UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE

DEPARTAMENTO DE BIOQUÍMICA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS – BIOQUÍMICA

A ADMINISTRAÇÃO AGUDA DE CAFEÍNA PREVINE O COMPROMETIMENTO DA MEMÓRIA PELA ESCOPOLAMINA EM CAMUNDONGOS ADULTOS

PAULO HENRIQUE SALDANHA BOTTON

Orientadora

Profª. Drª. Lisiane de Oliveira Porciúncula

PORTO ALEGRE – RS

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PORTO ALEGRE – RS

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RESUMO

A cafeína é a substância psicoestimulante mais consumida no mundo todo. Muitos estudos já foram realizados avaliando os seus benefícios sobre as funções cognitivas. Algumas evidências sugerem a participação do sistema colinérgico nos efeitos da cafeína, mas os estudos ainda são incipientes. O objetivo desse estudo foi verificar os efeitos da administração aguda de cafeína frente ao bloqueio dos receptores colinérgicos muscarínicos pela administração do antagonista não-seletivo escopolamina. Camundongos adultos machos receberam cafeína (10 mg/kg, i.p.) uma vez ao dia durante 4 dias. No quinto dia, a escopolamina (2 mg/kg, i.p.) foi administrada imediatamente após a sessão de treino na tarefa de reconhecimento de objetos. Na tarefa de esquiva inibitória a escopolamina foi administrada 15 minutos antes ou imediatamente após a sessão de treino para avaliar aquisição e consolidação da memória. Na tarefa de reconhecimento de objetos, a cafeína preveniu o comprometimento da memória de reconhecimento pela administração pós-treino de escopolamina. A cafeína também foi efetiva em prevenir o comprometimento da consolidação da memória de curta e longa duração pela administração pós-treino de escopolamina. Entretanto, a cafeína só foi efetiva em prevenir o comprometimento da memória pela administração pré-treino da escopolamina quando o teste foi realizado 90 minutos após o treino (memória de curta duração). Esses resultados sugerem que os efeitos preventivos da cafeína observados em modelos de déficit mnemônico podem envolver a participação do sistema colinérgico.

ABSTRACT

Caffeine is the psychostimulant most consumed worldwide. Many studies have been performed evaluating its benefits on the cognitive functions. There is evidence to suggest the participation of cholinergic system on the effects of caffeine, but studies are still incipient. The aim of this study was to investigate the effects of acute administration of caffeine on memory impairment by the blockade of muscarinic cholinergic receptors. Adult male mice received caffeine (10 mg/kg, i.p.) once daily during 4 consecutive days. On the fifth day, the non selective antagonist for muscarinic receptors, scopolamine (2 mg/kg, i.p.) was administered immediately after training session on the novel object recognition task. In the inhibitory avoidance task, scopolamine was administered 15 minute

s prior to or immediately after training session to evaluate acquisition and consolidation of memory. In the novel object recognition task caffeine was effective in preventing the impairment of short- and long-term memory consolidation by post training administration of scopolamine. In the inhibitory avoidance task, caffeine was effective in preventing short-term memory impairment by administration of scopolamine prior to training. When scopolamine was administered after training, caffeine was able to prevent both short- e long-term memory impairments. These results suggest that the preventive effects of caffeine against mnemonic deficits may involve participation of the cholinergic system.

LISTA DE ABREVIATURAS

ACh	Acetilcolina
AChE	Acetilcolinesterase
AMPc	Adenosina monofosfato cíclica
ChAT	Colina-aciltransferase
DA	Doença de Alzheimer
i.p.	Intra-peritoneal
LTM	Memória de longa duração
PLC	Fosfolipase C
SNC	Sistema nervoso central
STM	Memória de curta duração

1. INTRODUÇÃO

1.1 CAFEÍNA

Conforme descrito por Weinberg e Bealer (Weinberg e Bealer, 2001) achados antropológicos sugerem que a cafeína já era consumida e apreciada cerca de 700 mil a.C. Atualmente, a cafeína pode ser considerada a substância psicoestimulante mais consumida no mundo (Fredholm *et al.,* 1999), e sua principal fonte na dieta é pelo consumo de café, cuja planta do gênero *Coffea* é originária da Etiópia. Após o surgimento das primeiras plantações na península arábica (no século XIV), o consumo de café se difundiu pela Europa no século XVI e pela América Latina no século XVIII.

A cafeína pode ser encontrada em fontes comuns da dieta como as bebidas a base de cola (40 mg / 350 mL), bebidas energéticas (80 mg/250 mL, em alguns casos) e chocolate. Além dessas fontes, a cafeína pode estar presente em mais de sessenta plantas (por exemplo, no guaraná).

Na América do Sul, a erva mate (*ilex paraguariensis*), ou *yerba mate*, em espanhol, era usada por indígenas para a preparação de uma infusão cujo consumo tinha fins sociais e medicinais. Suas propriedades estimulantes eram atribuídas à presença de metilxantinas, entre elas a cafeína (Heck e de Mejia, 2007).

Quimicamente a cafeína é denominada 7-diidro-1,3,7-trimetil-1H-purina-2,6-diona ou mais comumente como 1,3,7-trimetilxantina. Por ser uma molécula hidrofóbica, a cafeína atravessa facilmente as membranas biológicas incluindo a barreira hematoencefálica. Em neonatos, por exemplo, a

concentração plasmática de cafeína chega a ser semelhante à encontrada no líquido cérebro-espinhal (Turmen, Louridase Aranda, 1979; Somani, Khanna e Bada, 1980). No trato gastrointestinal de humanos e roedores ela é totalmente absorvida, porém a diferença no tempo de meia-vida, bastante menor em ratos (0,7 a 1,2 h) implica em uma correção da concentração pela massa corporal, em que a dose de 3,5 mg/kg em humanos representa uma dose de 10 mg/kg em ratos, o que equivale ao conteúdo de 2 a 3 copos de café (Fredholm *et al.,* 1999).

A cafeína é metabolizada no fígado principalmente pelas enzimas do sistema citocromo P-450 e entre seus metabólitos destacam-se a paraxantina, teobromina e teofilina (Lelo *et al.,* 1986, Arnaud, 1987). Em roedores, o principal metabólito encontrado no plasma é a paraxantina, mas os níveis de teofilina também são elevados (Fuhr, *et al*., 1996, Miners e Birketh, 1996). Esses metabólitos apresentam atividade farmacológica, logo isso deve ser considerado quando os efeitos da cafeína estão sendo estudados (Fredholm *et al.,* 1999).

Farmacologicamente, a cafeína é um antagonista não-seletivo dos receptores A_1 e A_{2A} de adenosina. Em doses moderadas, o que equivale a uma ingestão de 300 mg/dia, a cafeína alcança níveis séricos na faixa do μM, competindo com a adenosina com Ki de 29 e 48 μM para os receptores A_1 e A_{2A}, respectivamente (Fredholm *et al.,* 1999; Kot e Daniel, 2008). No entanto, em doses elevadas, a cafeína aumenta a liberação de cálcio intracelular, interfere nos receptores $GABA_A$ e inibe as fosfodiesterases (Cunha e Agostinho, 2010). Portanto, esses efeitos, que requerem concentrações de cafeína de 10 a 100 vezes maiores que as encontradas na dieta (Daly, 1999),

não são os responsáveis pelos efeitos psicoestimulantes da cafeína em humanos.

Nos últimos anos muitos trabalhos vêm investigando os efeitos da administração de cafeína sobre o desempenho cognitivo tanto em animais quanto em humanos. Os efeitos psicoestimulantes decorrentes do antagonismo dos receptores de adenosina incluem aumento da atenção e do estado de alerta, e diminuição da fatiga (Griffiths *et al.,* 1990; Fredholm *et al.,* 1999; Huang *et al*., 2005). Apesar de não haver um consenso a respeito dos efeitos da cafeína sobre o aprendizado e memória, muitos estudos têm mostrado efeitos positivos da cafeína sobre o desempenho cognitivo de animais e humanos (Jarvis, 1993; Costa *et al.,* 2008a).

