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Uso do Canabidiol como protetor contra disfunções cognitivas associadas ao acúmulo de ferro cerebral em ratos Wistar

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USO DO CANABIDIOL COMO PROTETOR CONTRA DISFUNÇÕES COGNITIVAS ASSOCIADAS AO ACÚMULO DE FERRO CEREBRAL EM RATOS WISTAR

Dissertação apresentada como requisito para obtenção do grau de Mestre pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociências.

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RESUMO

O canabidiol é o principal constituinte não-psicotrópico da *Cannabis sativa* e possui uma ampla variedade de efeitos farmacológicos, incluindo efeito anticonvulsivante, sedativo, hipnótico, antipsicótico, antiinflamatório e neuroprotetor como demonstrado em estudos clínicos e pré-clínicos. Muitas doenças neurodegenerativas envolvem déficits cognitivos e isto tem levado ao questionamento sobre a possibilidade de utilização do canabidiol no tratamento dos danos de memória associado a essas patologias. No presente trabalho utilizou-se um modelo animal de dano cognitivo induzido pela sobrecarga de ferro a fim de investigar os efeitos do canabidiol na disfunção de memória. Ratos Wistar machos receberam veículo ou 10.0 mg/kg Fe^{+2} por via oral nos dias 12-14 pós-natal. Quando os animais completaram 2 meses de idade (idade adulta), receberam uma injeção intraperitonial aguda de veículo ou canabidiol (5.0 ou 10.0 mg/kg) imediatamente após a sessão de treino da tarefa de reconhecimento do objeto. Para investigar os efeitos do uso crônico de canabidiol os ratos tratados com ferro no período neonatal receberam injeções diárias intraperitoniais de canabidiol (5.0 ou 10.0 mg/kg) durante 14 dias. Vinte e quatro horas após a última injeção eles foram submetidos ao treino de reconhecimento de objeto. As sessões de teste de retenção foram realizadas 24 horas após o treino. Os resultados indicaram que os animais que receberam ferro no período neonatal apresentaram déficits severos de memória. Uma única injeção aguda de canabidiol na sua dose mais alta foi capaz de recuperar parcialmente a memória dos ratos tratados com ferro. O uso crônico de canabidiol melhorou a memória de reconhecimento dos ratos tratados com ferro de forma dose dependente. O uso agudo ou crônico de canabidiol não afeta a memória dos ratos controles. Os resultados do presente trabalho fornecem evidências que apontam para o uso do canabidiol no tratamento de déficit cognitivo associados às doenças neurodegenerativas. Investigações futuras, envolvendo ensaios clínicos seriam necessárias para determinar a utilidade deste fármaco no tratamento de seres humanos acometidos por doenças neurodegenerativas.

Palavras-chave: canabidiol; memória; doenças neurodegenerativas; neuroproteção; ferro.

ABSTRACT

Cannabidiol, the main non-psychotropic constituent of *Cannabis sativa*, has a large number of pharmacological effects including anticonvulsant, sedative, hypnotic, antipsychotic, antiinflammatory and neuroprotective, as demonstrated in both clinical and pre-clinical studies. Many neurodegenerative disorders involve cognitive deficits, and this has led to interest in whether cannabidiol could be useful in the treatment of memory impairment associated to these diseases. Here, we used an animal model of cognitive impairment, induced by iron overload in order to test the effects of cannabidiol in memory-impaired rats. Rats received vehicle or 10.0 mg/kg Fe^{+2} orally at postnatal days 12-14. When animals reached the age of 2 months, they received an acute intraperitoneal injection of vehicle or cannabidiol (5.0 or 10.0 mg/kg) immediately after the training session of the novel object recognition task. In order to investigate the effects of chronic cannabidiol, neonatally iron-treated rats received daily intraperitoneal injections of cannabidiol (5.0 or 10.0 mg/kg) for 14 days. Twenty-four hours after the last injection, they were submitted to object recognition training. Retention test sessions were performed 24 hours after training. Results indicated that animals that received iron in the neonatal period show severe memory deficits. A single acute injection of cannabidiol at the highest dose was able to partially recover memory in iron-treated rats. Chronic cannabidiol improved recognition memory in iron-treated rats in a dose-dependent manner. Acute or chronic cannabidiol does not affect memory in control rats. The present findings provide evidence that suggest the potential use of cannabidiol for the treatment of cognitive decline associated to neurodegenerative disorders. Further studies, including clinical trials are warranted in order to determine the usefulness of cannabidiol in humans suffering from neurodegenerative disorders.

Keywords: cannabidiol; memory; neurodegenerative disorders; neuroprotection; iron.

