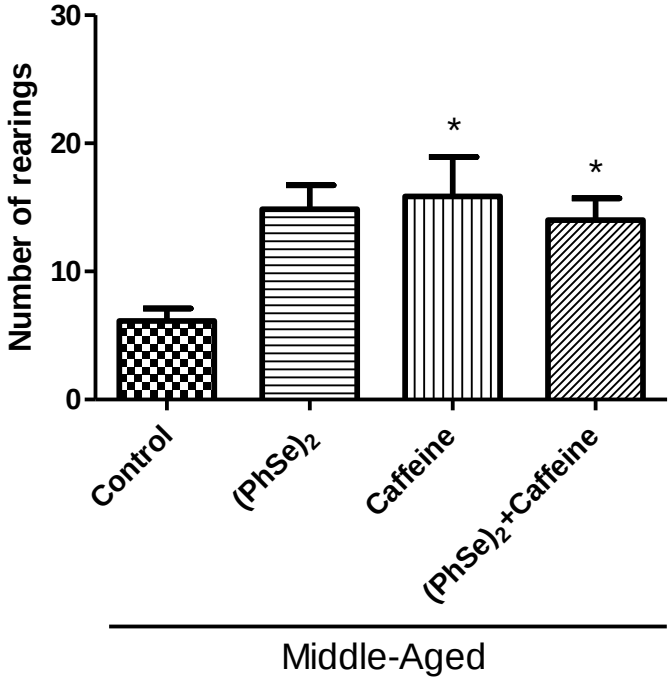

30
20
10
0
Number of rearings
*
*
Control
(PhSe)2
Caffeine
(PhSe)2+Caffeine
Middle-Aged

Fig. 4B

4. DISCUSSÃO

Nas últimas décadas os seres humanos têm conseguido um notável sucesso no prolongamento da vida. Entretanto, um problema emergente do século 21 é o número crescente de indivíduos com prejuízo cognitivo e demência na população idosa. Estima-se que um entre dois indivíduos com mais de 85 anos de idade sofrerão de algum tipo de demência (Deak e Sonntag, 2012). O SNC é extremamente afetado pelo processo de envelhecimento, que é caracterizado por alterações morfofuncionais as quais podem estar as mudanças nos processos bioquímicos.

Sabe-se que o estresse oxidativo aumenta durante o envelhecimento no cérebro. O SNC é particularmente mais afetado pelo dano oxidativo devido ao cérebro possuir elevado conteúdo de ácidos graxos polinsaturados facilmente oxidáveis, alto conteúdo de ferro e ascorbato (ingredientes chaves que influenciam na oxidação de lipídios de membrana), alto consumo de oxigênio por unidade de peso (cerca de 20% da quantidade total utilizada por humanos) além de não ser altamente enriquecido com defesas antioxidantes (Floyd, 1999). Todos esses fatores contribuem para a ocorrência do estresse oxidativo, gerado pelo aumento excessivo de EROS. Estudos anteriores têm demonstrado que a produção excessiva de EROS pode implicar no prejuízo cognitivo e distúrbios neuropatológicos tanto em animais experimentais velhos quanto em humanos idosos (Liu et al., 2003).

A cafeína apresenta efeitos antioxidantes (Gómez-Ruiz et al., 2007) além de ser efetiva em prevenir o prejuízo da memória. Aqui, neste primeiro estudo, foi demonstrado que tanto a cafeína quanto o SCH58261, um antagonista seletivo de receptores A_{2A}, revertem o déficit da memória de longo prazo de animais velhos quando avaliados no teste do reconhecimento do objeto. Além disso, a memória de curto prazo dos animais velhos foi melhorada. Também foi observada uma melhora na memória de curto e longo prazo nos animais jovens tratados com ambas cafeína e SCH58261. A adenosina, via RA A_1, inibe tanto a depressão de longa duração (de Mendonça et al., 1997) quanto potenciação de longa duração (de Mendonça e Ribeiro, 1994), eventos relacionados à plasticidade sináptica. A plasticidade sináptica tem um papel de extrema importância para os processos de aprendizado e memória. Além disso, a redução da ativação de RA A_{2A} parece ser relevante para a melhora da cognição (Wang et al., 2006). Assim, com base nestas considerações e nos resultados obtidos, cogita-se a possibilidade de que o antagonismo de RA A_1 e A_{2A} por parte da cafeína e do SCH58261 pode ser benéfico para impedir o prejuízo cognitivo influenciado pelo envelhecimento.

Adicionalmente, este estudo também mostrou uma diminuição parcial dos níveis de ER no cérebro de animais velhos tratados com cafeína e SCH5826. Uma vez que o declínio da memória com a idade está relacionado ao aumento de espécies reativas no cérebro (Head, 2009), o efeito na melhora da memória com cafeína e SCH58261 pode estar ligado, pelo menos em parte, a sua capacidade de diminuir as espécies reativas.