Em um trabalho realizado com voluntários humanos a cafeína foi administrada sob dois regimes diferentes: um deles simulando o consumo real, ou seja, várias pequenas doses (65 mg) em um total de 5 horas, ou então, em uma única dose (200 mg). Em ambos os regimes de administração a cafeína aumentou a atenção dos voluntários e melhorou o desempenho em diferentes tarefas cognitivas (Brice e Smith, 2002). Em roedores, resultados do nosso grupo mostraram que a administração de cafeína (10 mg/kg, i.p.) sob o mesmo regime adotado nesse trabalho foi capaz de melhorar o desempenho de camundongos adultos na tarefa de reconhecimento de objetos (Costa *et al.,* 2008b).

Além disso, trabalhos demonstraram que o consumo de cafeína pode estar relacionado a um menor risco de desenvolvimento de doença de Alzheimer (DA) (Maia e Mendonça, 2002; Ritchie *et al*., 2007) e Parkinson (Xu, Bastia e Schwarzschild, 2005). Em um modelo experimental da DA, a cafeína

foi capaz de prevenir o déficit cognitivo provocado pelo peptídeo β-amilóide, (Dall'Igna *et al.,* 2007) cujo acúmulo em placas senis é uma das características fisiopatológicas da DA (Castelllani, Rolston e Smith, 2010). A cafeína também mostrou-se benéfica em modelos experimentais da doença de Parkinson (Chen *et al.,* 2001; Xu, Xu e Chen, 2002)

Por outro lado, outros estudos mostram que a administração de cafeína pode provocar apenas efeitos discretos sobre a memória, ou mesmo nenhum efeito (Angelucci et al., 1999; van Boxtel et al*.,* 2003; Childs e de Wit, 2006).

1.2. SISTEMA ADENOSINÉRGICO

A transmissão purinérgica foi proposta por Burnstock no início da década de 70 do século passado, após a observação da ação do ATP como neurotransmissor em *Taenia coli* de roedores (Burnstock, 1972). A sinalização intercelular realizada por purinas é encontrada desde cedo na evolução, tornando-se uma rota amplamente distribuída e ligada à comunicação célula-célula (Burnstock, 2008).

Vários tecidos apresentam rotas de sinalização intercelulares nas quais nucleosídeos e nucleotídeos derivados das purinas exercem papel fundamental (Burnstock e Knight, 2004) e, em especial no SNC, diversos trabalhos vêm demonstrando a importância do papel desempenhado pelo ATP e pela adenosina.

A adenosina é uma purina onipresente. Além de fazer parte do *pool* energético celular, essa purina funciona ainda como um mensageiro

extracelular. Além disso, a adenosina está envolvida em funções essenciais para as células, como a síntese de ácidos nucléicos e metabolismo de aminoácidos (Lloyd, Lindström e Fredholm, 1993).

Apesar de não poder ser classificada como um neurotransmissor clássico como o ATP, já que não é armazenada em vesículas, não é liberada por exocitose e não atua predominantemente em sinapses (Cunha, Almeida e Ribeiro, 2001), a adenosina é classificada como um neuromodulador, exercendo influência sobre algumas funções do SNC, tais como liberação de neurotransmissores e excitabilidade neuronal (Dunwiddie & Hoffer 1980, Kocsis *et al* 1984, Fredholm et al., 2005; Ferré, 2008). Além disso, a adenosina atua mantendo a homeostase intracelular no SNC e em todas as células em que esteja presente (Cunha, 2001 e 2005),

A adenosina encontrada intracelularmente pode ser oriunda de duas fontes: da clivagem da S-adenosil-homocisteína catalisada pela enzima S-adenosil-homocisteína hidrolase (Schrader et al*.,* 1981), ou pela degradação do monofosfato de adenosina (AMP), pela enzima 5'-nucleotidase (Colgan *et al.,* 2006). Já a adenosina encontrada no meio extracelular pode ser formada por três vias distintas: (1) a sua liberação como tal, através de transportadores de nucleosídeos (Geiger e Frida, 1991) quando ocorre uma aumento da adenosina intracelular; (2) a partir da adenosina monofosfato cíclica (AMPc) após sua liberação no meio extracelular; e (3) por meio da ação das ectonucleotidases, quando ocorre liberação de nucleotídeos de adenina (principalmente o ATP) (Latini e Pedata, 2001). A concentração extracelular de adenosina, assim como a origem do ATP a partir do qual ela é originada, pode determinar sobre qual o receptor ela vai exercer suas ações e, conseqüentemente, as respostas

farmacológicas desencadeadas (Correia de Sá, Timóteo e Ribeiro 1996; Cunha et al 1996, Cunha, 2008a).

Os receptores responsáveis pelas ações do sistema purinérgico são classificados em dois grandes grupos: os P2, subdivididos em P2X e P2Y, através dos quais o ATP exerce sua sinalização; e os P1, responsáveis pela sinalização da adenosina (Sebastião e Ribeiro, 2009). Os receptores adenosinérgicos foram identificados na década de 70, do século XX, principalmente devido ao antagonismo exercido por metilxantinas como teofilina e cafeína. Até os anos 80, a existência de ao menos dois receptores adenosinérgicos estava confirmada, os A_1 e os A_{2A} (van Calke, Muller e Hamprecht, 1979; Londos, Cooper e Wolff, 1980). Até o presente momento, quatro subtipos de receptores P1 foram clonados: A_1, A_{2A}, A_{2B} e A_3, sendo todos acoplados a proteínas G. Os receptores A_1 e A_{2B} se ligam à família das proteínas G inibitórias (Gi), inibindo a produção do segundo mensageiro AMPc, enquanto os receptores A_{2A} e A_3 estão acoplados a proteínas G estimulatórias (Gs), que, por ativar a enzima adenilato ciclase, aumentam o AMPc intracelular. No estriado, os A_{2A} estão acoplados a proteínas G_{olf} (Kull, Svenningsson e Fredholm, 2000; Fredholm et al*.,* 2001; Borea et al., 2009). Enquanto a localização dos receptores A_1 e A_{2A} está bem caracterizada, o mesmo não pode ser dito em relação aos A_{2B} e A_3, cujos estudos são menos conclusíveis. O receptor A_1 está amplamente distribuído tanto no encéfalo humano quanto no de roedores (Mahan et al*.,* 1991; Reppert et al*.,* 1991). O receptor A_{2A} também pode ser encontrado em todo o encéfalo, porém sua expressão é maior no estriado (Schiffmann et al*.,* 1991; Svenningsson et al*.,* 1997; Fredholm et al*.,* 2005).

Concentrações de cafeína obtidas após a ingestão de apenas um copo de café (40–180 mg de cafeína) já são suficientes para bloquear os receptores pré e pós-sinápticos A_1 e A_{2A}, diminuindo o tônus inibitório da adenosina e resultando em uma ação psicoestimulante (Daly, 2007; Fredholm et al*.,* 2005). De maneira interessante, diversas evidências sugerem que os efeitos da adenosina sobre o sono são mediados via receptores A_1 (Basheer et al*.,* 2004). No entanto, estudos com abordagens farmacológicas (Nehlig, Daval e Debry, 1992; Fredholm 1995) ou com deleção genética mostraram que os efeitos psicoestimulantes da cafeína estão mais relacionados ao bloqueio seletivo do receptor A_{2A} (Fredholm et al*.,* 1999; El Yacoubi et al*.,* 2000; Chen et al*.,* 2001, Huang et al*.,* 2005),

Embora os estudos sobre o papel da adenosina nos processos que envolvam o aprendizado e memória sejam mais escassos, foi observado que a ativação dos receptores A_1 e A_{2A} parece prejudicar a memória (Suzuki et al*.,* 1993; Kopf et al*.,* 1999, Corodimas e Tomita, 2001). A estimulação desses receptores por meio da infusão respectiva dos agonistas CPA e CGS 21680 no córtex cingulado posterior comprometeu a evocação da memória de ratos submetidos à tarefa de esquiva inibitória (Pereira et al., 2005). Entretanto, a cafeína e o bloqueio seletivo de ambos os receptores parece melhorar o desempenho cognitivo de roedores em diferentes tipos de tarefas como esquiva inibitória (Pereira et al*.,* 2002), labirinto aquático de Morris (Angelucci et al*.,* 2002) e reconhecimento de objetos (Costa et al*.,* 2008b).

O sistema adenosinérgico vem ganhando destaque no estudo de patologias do SNC, uma vez que particularmente os receptores adenosinérgicos A_1 e A_{2A} têm sido considerados alvos promissores para o

tratamento de doenças neurodegenerativas agudas e crônicas no sistema nervoso central (SNC) (Cunha, 2008b; Jenner *et al.,* 2010; Chen e Chern, 2010).