SUMÁRIO

1. CAPÍTULO 1

1.1 INTRODUÇÃO

Devido ao envelhecimento populacional, a prevalência e a incidência de doenças neurodegenerativas comuns em idosos, como por exemplo, as doenças de Alzheimer (DA) e de Parkinson (DP), vem crescendo de forma significante mundialmente (Castellani et al., 2010; Hindle, 2010). No Reino Unido cerca de 5% da população acima de 65 anos apresenta algum tipo de demência, sendo que a prevalência é crescente à medida que a idade aumenta, chegando a 20% nos idosos acima de 80 anos (Edwardson & Kirkwood, 2002). A DA é a principal doença neurodegenerativa e constitui aproximadamente 70% de todos os casos de demência, afetando 25 milhões de pessoas no mundo. A sua incidência aumenta com a idade e tem dobrado a cada 5-10 anos. Nos Estados Unidos, a prevalência foi estimada em 5 milhões em 2007 e até 2050 está projetada a aumentar para 13 milhões (Castellani et al., 2010). No Brasil, apesar das grandes lacunas estatísticas, estima-se que cerca de 500 mil pessoas sejam acometidas pela doença. Embora essas cifras sejam questionáveis, o impacto da DA em nossa sociedade tem se revelado importante (MACHADO, 2006). Globalmente, o número de adultos na faixa etária de 65 anos está projetado a aumentar drasticamente, de 420 milhões de pessoas em 2000 para 973 milhões em 2030. Levando em consideração o fato de que a idade avançada é o maior fator de risco para DA, não podemos subestimar o problema que esta doença ocasiona também com relação aos custos financeiros e humanos (Castellani et al., 2010).

Assim como a DA, a DP tem sua prevalência e incidência aumentada à medida que a idade da população avança (Hindle, 2010; Chen, 2010; Pahwa & Lyons, 2010), acometendo aproximadamente 1% da população afetada com 60 anos ou mais, aproximadamente 4% dos idosos com 80 anos ou mais e aproximadamente 5,2% dos indivíduos internados em clínicas. Estima-se que a DP afete mais de 1 milhão de pessoas nos Estados unidos e mais de 5 milhões no mundo inteiro (Chen, 2010). Sua taxa anual de incidência tem sido estimada em torno de 16 – 19 por 100.000 pessoas quando o diagnóstico é realizado por especialista em desordens de movimento (Pahwa & Lyons, 2010). Devido à crescente população idosa nos Estados Unidos, estima-se que o número de pessoas com DP até o ano de 2030 terá dobrado. Essa patologia é a segunda

doença neurodegenerativa mais comum e seu impacto econômico anual nos Estados Unidos esta estimado em 10,8 bilhões de dólares, sendo 58% desse valor relacionado a custos médicos diretos. As prescrições de medicamentos contabilizam aproximadamente 14-22% dos custos e as clínicas de cuidado ao paciente, aproximadamente 41%. Anualmente, os custos indiretos, que incluem os dias de trabalho perdido pelos pacientes e profissionais da saúde, são estimados em 9.135 dólares (Chen, 2010).

O estudo dos mecanismos envolvidos no desenvolvimento dessas patologias neurodegenerativas, assim como, de medidas preventivas e terapêuticas, torna-se muito importante, uma vez que esses ainda não foram completamente elucidados. Além disso, esse tipo de doença gera uma profunda sobrecarga emocional, social e econômica, o que prejudica o estabelecimento de um envelhecimento bem sucedido entre a população de idosos.

1.1.1 Acúmulo de ferro cerebral associado às disfunções cognitivas

Um crescente corpo de evidências clínicas e experimentais sugere a participação do ferro em doenças neurodegenerativas, particularmente no mecanismo de morte celular na DP, pois a maioria das reações de formação de radicais hidroxil, induzidas pelo metabolismo da dopamina, envolve a presença de ferro. Além disso, evidências sugerem que o estresse oxidativo participa no mecanismo de morte neuronal devido à formação excessiva de peróxido de hidrogênio e radicais livres derivados de oxigênio que podem causar danos à célula através de reações de peroxidação lipídica e alterações na fluidez da membrana (Polla et al., 2003).

O período neonatal é crítico para o estabelecimento do conteúdo de ferro no encéfalo adulto. Investigações a respeito da captação de ferro pelo cérebro indicaram que o transporte de ferro ao órgão atinge seus níveis máximos durante o período pós-natal de rápido crescimento cerebral (Taylor & Morgan, 1990; Taylor et al., 1991). Além disso, a distribuição cerebral de ferro altera-se com o envelhecimento, podendo ter alguma relação com disfunções nas vias de manutenção da homeostasia desse metal e, conseqüentemente, promovendo os

depósitos nas regiões onde seu metabolismo é mais alto, podendo, desse modo, participar de eventos neurodegenerativos (Zecca et al., 2004; 2001; Martin et al., 1998).

Enquanto no passado a ênfase havia sido dada ao combate à deficiência de ferro (anemia), a aplicação indiscriminada de suplementação de ferro às crianças durante seu primeiro ano de vida tornou importante estudar os mecanismos através dos quais o organismo pode se proteger contra o excesso desse metal (Bothwell, 1995).