A Na^+K^+ATPase possui diversas funções neurais, modulando direta ou indiretamente a sinalização, liberação e captação de neurotransmissores bem como a neurogênese (Choi 1988; Xie e Askari 2002; Deisseroth et al. 2004). O tratamento com CAF e SCH58261 foi efetivo em normalizar a atividade da Na^+ K^+ ATPase em ratos velhos. No SNC, o aumento da excitabilidade neuronal consome mais energia célular. Tem sido demonstrado que cerca de metade ou mais do ATP total gerado no cérebro, no estado de repouso, é consumido pela Na^+K^+ ATPase para manter gradiente iônico transmembranar apropriado (Erecinska e Silver, 1989), mantendo as concentrações de K^+ altas e de Na^+ baixas no citoplasma, pois, esta condição é necessária para que potenciais de membrana e diversas atividades celulares mantenham-se normais. Levando isso em consideração, o decréscimo na atividade da Na^+K^+ATPase, consequentemente, reduz o gradiente eletroquímico Na^+/K^+, perturbando assim a homeostase iónica e as funções fisiológicas dos neurônios. Isto corrobora com os resultados demonstrados por Zhan et al. (2004), que observou um efeito de prejuízo no aprendizado e memória no labirinto aquático de Morris com a inibição da Na^+K^+ATPase por ouabaína. Assim o aumento da atividade da Na^+K^+ATPase em ratos velhos causado pela cafeína e SCH58261 também pode influenciar na melhora da memória espacial no teste do reconhecimento do objeto.

Após a confirmação dos efeitos benéficos da cafeína e SCH58261 em melhorar a memória de ratos velhos e considerando-se trabalhos anteriores que relatam a eficácia do $(PhSe)_2$ em melhorar a memória em modelos de déficit cognitivo (Souza et al., 2010; da Rocha et al., 2011), foi realizado um tratamento onde ratos de meia idade foram suplementados durante 30 dias com $(PhSe)_2$ (10 p.p.m. na ração) e cafeína (15 mg/kg, intragastrica) a fim de observar se estas drogas seriam capazes de melhorar a memória destes animais.

Utilizando o teste do reconhecimento de objeto foi observado que os animais de meia idade apresentavam um déficit na memória de curto e longo prazo quando comparados a animais jovens. Quando foi avaliada a memória de curto prazo, somente o grupo de animais tratados com $(PhSe)_2$ mostrou uma melhora quando comparados a animais jovens. Estranhamente, quando a memória de longo prazo foi avaliada, não foi observado nenhum efeito por parte do $(PhSe)_2$ em melhorar a memória dos animais de meia idade. Entretanto, os

ratos tratados com $(PhSe)_2$ e CAF mostraram uma melhora da memória de longo prazo. O tratamento somente com a cafeína não foi eficaz em melhorar a memória de curto e longo prazo. Aparentemente, a dose de 15 mg/kg ,administrada pelo período de 30 dias, não foi suficiente para reverter o déficit de memória causado pelo envelhecimento. Tanto a locomoção quanto a atividade exploratória dos ratos de meia idade foi prejudicada. Contudo, o tratamento com $(PhSe)_2$ e $(PhSe)_2$ associado a cafeína reverteu o déficit locomotor. Além disso, a atividade exploratória dos ratos de meia idade foi aumentada pela cafeína e $(PhSe)_2$ associado a cafeína.

Diversos trabalhos relatam os efeitos do $(PhSe)_2$ na melhora da cognição em roedores (Stangherlin et al., 2008; Souza et al., 2010; da Rocha et al., 2011). Entretanto o exato mecanismo pelo qual este composto orgânico de selênio proporciona a melhora da memória é desconhecido. Um dos seus possíveis efeitos na melhora da memória é sua capacidade antioxidante (Burger et al., 2004), pois, é bem relatada a influencia de espécies reativas no prejuízo da memória. Além disso, Rosa et al. (2003) propôs que o $(PhSe)_2$ poderia exercer suas ações na melhora da memória via estimulação da adenilato ciclase e/ou proteína quinase ativada por mitógeno (MAPK). É bem estabelecido que a via de sinalização AMPc/ proteína quinase A (PKA)/CREB, estimulada pela adenilato ciclase, e a via da MAPK no hipocampo e outras áreas do cérebro desempenham um papel crucial na formação da memória. Possivelmente, estes efeitos do $(PhSe)_2$ podem ter influenciado na melhora da memória de curto prazo em ratos de meia idade, embora não se tenha observado os mesmos resultados para a memória de longo prazo. Aparentemente, um efeito aditivo da CAF mais $(PhSe)_2$ foi suficiente para que os animais melhorassem a memória de longo prazo.

Apesar dos resultados obtidos e dos mecanismos considerados aqui, mais pesquisas se fazem necessárias para esclarecer os processos neuroquímicos envolvidos na melhora da memória por ação do SCH58261, CAF e $(PhSe)_2$.

5. CONCLUSÃO

Podemos concluir com os resultados apresentados nesta dissertação que

► Tanto a cafeína quanto o SCH58261 são capazes de reverter o prejuízo da memória de longo prazo em animais velhos. Além disso, estes dois compostos são capazes de melhorar a memória de ratos jovens e velhos. O estresse oxidativo parece ser um dos mecanismos envolvidos no efeito da cafeína e SCH58261 em melhorar a memória de ratos envelhecidos.

►A memória de curto e longo prazo foram melhoradas pela suplementação com $(PhSe)_2$ e $(PhSe)_2$ associado a cafeína, respectivamente, em ratos de meia idade, além destes tratamentos melhorarem a atividade locomotora e exploratória.

6. PERSPECTIVAS

Com base nos resultados obtidos neste trabalho, as perspectivas para trabalhos posteriores são:

- Estudar possíveis mecanismos envolvidos no efeito do $(PhSe)_2$ e da cafeína na melhora da memória em ratos de meia idade.

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