1.3.SISTEMA COLINÉRGICO

O conceito de sinapse foi proposto por Sherrington no final do século XIX, que o referia como uma propriedade particular da zona entre dois neurônios ou entre um neurônio e suas células efetoras (Sherrington, 1897; Shepherd e Erulkar, 1997). Porém, as limitações das ferramentas de microscopia da época limitavam a elucidação dos componentes e da estrutura da sinapse. Em 1921, Loewi estimulou o nervo vago de um coração isolado de sapo e verificou que tal estímulo cessava os batimentos. Ao perfundir outro coração com a solução fisiológica coletada do primeiro, o mesmo aconteceu, porém, sem estímulo prévio ao nervo vago, o que o levou a concluir que a substância liberada dos terminais do nervo vago era a responsável pela parada dos batimentos. A partir disso, a acetilcolina (ACh) foi identificada como a substância responsável pelos efeitos observados nos experimentos de Loewi (Anglade e Larabi-Godinot, 2010), sendo então o primeiro neurotransmissor identificado. Ela é amplamente difundida no SNC, sistema nervoso periférico (SNP), autônomo e entérico. Sua síntese ocorre na extremidade de neurônios colinérgicos e é catalisada pela colina-acetiltransferase (ChAT), que transfere um grupo acetil oriundo da acetil-coenzima A para a colina. A colina deve ser fornecida pela dieta, pois os neurônios não são capazes de sintetizá-la. No

entanto, a administração de colina, apesar de aumentar sua disponibilidade no cérebro, não necessariamente aumenta a síntese ou a liberação de ACh (Amenta e Tayebati, 2008).

Em 1914, Dale classificou os efeitos da ACh como semelhantes aos da nicotina ou semelhantes aos da muscarina. Atualmente se sabe que, quando liberada na fenda sináptica, a ACh se liga a duas famílias de receptores: os nicotínicos (ionotrópicos) e o muscarínicos (metabotrópicos) (Brown, 2010). Os primeiros, ao serem ativados, permitem o influxo de íons como Na^+, K^+ e Ca^+. Já os receptores muscarínicos, que são os mais abundantes e funcionalmente predominantes (Brown, 2010), pertencem à classe dos receptores acoplados à proteína G (Caulfield, 1993).

Os estudos de clonagem conseguiram identificar cinco subtipos de receptores muscarínicos: M_1-M_5. Os ímpares, M_1, M_3 e M_5, ligam-se preferencialmente a proteínas G da família Gq/11, que ativam a fosfolipase C (PLC). Já os M_2 e M_4 se ligam a proteínas G da família $G_{i/o}$ e podem afetar a adenilato ciclase e vários canais iônicos (Conn, Jones e Lindsley, 2009). Muitos neurônios colinérgicos podem expressar mais de um tipo de receptor muscarínico (Hassal *et al.,* 1993), sendo que a distribuição dos cinco subtipos no SNC e em tecidos periféricos é heterogênea (tabela 1). Em áreas como hipocampo e córtex, reconhecidamente importantes para os processos de aprendizado e memória, o receptor mais expresso é o M_1. No entanto, outros receptores muscarínicos também podem ser encontrados nessas regiões (Klinkengerg e Blokland, 2010).

A acetilcolinesterase (AChE) é a enzima responsável pela degradação da ACh na fenda sináptica, o que provoca a eliminação dos efeitos desse

neurotransmissor. Na DA, em que o sistema colinérgico é um dos principais alvos da neurodenegeração característica da patologia, o uso de inibidores da AChE é uma das principais estratégias no manejo dos sintomas (Castellani *et al.,* 2010).

SNC					
Área do Encéfalo	**M1**	**M2**	**M3**	**M4**	**M5**
Nucleos da base/septo		xx			
Córtex	xx		x	x	
Hipocampo	xx	x	x	x	x
Amígdala	x				
Estriado	x	x	x	xx	
Tecidos periféricos					
Órgão	**M1**	**M2**	**M3**	**M4**	**M5**
Coração		xx			
Pulmão		x	x	xx	
Íleo		x	xx		
Glândulas exócrinas	x		xx		

Tabela 1: Distribuição central e periférica dos diferentes subtipos de receptores muscarínicos. x: baixa/fraca expressão de RNAm ou imunoprecipitação; xx: alta/forte expressão de RNAm ou imunoprecipitação; (Adaptada de Klinkenberg e Blokland, 2010).

As investigações sobre mudanças em processos cognitivos após manipulação dos receptores colinérgicos é bastante focada nos campos de memória e atenção. Existem muitas evidências que apontam para a

participação de receptores muscarínicos em processos de aprendizado e memória. Em humanos, por exemplo, a administração de fisostigmina, um inibidor da AChE, tem mostrado efeitos positivos sobre a memória de trabalho (Furer *et al.,* 1997; Kirrane *et al.,* 2001). Enquanto isso, o bloqueio não-seletivo dos receptores muscarínicos pela escopolamina tem se mostrado prejudicial ao desempenho cognitivo tanto de humanos (Koller *et al.,* 2003; Green *et al.,* 2005; Ellis *et al.,* 2006) quanto de animais (Bymaster *et al.,* 1993; Wilson e King, 2000).

1.4. ESCOPOLAMINA

Na antigüidade já se sabia que a ingestão de extratos de algumas plantas poderia influenciar o estado mental dos indivíduos. Os extratos de plantas como a Belladona e a Datura, entre outros, eram usados como “poções mágicas” para provocar alucinações. Após a ingestão, os indivíduos perdiam o senso da realidade e não eram capazes de recordar o que acontecera durante a intoxicação. Já na idade média, a Belladona passou a ser usada por mulheres que queriam ficar mais atraentes por suas pupilas dilatadas. Mais tarde, descobriu-se que essas plantas eram ricas em alcalóides tropânicos que eram responsáveis por tais efeitos, dentre eles, a escopolamina.

A escopolamina é um antagonista colinérgico, não-seletivo, dos receptores muscarínicos. Suas propriedades amnésicas têm sido reportadas desde o início do século XX (Gaus, 1906; Thompson e Cotterril, 1909). Dessa forma, a escopolamina vem sendo aplicada no campo da

neuropsicofarmacologia como uma droga de referência na indução de modelos de prejuízo cognitivo relacionado à idade ou à demência, característicos do déficit colinérgico tanto em humanos quando em animais (Klinkenberg e Blokland, 2010). Tais efeitos podem ser revertidos por determinadas drogas, o que torna o modelo de déficit cognitivo induzido por escopolamina uma ferramenta muito utilizada na avaliação da eficácia e efetividade de novas drogas colinérgicas.

Diversas tarefas comportamentais são aplicadas para se avaliar os efeitos da escopolamina sobre o desempenho mnemônico de animais, entre elas estão o reconhecimento de objetos e a esquiva inibitória. A infusão de escopolamina no córtex perirrinal ou no córtex insular foi capaz de prejudicar o desempenho de ratos submetidos ao reconhecimento de objetos (Warburton *et al.,* 2003; Abel, Ishida e Iwasaki 2004; Bermudez-Ratoni *et al.,* 2005). Em testes de esquiva inibitória, a administração de escopolamina no córtex frontal (Santucci e Shaw, 2003) e no hipocampo (Wiener e Messer, 1973) tende a prejudicar o desempenho dos animais.

O efeito amnésico característico da administração de escopolamina a torna uma ferramenta farmacológica bastante interessante para estudos de mecanismos e novos fármacos associados a patologias do SNC, principalmente àquelas relacionadas ao sistema colinérgico, uma vez que contempla déficit cognitivo e perda da memória característicos de demência, e, até mesmo, mimetiza sintomas da DA (Deiana *et al*., 2009).

2. OBJETIVOS

2.1. OBJETIVO GERAL

Considerando a participação do sistema adenosinérgico em processos de aprendizado e memória, sendo que a cafeína tem sido apontada como uma substância que exerce efeitos positivos sobre o desempenho cognitivo de humanos e animais, e o sistema colinérgico é um dos principais alvos de neurodegeneração em patologias no SNC, incluindo a DA, o objetivo desse trabalho foi verificar se um tratamento com cafeína seria capaz de prevenir o prejuízo cognitivo induzido pela administração de escopolamina, um antagonista colinérgico, em camundongos machos adultos.