De fato, Fredriksson e colaboradores, em um estudo utilizando camundongos, descreveram pela primeira vez que o tratamento sistêmico com ferro durante o período de rápido desenvolvimento cerebral (período que ocorre em humanos, desde o último trimestre da gravidez até um ano de vida) produz acúmulo seletivo de ferro nos gânglios da base, além de causar disfunções neurocomportamentais (Fredriksson et al., 1999). Os resultados mostraram ainda, que camundongos (Fredriksson et al., 2000) e ratos (Schröder et al., 2001) tratados com ferro do 10° ao 12° dia de vida pós-natal apresentam hipoatividade motora, bem como déficits no aprendizado e memória em duas diferentes tarefas comportamentais, o labirinto radial de oito braços e a esquiva inibitória.

Recentemente, foi verificado que ratos tratados com ferro do 12° ao 14° dia de vida pós-natal apresentam déficits de memória de reconhecimento quando adultos (de Lima et al., 2005a). Foi observado também que a administração de ferro no período neonatal induz um aumento significativo na peroxidação lipídica na substância negra (SN), no córtex e no hipocampo, bem como um aumento de danos oxidativos às proteínas nestas mesmas regiões cerebrais de ratos adultos. Adicionalmente, a análise revelou que ocorre uma diminuição da atividade da superóxido dismutase (enzima antioxidante) na SN, no córtex e no hipocampo. Esses resultados sugerem que o ferro possa estar exercendo seus efeitos deletérios sobre a cognição através do aumento dos níveis de estresse oxidativo cerebral (de Lima et al., 2005a).

1.1.2 Canabinóides

Os canabinóides compõem um grupo heteromórfico de moléculas que apresentam atividade sob receptores específicos, denominados receptores de canabinóides. São diferenciados em três grupos: endógenos ou endocanabinóides, canabinóides sintéticos e fitocanabinóides. Este último engloba compostos terpenólicos naturais derivados da planta *Cannabis sativa* (Russo, 2005).

1.1.2.1 Canabidiol (CBD)

Canabidiol (CBD) (Fig. 1) é o principal composto não psicotrópico presente nas glândulas dos tricomas da *Cannabis sativa*. Foi isolado da maconha na década de 1930, mas teve sua estrutura e estereoquímica elucidada somente no ano de 1963. Os estudos farmacológicos relacionados a esse canabinóide começaram a partir de 1970, e em 1981 os brasileiros Carlini e Cunha apresentaram a sua ação hipnótica e anticonvulsivante. Desde então, muitas propriedades farmacológicas têm sido demonstradas, incluindo propriedades sedativas, antipsicóticas, antioxidantes, ansiolíticas, anticonvulsivantes, antiinflamatórias e neuroprotetoras (Scuderi et al., 2008).

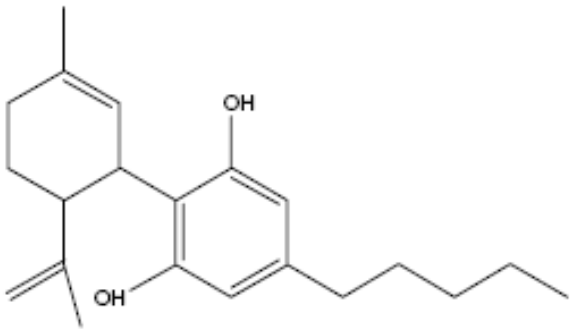

***Figura 1*. Estrutura do CBD (Russo, 2005)**

A maioria dos efeitos exercidos pelos fitocanabinóides são mediados através de ação antagonista ou agonista em receptores específicos. Porém, os mesmos podem exercer alguns efeitos que não são intermediados por receptores, tais como: efeitos no sistema imunológico, no sistema circulatório e efeitos neuroprotetores.

Os tecidos de mamíferos expressam pelo menos dois tipos de receptores para canabinóides: CB_1 e CB_2. Ambos estão acoplados à proteína G inibitória (proteína Gi), negativamente à adenilato ciclase e positivamente à proteína quinase ativada por mitógeno. A ativação de proteínas Gi ocasiona a inibição da adenilato ciclase, inibindo dessa forma a conversão de AMP à AMP cíclico (Grotenhermen, 2003; Pertwee, 2006; Ryberg et al., 2007).

Atualmente se conhece muito pouco sobre os mecanismos moleculares de ação do CBD. Até o início do século XXI os autores propuseram, através de suas pesquisas, que ao contrário do Δ^9-tetrahidrocanabidiol (Δ^9-THC), o CBD tem uma baixa afinidade pelos dois subtipos de receptores CB_1 e CB_2. Como consequência, alguns autores têm investigado se o CBD interage com proteínas do "sistema de sinalização endocanabinóide" (exceto receptores CB_1/CB_2). Essas proteínas citadas são: 1) amida hidrolase de ácido graxo (FAAH), enzima intracelular que catalisa a hidrólise do ligante canabinóide endógeno anandamida (araquidonoiletanolamida, AEA); e 2) "transportador de membrana anandamida" (AMT), responsável por facilitar o transporte de AEA através da membrana celular e, subseqüentemente, sua degradação intracelular. Através desses estudos descobriu-se que o CBD inibe tanto a hidrólise de AEA através de preparações de membrana contendo FAAH como a absorção de AEA pelas células RBL - 2H3 via AMT. Embora esses efeitos tenham sido observados em altas concentrações (na ordem de concentração μM), tais descobertas incluem a possibilidade de que algumas ações farmacológicas do CBD podem ser devido à inibição da degradação de AEA, e posterior aumento dos níveis endógenos desse mediador, para o qual as propriedades neuroprotetoras foram sugeridas. Outros estudos indicam que muitas das atividades farmacológicas do CBD foram encontradas apenas *in vivo*, logo, algumas delas podem ocorrer em decorrência de metabólitos formados a partir do CBD (Bisogno et al., 2001). No entanto, no ano de 2007, Thomas e colaboradores publicaram um estudo feito *in vitro,* onde mostraram a ação antagonista de alta potência do CBD diante dos agonistas de receptores CB_1 e CB_2 presentes em membranas de cérebro de ratos e em membranas preparadas a partir de células transfectadas com receptores hCB_2 de ovário de hamster chinês (CHO) (Thomas et al., 2007). Baseado nesses dados, Pertwee complementou em um estudo posterior, que esta interação antagônica ocorre com baixas concentrações (nanomolar) de substrato e que a mesma é essencialmente não competitiva por natureza (Pertwee, 2008).

Estudos já evidenciaram a existência de um ou mais subtipos de receptores canabinóides, como exemplo, o receptor vanilóide VRI (TRPV1) (Grotenhermen, 2003; Pertwee, 2006; Bisogno et al., 2001; Howlett et al., 2002). Em um trabalho realizado por Bisogno e colaboradores, os autores mostraram que alguns efeitos farmacológicos do CBD são similares aos apresentados pela capsaicina e por agonistas sintéticos de VR1. Assim como a capsaicina, CBD induz efeitos antiinflamatórios, o que possivelmente explica a sua capacidade de modular a liberação de mediadores antiinflamatórios e pró-inflamatórios. Essas duas substâncias também têm em comum efeito anticonvulsivante e anti-artrite reumatóide. Nesse estudo verificou-se que o CBD, quando comparado com a capsaicina, é um agonista total, embora fraco, de VR1 humano em concentrações que podem ser alcançadas após a administração do mesmo em doses usuais *in vivo* (10 ± 50 mg/kg^{-1} em homens), e mais baixas do que as doses de CBD necessárias para ligá-lo aos receptores de canabinóides. O CBD parece dessensibilizar o receptor VR1 à ação da capsaicina, deste modo, reafirma a hipótese de que canabinóides exercem mecanismo antiinflamatório em parte, por dessensibilização de nociceptores sensoriais (Bisogno et al., 2001). Outros estudos realizados por diferentes autores afirmam que o CBD também apresenta afinidade de ligação pelo receptor serotoninérgico $5\text{-}HT_{1A}$ e essa interação estaria relacionada tanto com a atenuação do tamanho do infarto cerebral quando ocorre uma isquemia, quanto aos seus efeitos ansiolíticos (Scuderi et al., 2008). Segundo Kathmann e colaboradores (2006) o CBD também pode comportar-se como um modulador alostérico nos receptores opióides μ e δ. Porém, como a atividade modulatória é alcançada somente na presença de altíssimas concentrações desse fitocanabidiol, o efeito *in vivo* da substância não pode ser atribuído a esse mecanismo (Kathmann et al., 2006). O último receptor considerado não-CB_1/CB_2 descoberto até a data presente é um receptor órfão acoplado à proteína G, ou melhor, GPR55. O estudo comprovou a sua habilidade de interagir e ser modulado por ligantes canabinóides de origem endógena, sintética e vegetal, sendo o CBD um ligante antagonista (Ryberg et al., 2007).

Alguns trabalhos tem evidenciado que o CBD apresenta função antioxidante independente de receptores para canabinóides, resultando em uma significativa diminuição no dano neuronal (Hampson et al., 1998; Hamelink et al., 2005; Esposito et al., 2006; Cassol-Jr et al., 2010). Esse efeito antioxidante tem sido demonstrado

através de estudos com diferentes modelos animais e técnicas *in vitro,* como por exemplo, no estudo realizado por Hampson e colaboradores (1998), no qual o CBD apresentou propriedades antioxidantes comparáveis ao butilhidroxitolueno (BHT) em células neuronais danificadas pela toxicidade glutamatérgica. E demonstrou igual, ou até mesmo maior proteção do que os conhecidos antioxidantes alfa-tocoferol e ascorbato (Hampson et al., 1998).

1.1.2.2 Possível uso do CBD no tratamento de doenças neurodegenerativas

Ainda que o mecanismo de ação dessa substância não esteja totalmente elucidado, é crescente o número de estudos que sugerem o CBD como um potencial agente terapêutico no tratamento de doenças neurodegenerativas como DA, DP, Huntington, e Esclerose Múltipla (Zuardi, 2008). No entanto, a caracterização desse efeito é limitada pela falta de estudos utilizando modelos animais adequados de disfunção cognitiva que reproduzam aspectos de doenças neurodegenerativas (Bisogno & Di Marzo, 2008). Alguns trabalhos publicados apresentam modelos animais de mamíferos roedores apropriados e diferentes técnicas que mostraram ser efetivas em patologias como DP e DA.