2.2. OBJETIVOS ESPECÍFICOS

- Verificar os efeitos da administração de quatro doses diárias de cafeína sobre o comprometimento causado pela escopolamina no desempenho da tarefa de reconhecimento de objetos;

- Analisar os efeitos da cafeína, sob o mesmo regime de administração acima citado, sobre o déficit induzido pela administração pré e pós-treino de escopolamina no desempenho da tarefa de esquiva inibitória.

ARTIGO CIENTÍFICO

Caffeine prevents disruption of memory consolidation in the inhibitory avoidance and novel object recognition tasks by scopolamine in adult mice.

Paulo Henrique Botton, Marcelo S. Costa, Ana Paula Ardais, Sabrina Mioranzza, Diogo O. Souza, João Batista Teixeira da Rocha, Lisiane O. Porciúncula.

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Research report

Caffeine prevents disruption of memory consolidation in the inhibitory avoidance and novel object recognition tasks by scopolamine in adult mice

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ABSTRACT

Caffeine is a psychostimulant with positive effects on cognition. Recent studies have suggested the participation of the cholinergic system in the effects of caffeine on wakefulness. However, there are few studies assessing the contribution of cholinergic system in the cognitive enhancer properties of caffeine. In the present study, the effects of a dose and schedule of administration of caffeine that improved memory recognition were investigated on scopolamine-induced impairment of memory in adult mice. Inhibitory avoidance and novel object recognition tasks were used to assess learning and memory. Caffeine (10 mg/kg, i.p.) was administered during 4 consecutive days, and the treatment was interrupted 24 h before scopolamine administration (2 mg/kg, i.p.). Scopolamine was administered prior to or immediately after training. Short-term and long-term memory was evaluated in both tasks. In the novel object recognition task, pre treatment with caffeine prevented the disruption of short- and long-term memory by scopolamine. In the inhibitory avoidance task, caffeine prevented short- but not long-term memory disruption by pre training administration of scopolamine. Caffeine prevented short- and long-term memory disruption by post training administration of scopolamine. Both treatments did not affect locomotor activity of the animals. These findings suggest that acute treatment with caffeine followed by its withdrawal may be effective against cholinergic-induced disruption of memory assessed in an aversive and non-aversive task. Finally, our results revealed that the cholinergic system is involved in the positive effects of caffeine on cognitive functions.

1. Introduction

Caffeine is one of the most consumed substance around the world. Its psychostimulant effects on the central nervous system (CNS) include promotion of wakefulness, increase of arousal and decrease in fatigue [22,27]. The blockade of adenosine A_1 and A_{2A} receptors is considered the primary pharmacological target of caffeine and this non-selective antagonism seems to be responsible for the psychostimulant effect of caffeine in the CNS [20,22].

The cholinergic system of the forebrain complex projecting to the cortex takes part in mediating attention, and degeneration of the cholinergic neurons of the Nucleus Basalis of Meynert (NBM) is thought to be part of the decline in cognitive functions observed in Alzheimer's disease (AD) [43]. Peripheral or intracerebral administration of scopolamine, a non-selective muscarinic acetylcholine receptor antagonist, produces deficits in a variety of spatial tests such as elevated T-maze maze, non-spatial working memory tests [15,33], aversive task as the inhibitory avoidance [31,39], and non-aversive tasks as the novel object recognition [17,41,42]. Thus, scopolamine is very suitable for investigating learning and memory studies with the involvement of cholinergic system, since the cholinergic activity takes part of this memory formation process.

Recent studies have pointed to the participation of other neurotransmitter systems in the stimulant effects of caffeine. In particular, caffeine seems to promote arousal by enhancing cortical cholinergic transmission [46], and when chronically administered caffeine increased the release of acetylcholine in the prefrontal cortex of adult rats [1]. Behavioral studies also have shown the participation of adenosinergic system in the mnemonic deficits caused by administration of cholinergic antagonists [11,40].

The positive effects of caffeine on cognition have been evidenced in human as well as in animal studies, albeit data from human studies present some divergences according to schedule, withdrawal and doses of administration [2,7,8,10,24,25,47]. Two

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epidemiological studies have evidenced preventive effects of caffeine against dementia associated to Alzheimer's disease [29,38]. Caffeine also prevented neuronal death and memory deficits in experimental models of AD [3,12,13]. Likewise, caffeine was effective in preventing aging cognitive decline in two different treatment regimen in rodents [9,34].

Despite of the documented importance of the cholinergic system in the cognitive functions, there are few studies investigating the participation of this system in the cognitive enhancer properties of caffeine [37]. In this study, effects of caffeine on scopolamine-induced memory deficits were assessed in a dose regimen that improved adult mice object recognition memory [10]. Thus, it was investigated whether the caffeine treatment mentioned above with its concomitant withdrawal could prevent scopolamine-induced amnesia in two different tasks used to evaluate learning and memory.

2. Material and methods

2.1. Animals

Male CF1 mice (3–4 months old) were obtained from Stated Foundation for Health Science Research (FEPPS, Porto Alegre/RS, Brazil). All experimental procedures were performed according to the NIH Guide for Care and Use of Laboratory Animals and to Brazilian Society for Neuroscience and Behaviour (SBNeC) recommendations for animal care. This work was approved by the ethical committee of Federal University of Rio Grande do Sul. A total number of 349 animals were used. Mice were housed in standard cages and kept 4 to a cage under a reversed 12/12 h-light/dark cycle with free access to food and water. Independent groups of mice were used for each behavioral test. All behavioral tests were performed between 8:00 a.m. and 5:00 p.m.

2.2. Treatment

Caffeine (10 mg/kg, i.p.) or saline (0.9 g%, i.p.) in a single injection per day were administered during 4 consecutive days in a dose equivalent to 2–3 cups of coffee [22]. At the fifth day, mice received a single injection of scopolamine hydrobromide (2 mg/kg, i.p.) (Sigma, São Paulo/SP, Brazil) administered in saline (0.9 g%) as a vehicle 15 min prior to (acquisition) or immediately after training (consolidation) in the inhibitory avoidance task. For the novel object recognition task, scopolamine was immediately administered after mice had been exposed to two similar objects (training session). Scopolamine was administered 90 min before placing mouse in the open field arena in order to discard effects on locomotor activity in both tasks. Scopolamine was administered at the dose of 2 mg/kg (i.p.) in order to assure memory deficits 24 h after training. The dose of scopolamine chosen was lower than previously used with this mice strain [14]. Independent groups of animals were used for each behavioral test.

2.3. Object recognition task

The apparatus consisted of a black-painted wood small chamber with the following dimensions: 50 cm × 25 cm × 25 cm. Mice had been acclimated in the apparatus during 10 min 24 h before starting the task. The training session consisted into placing a mouse in the apparatus containing two similar objects and allowed it to explore for 10 min. Each mouse was always placed in the apparatus facing the wall and after the test mouse was put back in its home cage. The objects were positioned in two adjacent corners, 9 cm from the walls. The objects presented similar textures, colors and sizes, but different shapes in the test session (Duplo Lego toys). The test session was performed 90 min (short-term memory) or 24 h after training (long-term memory) and two dissimilar objects were present, a familiar and a novel one. Discrimination ratio for each mouse was expressed by $T_N/(T_N + T_F)$ ratio [T_F = time spent exploring familiar object; T_N = time spent exploring the novel object]. The objects were cleaned with 10% ethanol solution between trials. Exploration was defined by directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose or forepaws. Sitting on the object was not considered exploratory behavior.

2.4. Inhibitory avoidance task

The inhibitory avoidance task was assessed in an apparatus consisted of an acrylic box (50 cm × 25 cm × 25 cm) whose floor contains parallel caliber stainless-steel bars (1 mm diameter) spaced 1 cm apart. A platform (2 cm high and 4 cm × 6 cm wide) was placed in the center of the box. In the training session, mice were placed on the platform and the latency to step-down onto the floor with the four paws was measured with an automatic device; immediately after stepping-down mice received a 0.5 mA, 2 s footshock. After they had received the footshock, mice were immediately placed in their home cage. The test session was carried out 90 min after training (short-term memory) or 24 h after training (long-term memory). No footshock was given in the test session, and step-down latencies (180 s ceiling) were taken as a measure of retention.