Na DP os autores afirmam que o papel do CBD é neutralizar o dano oxidativo aos neurônios do sistema nervoso (Sevcik & Masek, 2000). Lastres-Becker e colaboradores (2005) realizaram um estudo com modelo animal (*in vivo* e *in vitro)* no qual 6-hidroxi-dopamina foi injetada no feixe medial do cérebro anterior de ratos. Depois de confirmada a depleção significativa de dopamina e tirosina hidroxilase, CBD (3mg/kg/dia) foi administrado durante 2 semanas e os resultados indicaram que o CBD exerce seus efeitos neuroprotetores através de sua ação antioxidante, um mecanismo que seria independente de sua ação sobre os receptores. Apesar disso os autores não descartaram a possibilidade de que a neuroproteção do composto seja resultante do seu potencial antiinflamatório (relacionado ao receptor CB_2). Através de estudos *in vitro*, os autores sugeriram que o canabinóide exerceu sua maior ação protetora através da regulação da função glial (Lastres-Becker et al., 2005). Em um estudo com ratos realizado por García-Arencibia e colaboradores, (2007), o CBD apresentou capacidade de reverter a depleção de dopamina causada

pela 6-hidroxi-dopamina, porém, somente quando administrado imediatamente após a lesão. Quando administrado uma semana depois, não foi encontrado resultado positivo. Neste mesmo estudo, o grupo descobriu que o efeito do CBD implicou na melhor regulação dos níveis de mRNA para Cu,Zn-superóxido dismutase (enzima chave de defesa endógena contra estresse oxidativo). Os mesmos concluíram que seus resultados indicam que o canabinóide que possui propriedade antioxidante independente de receptor fornece neuroproteção contra a degeneração progressiva dos neurônios dopaminérgicos nigroestriatais (García-Arencibia et al., 2007).

Na DA, outra demência senil muito pesquisada, demonstrou-se que o CBD aumenta os níveis de pró-caspase 3, e em paralelo reduz os níveis de caspase 3 em células PC12 tratadas com Aβ. Estes achados indicam que o composto está intimamente relacionado com a inibição da apotose neuronal, visto que o mesmo impede a fragmentação do DNA induzida por placas Aβ (processo típico de morte celular programada) (Iuvone et al., 2004). Outro grupo, que também verificou a ação do CBD em um modelo animal da DA, injetou em ratos, via intrahipocampal, o fragmento humano de Aβ para induzir neuroinflamação. Os resultados mostraram que CBD impediu a liberação de IL-1β, diminuindo assim a prejudicial cascata neuroinflamatória presente na patologia (Esposito et al.,2007).

Embora estudos já tenham sido concluídos e muitos outros estão em andamento, ainda há um obstáculo encontrado pelo meio científico: a deficiente caracterização dos efeitos do CBD sobre os processos de plasticidade sináptica, que são os mais prejudicados em pacientes com doenças neurodegenerativas, notadamente memória de longa duração com conteúdo espacial, contextual, afetivo e de reconhecimento. Estudos comportamentais com ratos demonstraram que os principais efeitos de canabinóides ocorrem em regiões cerebrais que desempenham importante papel na ansiedade e aprendizado aversivo, como amígdala e hipocampo. Foi demonstrado que o inibidor da recaptação da anandamida (AM404) e o CBD facilitaram a extinção da memória contextual de medo e também proporcionaram efeitos ansiolíticos aos animais (Bitencourt et al., 2008). Por outro lado, um estudo realizado com roedores que receberam via intraperitoneal extrato rico em Δ^9-THC ou extrato rico em CBD ou ambos paralelamente assegurou que os animais que receberam Δ^9-THC expressaram significantes déficits quanto à memória de trabalho espacial e à memória de curta duração. Todavia, o CBD não apresentou efeito nestes dois tipos de memória, mesmo quando administrado em doses acima

do usual (50 mg/kg). Quando administrado concomitantemente com Δ^9-THC, o CBD não reverteu os déficits de memória ocasionados pelo mesmo. O grupo concluiu que a memória de trabalho espacial e a memória de curta duração não são sensíveis ao CBD (Fadda et al., 2004).

Tendo em vista os trabalhos já publicados, aliados à necessidade de estudos adicionais em modelos animais adequados para o avanço da ciência diante de tais patologias, na presente dissertação investigamos os efeitos da administração sistêmica de CBD sobre a memória de reconhecimento de longa duração em um modelo animal de disfunção cognitiva associada ao envelhecimento e às doenças neurodegenerativas.

1.3 OBJETIVOS

1.3.1 Objetivo Geral

Avaliar os efeitos do tratamento agudo com CBD sobre a memória em ratos normais e dos tratamentos agudo e crônico com CBD sobre o déficit de memória induzido pelo tratamento com ferro no período neonatal.

1.3.2 Objetivos Específicos

- Avaliar o efeito do tratamento agudo com CBD sobre a memória de reconhecimento em animais adultos (dois meses de vida) normais.

- Avaliar o efeito do tratamento agudo com CBD sobre a memória aversiva em animais adultos (dois meses de vida) normais.