2.5. Open field task

The open field test represents a widely used model for the evaluation of locomotor activity. The apparatus was made of black-painted Plexiglas measuring 50 cm × 50 cm and was surrounded by 50 cm high walls. The experiments were conducted in a sound-attenuated room under low-intensity light (12 lx). Each mouse was placed in the center of the arena and the distance traveled was recorded during 10 min. The experiment was recorded with a video camera positioned above the arena and monitored in an adjacent room by an observer blind to the drug treatment of the animals. The analysis was performed using a computer-operated tracking system (Any-maze, Stoelting, Woods Dale, IL).

2.6. Statistical analysis

Step-down latencies are expressed as medians (interquartile ranges). Wilcoxon test was used to analyze differences between training and test latencies of the same group and Mann–Whitney U test (two-tailed) was used to compare treatments. For object recognition test, three-way ANOVA pre treatment × treatment × repeated measures (as independent variables) was performed. For the open field test, differences between groups were analyzed by using one-way ANOVA and Newman–Keuls for multiple comparisons as a post hoc test. Graphpad Prism 4 and SPSS were softwares used and significant differences were considered when $P < 0.05$.

3. Results

3.1. Object recognition task

The influence of pre treatment with caffeine on scopolamine-induced memory impairment was initially investigated in the novel object recognition task that consists on a non-aversive task. As a normal behavior, mice spent less time on the familiar object in the test session when comparing to training. Three-way ANOVA pre treatment × treatment × trials (as repeated measures) revealed a significant main effect of trials [F (1,49) = 140.9; $P < 0.001$] and a significant three-way interaction [F (1,49) = 7.53; $P = 0.0084$] (Fig. 1A). Thus, scopolamine caused a decrease in the difference between training and test score, whereas pre administration of caffeine abolished the partial amnesic effect of scopolamine. For the object recognition index, three-way ANOVA revealed a significant main effect of trials [F (1,49) = 49.2; $P < 0.001$] and significant effect of pre treatment × trials [F (1,49) = 8.40; $P = 0.0055$] and treatment × trials [F (1,49) = 5.269; $P = 0.026$] interactions (Fig. 1B). As observed, scopolamine caused impairment in the novel object recognition and pre treatment with caffeine was able to prevent it (Fig. 1B). Furthermore, according to our previous data, mice pre treated with caffeine that received saline in the training session presented higher recognition index than saline group in the test session [F (7, 105) = 11.83, $P < 0.0001$] (Fig. 1B).

Long-term memory was also assessed for recognition object memory. Three-way ANOVA revealed a significant main effect of trials [F (1,45) = 36.44 = $P < 0.001$] and significant effect of treatment × trials [F (1,45) = 6.35, $P = 0.015$] (Fig. 2A). Different from other mice groups, only mice pre treated with saline that received scopolamine after training did not show differences between training and test sessions on the time spent in the familiar object. For object recognition index, three-way ANOVA revealed a significant main effect of trials [F (1,45) = 31.96 = $P < 0.001$] and a significant three-way interaction [F (1,45) = 15.30; $P = 0.000306$]. Thus, mice pre treated with saline that received scopolamine immediately after training did not present difference between training and test session indexes (Fig. 2B). However, mice pre treated with caffeine that received scopolamine after training showed significant difference in the recognition index between training and test session (Fig. 2B).

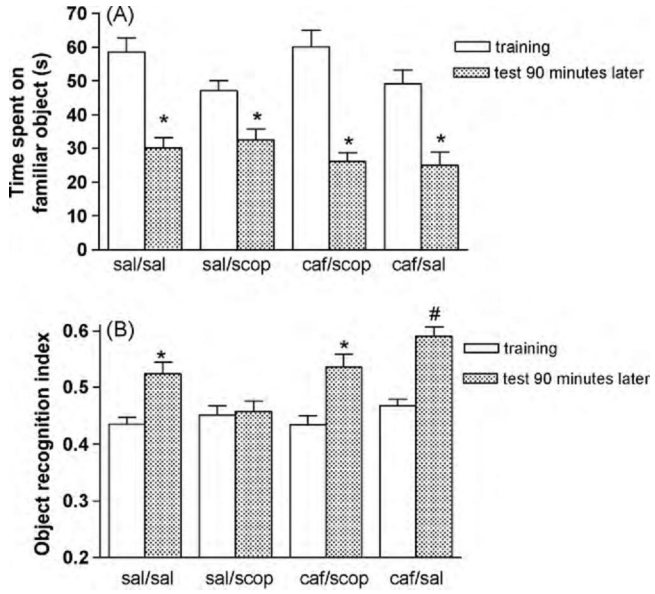

Fig. 1. Caffeine treatment on scopolamine-induced memory impairment on object recognition task–short-term memory. sal/sal: pre-treatment with saline (0.9 g%, i.p.) during 4 days before a single injection of saline (0.9 g%, i.p.) immediately after training; sal/scop: pre-treatment with saline (0.9 g%, i.p.) during 4 days before a single injection of scopolamine (2 mg/kg, i.p.) immediately after training; caf/scop: pre-treatment with caffeine (10 mg/kg, i.p.) during 4 days before a single injection of scopolamine (2 mg/kg, i.p.) immediately after training; caf/sal: pre-treatment with caffeine (10 mg/kg, i.p.) during 4 days before a single injection of saline (0.9 g%, i.p.) immediately after training; (A) time spent on familiar object in seconds. Results are presented as media + S.E.M. of 12–14 mice. $^{*}P < 0.01$, differences between time spent on the familiar object in the training and test session. (B) Object recognition index. Results are presented as mean + S.E.M. of 12–14 mice. $^{*}P < 0.01$, differences between recognition index in the training and test session. $^{\#}P < 0.05$, differences between recognition index of sal/sal and caf/sal groups in the test session.

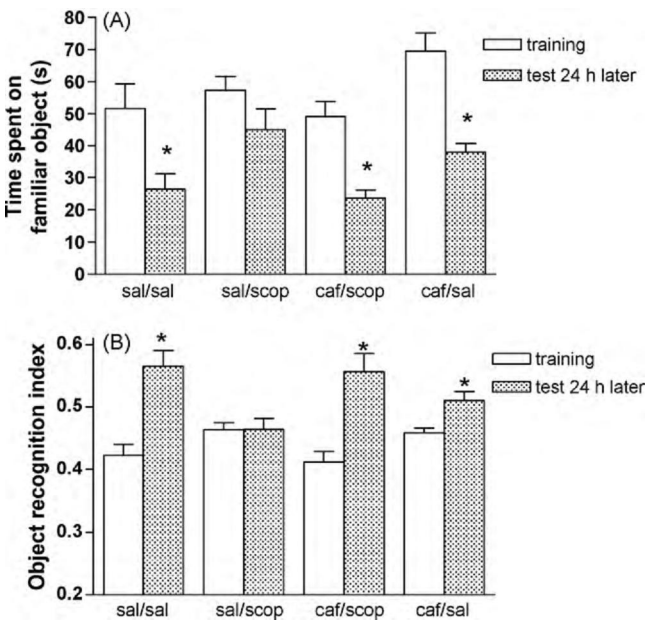

Fig. 2. Caffeine treatment on scopolamine-induced memory impairment on object recognition task–long-term memory. sal/sal: pre-treatment with saline (0.9 g%, i.p.) during 4 days before a single injection of saline (0.9 g%, i.p.) immediately after training; sal/scop: pre-treatment with saline (0.9 g%, i.p.) during 4 days before a single injection of scopolamine (2 mg/kg, i.p.) immediately after training; caf/scop: pre-treatment with caffeine (10 mg/kg, i.p.) during 4 days before a single injection of scopolamine (2 mg/kg, i.p.) immediately after training; caf/sal: pre-treatment with caffeine (10 mg/kg, i.p.) during 4 days before a single injection of saline (0.9 g%, i.p.) immediately after training; (A) time spent on familiar object in seconds. Results are presented as media + S.E.M. of 11–13 mice. $^{*}P < 0.01$, differences between time spent on the familiar object in the training and test session. (B) Object recognition index. Results are presented as mean + S.E.M. of 11–13 mice. $^{*}P < 0.01$, differences between recognition index in the training and test session.

3.2. Inhibitory avoidance task

Previous administration of caffeine during four days before scopolamine was evaluated in the inhibitory avoidance task in two memory phases: acquisition and consolidation. For the acquisition memory process, scopolamine was administered 15 min before training session. Scopolamine caused impairment on the acquisition of memory since the latencies between training and test session were not statistically different for short-term as well as for long-term memory (Fig. 3A and B). Previous treatment with caffeine was effective in preventing the impairment of short-term memory caused by pre training treatment with scopolamine (Fig. 3A). However, mice previously treated with caffeine that received pre training injection of scopolamine did not present difference between training and test latencies for the long-term memory (Fig. 3B). Thus, pre treatment with caffeine did not prevent scopolamine-induced impairment in the acquisition phase when long-term memory was assessed.

For the memory consolidation, the effects of pre treatment with caffeine were evaluated in mice that received saline or scopolamine immediately after training session. The latencies between training and test session were not statistically different in mice pre treated with saline that received scopolamine immediately after training session (Fig. 4A and B). Pre treatment with caffeine was able to prevent the amnesic effects caused by scopolamine, since the latencies between training and test session were statistically different for short-term memory (Fig. 4A) as well as long-term memory (Fig. 4B). For consolidation memory, pre treatment with caffeine was able to prevent the disruption of short- and long-term memory caused by scopolamine.

3.3. Open field

In order to assess the general mobility of mice according to the treatment, mice were exposed to an open field and traveled distance was recorded during 10 min. Scopolamine administered 90 min before placing mouse in the open field did not cause any effect on the traveled distance during 10 min of video recordings. Likewise, mice pre treated with caffeine and saline or scopolamine did not present any alterations in their locomotor activity (Fig. 5).

4. Discussion

In this study, scopolamine efficiently disrupted the recognition memory for the novel object recognition, a task widely used to assess recognition memory performance in rodents [19]. Comparing to other tasks, the object recognition taxes memory after only one trial, which makes this test very sensitive to memory impairment by pharmacological interventions [16]. Pre treatment with caffeine was effective in preventing the impairment of short-term as well as long-term memory by scopolamine for novel object recognition. In addition, caffeine at the same dose and schedule of administration confirmed its positive effects in the object recognition task when short-term memory was assessed [10].

Given that acute administration of the same dose of caffeine used here usually causes hyperlocomotion in rodents [18], the treatment was interrupted 24 h before scopolamine administration. Additionally, open field test was carried out with the same treatment regimen used to evaluate the novel object recognition task and data confirmed that all groups of mice did not differ in the traveled distance. Thus, the preventive effects of caffeine on

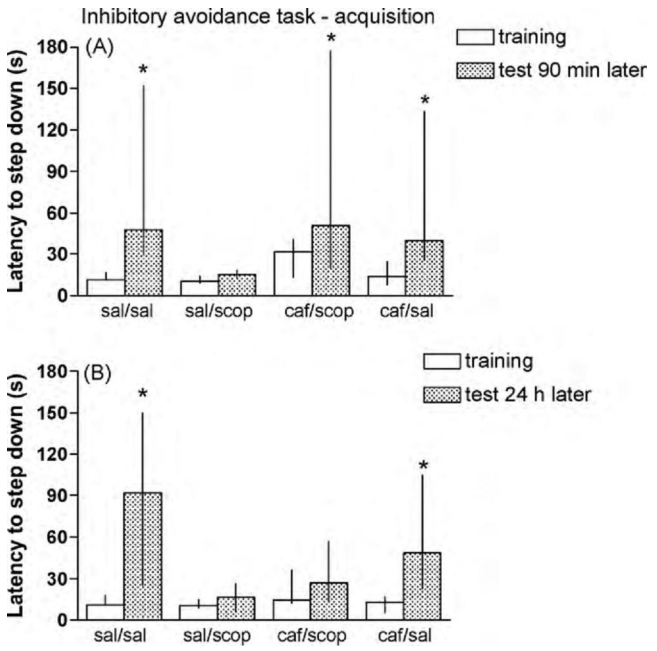

Fig. 3. Pre administration of caffeine on scopolamine-induced memory impairment in the inhibitory avoidance task: scopolamine administered pre training. sal/sal: pre-treatment with saline (0.9 g%, i.p.) during 4 days before a single injection of saline (0.9 g%, i.p.) 15 min prior to training; sal/scop: pre-treatment with saline (0.9 g%, i.p.) during four 4 before a single injection of scopolamine (2 mg/kg, i.p.) 15 min prior to training; caf/scop: pre-treatment with caffeine (10 mg/kg, i.p.) during 4 days before a single injection of scopolamine (2 mg/kg, i.p.) 15 min prior to training; caf/sal: pre-treatment with caffeine (10 mg/kg, i.p.) during 4 days before a single injection of saline (0.9 g%, i.p.) 15 min prior to training; (A) short-term memory assessed 90 min after training. Results are presented as median and interquartile range of 15–18 mice. $^{*}P < 0.05$, differences between latencies of training and test session (Wilcoxon test) and different from sal/scop test latency (Mann–Withney *U* test). (B) Long-term memory assessed 24 h after training. Results are presented as median and interquartile range of 13–15 mice (Wilcoxon test) and different from sal/scop and caf/scop test latencies (Mann–Withney *U* test).

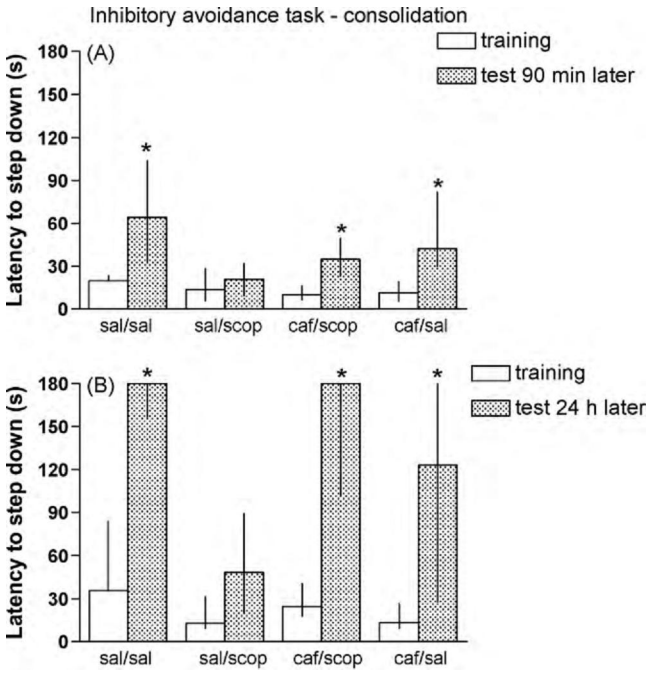

Fig. 4. Pre-administration of caffeine on scopolamine-induced memory impairment in the inhibitory avoidance task: scopolamine administered post training. sal/sal: pre-treatment with saline (0.9 g%, i.p.) during 4 days before a single injection of saline (0.9 g%, i.p.) immediately after training; sal/scop: pre-treatment with saline (0.9 g%, i.p.) during 4 days before a single injection of scopolamine (2 mg/kg, i.p.) immediately after training; caf/scop: pre-treatment with caffeine (10 mg/kg, i.p.) during 4 days before a single injection of scopolamine (2 mg/kg, i.p.) immediately after training; caf/sal: pre-treatment with caffeine (10 mg/kg, i.p.) during 4 days before a single injection of saline (0.9 g%, i.p.) immediately after training; (A) short-term memory assessed 90 min after training session. Results are presented as median and interquartile range of 10–11 mice. $^{*}P < 0.05$, differences between latencies of training and test session (Wilcoxon test) and different from sal/scop test latency (Mann–Withney *U* test). (B) Long-term memory assessed 24 h after training session. Results are presented as median and interquartile range of 9–15 mice. $^{*}P < 0.05$, differences between latencies of training and test session (Wilcoxon test) and different from sal/scop test latency (Mann–Withney *U* test).

scopolamine-induced amnesia should not be related to interferences in the locomotor activity of the animals.

Over the past years, the blockade of muscarinic receptors by scopolamine has been widely used in order to evaluate novel pharmacological strategies to prevent memory impairment with participation of cholinergic system. However, the state-dependency may be responsible for amnesic effects caused by drugs such as scopolamine [36]. In this phenomenon, a drug that impair memory when administered before training session lacks its amnesic effects when it is administered again before test session. Consequently, the retrieval of an engram from memory requires that the organism be in a state similar to that in which the engram was initially acquired. However, scopolamine was described to induce state-dependency on memory when it was infused directly in different brain regions in order to assess the role of cholinergic system on memory formation process [4,23]. Nevertheless, an elegant study was designed to discard state-dependency triggered by systemic administration of scopolamine. In this study, scopolamine systemically administered pre training and/or pre test equally disrupted acquisition and recall of the passive avoidance task in rats [48]. Based on this report, it is unlikely that amnesic effects of scopolamine observed here had been related to state-dependency.