- Avaliar o efeito do tratamento agudo com CBD sobre os prejuízos de memória de reconhecimento induzidos pelo tratamento com ferro no período neonatal.

- Avaliar o efeito do tratamento crônico com CBD sobre os prejuízos de memória de reconhecimento induzidos pelo tratamento com ferro no período neonatal.

- Avaliar o efeito do tratamento crônico com CBD sobre a atividade em campo aberto (medida de atividade locomotora) em ratos tratados com ferro no período neonatal.

2. CAPÍTULO 2

Artigo científico

Title:

Memory rescuing properties of cannabidiol in an animal model of cognitive impairment relevant to neurodegenerative disorders

Running title: Cannabidiol in memory impairment

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Abstract

Cannabidiol, the main non-psychotropic constituent of *Cannabis sativa*, possesses a large number of pharmacological effects including anticonvulsive, sedative, hypnotic, anxiolytic, antipsychotic, anti-inflammatory, and neuroprotective, as demonstrated in clinical and pre-clinical studies. Many neurodegenerative disorders involve cognitive deficits, and this has led to interest in whether cannabidiol could be useful in the treatment of memory impairment associated to these diseases. Here, we used an animal model of cognitive impairment, induced by iron overload in order to test the effects of cannabidiol in memory-impaired rats. Rats received vehicle or iron at postnatal days 12-14. At the age of 2 months, they received an acute intraperitoneal injection of vehicle or cannabidiol (5.0 or 10.0 mg/kg) immediately after the training session of the novel object recognition task. In order to investigate the effects of chronic cannabidiol, iron-treated rats received daily intraperitoneal injections of cannabidiol (5.0 or 10.0 mg/kg) for 14 days. Twenty-four hours after the last injection, they were submitted to object recognition training. Retention tests were performed 24 hours after training. A single acute injection of cannabidiol at the highest dose was able to partially recover memory in iron-treated rats. Chronic cannabidiol improved recognition memory in iron-treated rats in a dose-dependent manner. Acute or chronic cannabidiol does not affect memory in control rats. The present findings provide evidence suggesting the potential use of cannabidiol for the treatment of cognitive decline associated with neurodegenerative disorders. Further studies, including clinical trials, are warranted to determine the usefulness of cannabidiol in humans suffering from neurodegenerative disorders.

Keywords: cannabidiol; memory; neurodegenerative disorders; neuroprotection; iron.

Introduction

Cannabidiol, the main non-psychotropic constituent of *Cannabis sativa*, was first isolated in the late 1930's. Since the early studies performed in the 1970's, a large number of cannabidiol's pharmacological effects have been described (Scuderi, *et al* 2009). A host of actions, including anticonvulsive, sedative, hypnotic, anxiolytic, antipsychotic, antiinflammatory and neuroprotective have been demonstrated in both clinical and pre-clinical studies (for a review see Zuardi, 2008).

Accordingly, Lastres-Becker and coworkers (2005) have demonstrated that cannabidiol was able to reverse dopamine depletion in a 6-hydroxydopamine (6-OHDA)-induced rat model of Parkinson's disease. Cannabidiol has also proven to protect differentiated PC12 neuronal cells from Aβ peptide exposure, through a combination of antioxidant, antiinflammatory and antiapoptotic effects (Iuvone, *et al* 2004). Additionally, cannabidiol significantly attenuated GFAP mRNA and protein expression and impaired iNOS and IL-1b protein expression in Aβ-injected animals, suggesting that it attenuated Aβ-induced inflammatory responses (Esposito, *et al* 2007). Neuroprotective effects of cannabidiol have also been demonstrated using in vitro models of hypoxic–ischemic immature brain (Castillo, *et al* 2010) and prion toxicity (Dirikoc, *et al* 2007).

Many neurodegenerative disorders involve cognitive deficits, and this has led to interest in whether cannabidiol may be useful in the treatment of memory impairment associated to these diseases (Crippa, *et al* 2010). We have previously demonstrated that iron, when administered to rodents in the neonatal period induces persistent memory deficits that are relevant to neurodegenerative disorders (Schröder, *et al* 2001; de Lima, *et al* 2005a; 2007; 2008a). It is currently known that

iron selectively accumulates in the brains of patients suffering from neurodegenerative disorders (for a review Zecca, *et al* 2004) and the disruption of iron homeostasis has been implicated in the pathogenesis of Alzheimer's, Parkinson's and Huntington's diseases, among others (Kell, 2010). Remarkably, recent studies have shown that iron content in brain regions has been positively correlated with poorer performance in cognitive testing in Alzheimer's patients (Ding, *et al* 2009). In addition, redox-active iron levels in the cerebrospinal fluid increased with the degree of cognitive impairment from normal to MCI subjects (Lavados, *et al* 2008). Iron-induced memory impairments are associated with increased oxidative stress markers in brain regions relevant to memory formation (de Lima, *et al* 2005a). Thus, we have proposed that at least in part iron-induced oxidative damage might play a role in iron's deleterious effects on cognition. According to Lavados and coworkers (2008), given the relevance of oxidative damage in neurodegeneration, it might be possible to associate the development of cognitive and functional decline with the presence of redox-active iron.