Pre treatment with caffeine caused distinct effects on scopolamine-induced impairment of learning and memory process in the inhibitory avoidance task. The preventive effect of caffeine was dependent on the treatment regimen for scopolamine. The preventive effect of caffeine against scopolamine-induced impairment of acquisition of the task could be observed 1.5 h but not 24 h

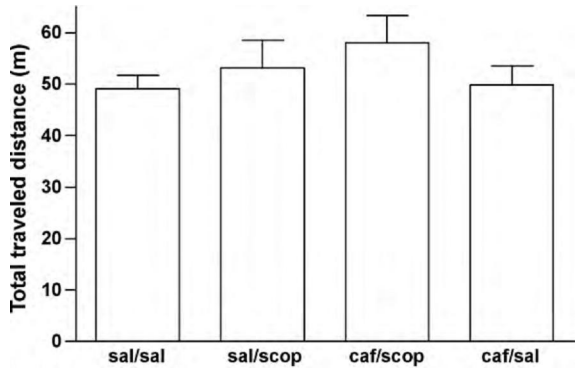

Fig. 5. Spontaneous locomotor activity in an open field 90 min after scopolamine (2 mg/kg, i.p.) injection. Locomotor activity was recorded during ten min. sal/sal: pre-treatment with saline (0.9 g%, i.p.) during 4 days before a single injection of saline (0.9 g%, i.p.) 90 min before open field exposure; sal/scop: pre-treatment with saline (0.9 g%, i.p.) during 4 days before a single injection of scopolamine (2 mg/kg, i.p.) 90 min before open field exposure; caf/scop: pre-treatment with caffeine (10 mg/kg, i.p.) during 4 days before a single injection of scopolamine (2 mg/kg, i.p.) 90 min before open field exposure; caf/sal: pre-treatment with caffeine (10 mg/kg, i.p.) during 4 days before a single injection of saline (0.9 g%, i.p.) 90 min before open field exposure; results are presented as media + S.E.M. of the traveled distance in meters (m) from 9 to 10 mice. No differences were found between groups.

after training. On the other hand, caffeine prevented scopolamine-induced impairment of memory consolidation. In our study, the effect of pre training administration of scopolamine in disrupting long-term memory appeared to be more robust for acquisition than consolidation. Besides, long-term memory was assessed 48 h after caffeine withdrawal. This difference could help to understand the lack of preventive effect for caffeine against scopolamine-induced impairment of acquisition of the task when long-term memory was assessed.

Considering that the consolidation of memory is a process that lasts few hours through which memories are transformed from a labile into a more stable state, probably a pharmacological intervention could be more effective for consolidation than acquisition. However, if the administration of a drug occurred many hours before or after training, such drug should be less effective in preventing memory disruption than shortly after training [30]. In this study, caffeine withdrawal was made prior to training and its preventive effect against scopolamine-induced amnesia persisted in two different tasks when memory consolidation process was assessed. From our knowledge it is the first report in which beneficial effects of caffeine were observed 48 h after withdrawal. One of the caffeine withdrawal symptoms that rodents present includes decrease on the locomotor activity [21,26], but in our study the preventive effects of caffeine against scopolamine-induced amnesia should not be related to withdrawal symptoms because the dose and treatment regimen differ from aforementioned studies.

Caffeine acutely administered at similar doses has presented beneficial effects against different models of mnemonic deficits that include two experimental models of Alzheimer's disease [3,13,35,45]. Besides, in a placebo-controlled double blind crossover study, caffeine administered as three cups of coffee attenuated the scopolamine-induced impairment of free recall from short- and long-term memory, and also quality and speed of retrieval from long-term memory in a word learning task [37]. In a chronic manner, recent epidemiological studies have shown that caffeine prevent cognitive decline associated to Alzheimer's disease [29,38]. Caffeine was also effective in preventing age-cognitive decline in rodents [9,34] and neuronal death caused by beta-amyloid peptide [12]. The blockade of adenosine A_{2A} receptors has been responsible for the neuroprotective effects against memory-induced impairment in experimental models of AD.

Although some studies pointed to the antagonism of adenosine A_{2A} receptors for the positive effects of caffeine on cognition, our study did not seek to characterize pharmacologically which adenosine receptors could be involved in this preventive effect of caffeine observed here. In this scenario, it seems that both adenosine receptors could be involved in the effects of caffeine on cholinergic system functioning since the selective antagonist of adenosine A_{2A} receptors was not able to prevent scopolamine-induce memory impairment in the Y-maze task [11]. Accordingly, *in vitro* studies demonstrated that the increase on caffeine-induced acetylcholine release to the extracellular medium is mediated by activation of adenosine A_1 receptors rather than adenosine A_{2A} receptors [6]. Besides, caffeine seems to promote arousal by enhancing cortical cholinergic transmission via adenosine A_1 and A_{2A} receptors [46]. The binding kinetic analysis of cortical membranes from mice treated during seven consecutive days with caffeine presented an increase on B_{max} values for nicotinic and muscarinic receptors with no alterations on the K_D values [44]. Based on these studies aforementioned, it is likely that this effect of caffeine on increasing cholinergic neurotransmission may also be responsible for its preventive effects observed in our study.

Currently much is known about the relationship between dopamine and acetylcholine as well as dopamine and adenosine. However, molecular, cellular and even behavioral evidence of how adenosinergic and cholinergic systems interact under normal conditions, i.e. functional relevance of this interaction remains still incipient mainly in learning and memory processes. Behavioral studies had suggested caffeine as adjunctive therapy to reduce doses and the associated cognitive impairment of anti-cholinergics currently prescribed for the treatment of Parkinson's disease [32].

Anti-cholinergic agents have been tested to refrain neuronal degeneration and to ameliorate the symptoms related to mnemonic deficits [5]. However, they usually presented side effects that become difficult to further clinical studies [28]. Thus, it remains to be determined if a chronic administration of caffeine could be effective against scopolamine-induced amnesia. Although caffeine is not considered an anti-cholinergic drug, our results suggest that the cholinergic system may contribute to its cognitive enhancer property.

Since caffeine is a usual diet component of almost all populations in the world, these findings may be relevant to provide new insights on the participation of the cholinergic system in the positive effects of caffeine on cognition.

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DISCUSSÃO

Nesse trabalho, os clássicos efeitos amnésicos da escopolamina foram confirmados em ambas as tarefas comportamentais utilizadas para avaliar o aprendizado e memória.

A tarefa de reconhecimento de objetos é bastante usada para acessar memórias declarativas. Dentre suas vantagens, destaca-se o fato dessa tarefa explorar o comportamento natural do animal, pois ela não requer um estímulo aversivo ou deprivação de água ou comida. Além disso, a memória de reconhecimento se forma após apenas uma exposição aos objetos, o que a torna bastante sensível a intervenções farmacológicas (Dere, Houston e de Souza Silva, 2007).

O pré-tratamento de quatro dias com cafeína foi capaz de prevenir o prejuízo tanto da consolidação da memória de curta duração (STM, do inglês, *short-term memory*) quanto da memória de longa duração (LTM, *long-term memory*) na tarefa de reconhecimento de objetos. Este efeito positivo da cafeína sobre o desempenho de camundongos nessa tarefa já havia sido observado em animais envelhecidos (Costa *et al*., 2008a), bem como em animais adultos (Costa *et al*., 2008b).

A administração aguda de cafeína pode estimular a atividade locomotora dos animais (El Yacoubi *et al*., 2000). Nesse protocolo, última dose de cafeína foi administrada 24 horas antes da sessão de treinamento e da administração de escopolamina. Para certificar que nenhum efeito locomotor influenciava os resultados observados, um grupo de animais que recebeu o mesmo tratamento com cafeína foi submetido à tarefa do campo aberto, que entre outras coisas,

permite a avaliação da atividade locomotora. Os resultados não apontaram diferenças na distância percorrida por todos os grupos. Portanto, os efeitos preventivos da cafeína observados nesse trabalho não estão relacionados a uma alteração na locomoção dos animais.