Recent studies have shown that cannabidiol may recover cognitive function in animals submitted to sepsis (Cassol-Jr, *et al* 2010) and to hepatic encephalopathy (Avraham, *et al* 2010). In humans, preliminary results suggest that CANNABIDIOL can have beneficial effects in the treatment of Parkinson Disorder (Zuardi, *et al* 2009) and a double-blind placebo controlled trial is currently underway to evaluate this possibility. However, it is still necessary to determine the precise mechanisms of action of cannabidiol on cognitive deficits associated to neurodegenerative disorders (Krishnan, *et al* 2009; Iuvone, *et al* 2009; Crippa, *et al* 2010) Thus, in the present study we aimed to evaluate the effects of cannabidiol on cognitive impairment associated to iron treatment.

Material and Methods

Animals

Sixty adult male Wistar rats (200-250 g) were obtained from the State Health Science Research Foundation (FEPPS-RS, Porto Alegre, Brazil).

For iron-induced memory impairment experiment, pregnant Wistar rats were obtained from the FEPPS-RS. After birth each litter was adjusted within 48 h to eight rat pups, and to contain offspring of both genders in about equal proportions. Each pup was kept together with its mother in a plastic cage with sawdust bedding in a room temperature of 21± 1ºC and a 12/12 h light/dark cycle. At the age of 3 weeks, pups were weaned and the males were selected and raised maintained in groups of three to five in individually ventilated cages with sawdust bedding. For postnatal treatments, animals were given standardized pellet food and tap water *ad libitum*.

All behavioral experiments were performed at light phase between 09:00 h and 16:30 h. All experimental procedures were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH publication No. 80-23 revised 1996) and approved by the Institutional Ethics Committee of the Pontifical Catholic University (CEUA 10/00145). All efforts were made to minimize the number of animals and their suffering.

Treatments

Cannabidiol

For the experiments investigating the effects of cannabidiol on memory in naïve animals, adult rats were trained and tested in the novel object recognition task. Ten days later, groups were semi-randomized, in order to guarantee that a rat would not receive the same previous treatment, and were trained and tested in the inhibitory avoidance task. Vehicle (Tween 80- saline solution 1:16 v/v) (Lastres-Becker, *et al* 2005), or cannabidiol (approximately 99.9% pure; kindly supplied by THC-Pharm, Frankfurt, Germany and STI-Pharm, Brentwood, UK, at the doses of 2.5, 5.0, and 10 mg/kg, n = 15 per group) (Cassol-Jr, *et al* 2010) were administered intraperitoneally immediately after the training session of either inhibitory avoidance or novel object recognition task.

For the investigation of the effects of cannabidiol on iron-induced memory impairments, adult (2 month-old) rats treated neonatally with vehicle or iron (as described in detail below) received an acute intraperitoneal injection of vehicle or cannabidiol (at the doses of 5 and 10 mg/kg) immediately after the training session of the object recognition task. For experiments investigating the chronic effects of cannabidiol on iron-induced memory impairments, adult (2 months-old) rats treated neonatally with vehicle or iron received a daily intraperitoneal injection of vehicle or cannabidiol (at the doses of 5 and 10 mg/kg) for 14 consecutive days. Drug solutions were freshly prepared immediately prior to administration.

Iron neonatal treatment

The neonatal iron treatment has been described in detail elsewhere (Perez, *et al* 2010; Rech, *et al* 2010; de Lima, *et al* 2008a). Briefly, 12-day-old rat pups received orally a single daily dose (10 ml/Kg solution volume) of vehicle (5% sorbitol in water) (control group) or 30 mg/Kg of body weight of Fe^{2+} (iron carbonyl, Sigma-Aldrich, São Paulo, Brazil) via a metallic gastric tube, over 3 days (postnatal days 12-14). In this model, iron is given orally during the period of maximal iron uptake by the brain, so that the model correlates with dietary iron supplementation to infants. We previously characterized that this treatment protocol induces a selective accumulation of iron in the rat basal ganglia (Schröder, *et al* 2001).

Behavioral procedures

Inhibitory avoidance task

We used the single-trial step-down inhibitory avoidance (IA) conditioning as an established model of fear-motivated, hippocampus-dependent memory (Izquierdo and Medina, 1997; Taubenfeld, *et al* 1999). In IA training, animals learn to associate a location in the training apparatus with an aversive stimulus (footshock). The IA behavioral training and retention test procedures were described in previous reports (Schröder, *et al* 2001; Quevedo, *et al* 2004). The IA apparatus was a 50 x 25 x 25-cm acrylic box (Albarsch, Porto Alegre, Brazil) whose floor consisted of parallel caliber stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7-cm wide, 2.5-cm high platform was placed on the floor of the box against the left wall. On the training trial,

rats were placed on the platform and their latency to step down on the grid with all four paws was measured with an automatic device. Immediately after stepping down on the grid, rats received a mild foot shock (0.4 mA) and were removed from the apparatus immediately afterwards. A retention test trial was carried out 24 after the training trial. The retention test trial was procedurally identical to training, except that no footshock was presented. Step-down latencies (s) on the retention test trial (maximum 180 s) were used as a measure of IA retention (Schröder, *et al* 2001; Quevedo, *et al* 2004).