Apesar de apresentar algumas restrições como efeitos periféricos, por exemplo, o modelo de prejuízo colinérgico induzido por escopolamina vem sendo empregado há bastante tempo. Alguns trabalhos apontam que os efeitos amnésicos desencadeados pela escopolamina possam ser decorrentes da indução de um fenômeno de dependência de estado, em que, por exemplo, uma droga que prejudica a memória quando administrada antes do treino perde seu afeito se administrada antes da sessão de teste. No entanto alguns fatores podem interferir na indução de dependência de estado pela escopolamina, como intensidade do treino e dose administrada (Quirarte *et al*., 1994). Além disso, esse fenômeno só foi verificado quando a escopolamina foi administrada centralmente, em diferentes regiões do encéfalo (Ghorbanalizadeh-Khalifeh-Mahaleh *et al*., 2008; Azami *et al*., 2010), o que torna improvável que a dependência de estado influencie os efeitos amnésicos da escopolamina no protocolo aplicado neste trabalho.

Quando a memória aversiva dos animais foi observada, na tarefa de esquiva inibitória, o tratamento com cafeína resultou em efeitos distintos de acordo com o regime de administração da escopolamina. Nessa tarefa, a escopolamina foi administrada em dois momentos distintos, 15 minutos antes do treino, para agir sobre a aquisição da memória, ou imediatamente após o treino, para prejudicar a sua consolidação. A cafeína foi capaz de prevenir os efeitos da escopolamina apenas sobre a aquisição da STM. Por outro lado, a

cafeína preveniu o prejuízo da consolidação tanto da STM quanto da LTM. De fato, tem sido demonstrado que a escopolamina é mais efetiva em bloquear a aquisição da memória (Winters *et al*., 2007 e 2008; Young, Bohenek e Fanselow, 1995). Corroborando com isso, um aumento de acetilcolina é favorável a aquisição de novas informações através de uma rede inclui regiões encefálicas como o córtex entorrial, hipocampo (CA3, CA1 e giro denteado) e o septo medial (Meeter, Murre e Talamini, 2004).

A retirada da cafeína 24 horas antes do treinamento pode ser um motivo pelo qual ela não tenha sido capaz de prevenir o prejuízo da LTM, já que no momento do teste a última dose de cafeína havia sido administrada ao animal 48 horas antes. Se uma droga é administrada muito tempo antes ou depois do treino, provavelmente ela será menos efetiva em prevenir um déficit mnemônico se comparada a uma administração mais próxima do treinamento (McGaugh e Roozendaal, 2009). No entanto, o tratamento com cafeína foi capaz de prevenir a consolidação da LTM, ou seja, 48 horas após a administração da sua última dose. Além disso, foi mostrado que nesse tipo de tarefa os efeitos benéficos da cafeína são mais pronunciados na fase de consolidação da memória (Angelucci *et al*., 1999). Esse trabalho é pioneiro em demonstrar da efeitos cafeína dois dias após sua última administração.

A retirada da cafeína pode provocar sintomas como hipolocomoção (Holtzman, 1983; Finn e Holtzman, 1986;). No entanto, os efeitos observados aqui provavelmente não estejam relacionados a uma síndrome de abstinência, já que a dose utilizada nesse estudo difere dos acima citados.

Doses similares de cafeína foram aplicadas em estudos de diferentes modelos de déficit cognitivo, incluindo dois que mimetizam a DA, e foram

efetivas em reverter os efeitos deletérios observados sobre a cognição (Arendash *et al*., 2006, Dall'Igna *et al*., 2007; Prediger, Fernandes e Takahashi 2005). Além disso, a cafeína preveniu a morte neuronal induzida pelo peptídeo β-amilóide (Dall'Igna *et al*., 2003). Em roedores envelhecidos, a cafeína foi capaz de prevenir o déficit cognitivo observado em decorrência da idade (Costa *et al*., 2008a; Prediger, Batista e Takahashi; 2005). Em humanos a cafeína também apresenta efeitos positivos. A administração de três copos de café foi capaz de melhorar a qualidade e a velocidade da evocação da memória em uma tarefa de aprendizado de palavras (Riedel *et al*., 1995).

Apesar de estudos apontarem os receptores A_{2A} como os responsáveis pelos efeitos da cafeína, o objetivo deste trabalho não foi caracterizar farmacologicamente o papel de cada receptor adenosinérgico. Atualmente vários estudos abordam a relação do sistema adenosinérgico com o sistema dopaminérgico, e, ainda, do sistema dopaminérgico com o colinérgico. No entanto, ainda não está elucidada a relação entre os sistemas adenosinérgico e colinérgico. Outros sistemas parecem estar por trás dos efeitos psicoestimulantes da cafeína, que parece aumentar o estado de alerta por meio de um aumento na transmissão colinérgica cortical (Van Dort, Baghdoyan e Lydic, 2009), e, quando administrada cronicamente, a cafeína aumenta a liberação de ACh no córtex pré-frontal de ratos (Acquas , Tanda e Di Chiara, 2002). Dessa forma, apesar de não ser uma droga colinérgica, há indícios que o sistema colinérgico possa contribuir para os efeitos positivos sobre a cognição característicos da cafeína.

Drogas anti-colinesterásicas vêm sendo utilizadas como estratégias para minimizar os prejuízos cognitivos em casos de demência, entre elas a DA.

Entretanto, elas apresentam muitos efeitos colaterais, e o desenvolvimento de alternativas se torna interessante. Os efeitos da administração crônica de cafeína ainda merecem mais estudos, porém dois deles (Maia e Mendonça, 2002; Ritchie *et al.*, 2007) mostraram uma diminuição na probabilidade de incidência de DA em pessoas que consumiram cafeína durante sua vida.

Assim, uma vez que a cafeína está presente na dieta da população mundial, e o sistema colinérgico é o alvo de neurodegeneração característica de diferentes patologias, como a DA, mais estudos devem ser realizados buscando um aprofundamento a respeito da relação do sistema adenosinérgico e da cafeína com o sistema colinérgico.

CONCLUSÕES

Apesar das controvérsias a respeito dos seus benefícios sobre o desempenho cognitivo de animais e humanos, inúmeros trabalhos vêm demonstrando efeitos positivos do consumo de cafeína em processos de aprendizado e memória. Nesse trabalho, a cafeína foi efetiva em prevenir a amnésia provocada pelo comprometimento do sistema colinérgico induzido pela administração de escopolamina em camundongos em duas tarefas comportamentais.

Nas tarefas de reconhecimento de objetos e esquiva inibitória a consolidação das memórias de curta e longa duração foi prevenida pela cafeína. Já no processo de aquisição da memória, que foi avaliado apenas na tarefa de esquiva inibitória, a cafeína só foi efetiva em prevenir o comprometimento da memória de curta duração. Além disso, esse estudo é pioneiro ao mostrar efeitos benéficos da cafeína sobe a memória 48 horas após a sua retirada.

Mesmo que os mecanismos não estejam totalmente esclarecidos, o consumo de cafeína parece ser benéfico na prevenção de doenças neurodegenerativas, entre elas a doença de Alzheimer, em que o principal alvo é o sistema colinérgico. Os achados desse trabalho mostram que, apesar de não ser uma droga colinérgica, os efeitos da cafeína nesses casos podem ser mediados por uma interação do sistema adenosinérgico com o sistema colinérgico. Dessa forma, mesmo que não se caracterize uma abordagem terapêutica efetiva, a cafeína pode ser ao menos como uma ferramenta de muita utilidade no estudo e desenvolvimento de novas drogas para essas

patologias do SNC, uma vez que as terapias disponíveis atualmente apenas retardam o agravamento dos sintomas e, ainda, apresentam diversos efeitos colaterais.

Por fim, a cafeína, que é conhecida por suas propriedades estimulantes, tem seu consumo amplamente difundido no mundo todo, estando presente em diferentes alimentos e bebidas. Dessa forma, se torna interessante a realização mais estudos a respeito suas propriedades, abordando os efeitos do seu consumo crônico ou agudo sobre parâmetros relacionados à cognição e, não menos importante, sobre efeitos periféricos relacionados à sua ingestão, para esclarecer os benefícios e malefícios dessa substância tão comum ao dia-a-dia da maioria da população.

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