Novel object recognition

The novel object recognition task was performed as previously described (de Lima, *et al* 2007; 2008a). Briefly, the novel object recognition task took place in an open field apparatus (45 x 40 x 60 cm) with sawdust covering its floor. On the first day, rats underwent a habituation session during which they were placed in the empty open field for 5 min. On the following day, rats were given one 5-min training trial in which they were exposed to two identical objects (A1 and A2). The objects were positioned in two adjacent corners, 9 cm from the walls. On the long-term memory (LTM) testing trial (24 h after the training session), rats were allowed to explore the open field for 5 min in the presence of two objects: the familiar object A and a novel object B. These were placed in the same locations as in the training session. In long-term retention test trial, the novel object was placed in 50% trials in the right side and 50% trials in the left side of the open field. All objects were made of plastic Duplo Lego Toys and had a height of about 10 cm. Objects presented similar textures, colors, and sizes, but distinctive shapes. Between trials the objects were washed with 10% ethanol solution. Object exploration was measured by an experimenter blind to group

treatment assignments; using two stopwatches to record the time spent exploring the objects during the experimental sessions. Exploration was defined as follows: sniffing or touching the object with the nose. Sitting on the object was not considered as exploration. A recognition index calculated for each animal was expressed by the ratio $T_N/(T_F+T_N)$ [T_F= time spent exploring the familiar object (A); T_N= time spent exploring the novel object (B)].

Open-field behavior

In order to control for possible sensorimotor effects induced by chronic cannabidiol, behavior during habituation to the open field prior to object recognition training was evaluated after chronic cannabidiol administration, as previously described (de Lima, *et al* 2005b; 2008b). The open field was a 40 X 45 X 60 cm arena surrounded by 50 cm high walls, made of plywood with a frontal glass wall. The floor of the arena was divided into 12 equal squares by black lines. Animals were placed in the rear left corner and left to explore the field freely for 5 min. Latency to start locomotion, line crossings, rearings and the number of fecal pellets produced were counted. The number of crossings and rearings were used, respectively, as measures of locomotor activity and exploratory behavior, whereas the latency to start locomotion and the number of fecal pellets were used as measures of anxiety.

Statistical analysis

Behavioral data were analyzed as previously described (de Lima, *et al* 2005a; 2005b; 2007; 2008a; 2008b). Data for latency to step-down and recognition indexes are expressed as mean ± S.E.M. Comparisons among experimental groups were performed using a Kruskal-Wallis analysis of variance followed by Mann-Whitney *U*-tests, two-tailed when necessary. Data from the experiment evaluating open field behavior were analyzed by one-way analysis of variance (ANOVA) and are expressed as mean ± S.E.M. In all comparisons, *p* values less than 0.05 were considered to indicate statistical significance.

Results

In the first experiment we sought to evaluate the acute effects of cannabidiol on memory consolidation in control rats using two different learning paradigms. Figure 1 shows the results of acute cannabidiol administration in adult rats immediately after training session of inhibitory avoidance task (Fig. 1A) and novel object recognition task (Fig 1B). Cannabidiol has not affected memory for inhibitory avoidance as no significant differences in latencies to step-down among groups were found in the training ($H_{(3)}$=4.75, p = 0.19) or testing ($H_{(3)}$=4.47, p = 0.21) sessions. Similar results were found when animals were tested in the novel object recognition task. Cannabidiol at all doses used has no significant effect on recognition indexes in the retention testing session ($H_{(3)}$=3.33, p = 0.95). No statistically significant differences were found among groups in recognition index ($H_{(3)}$=6.95, p = 0.073) and total time exploring objects ($H_{(3)}$=1.25, p = 0.74) in the training session.

Using a model of cognitive impairment induced by iron administration in the neonatal period we aimed to investigate the effects of a single acute injection of

cannabidiol immediately after training in ameliorating memory impairment (Figure 2). Statistical comparison of recognition indexes using Kruskal-Wallis analyses of variance showed significant difference among groups in long-term retention test ($H_{(5)}$ = 48.75, $p < 0.0001$), but not in training session ($H_{(5)}$ = 10.21, $p = 0.07$). Further analyses with Mann-Whitney U tests showed that rats neonatally treated with iron that received vehicle in adulthood present significantly lower recognition indexes than the control group veh-veh ($p < 0.0001$) in long-term retention test, indicating that iron given in the neonatal period induces severe recognition memory impairment (Fig. 2).

Acute administration of cannabidiol at the lowest dose had no effect on iron-induced recognition memory deficits, as recognition index of animals treated neonatally with iron and cannabidiol 5.0 mg/kg did not significantly differ from the group treated with iron in the neonatal period and vehicle ($p = 0.125$), and presented a significantly lower recognition index when compared to controls (veh-veh; $p < 0.0001$) (Fig.2).

Iron-treated rats that received a single administration of cannabidiol at the dose of 10 mg/kg showed significantly higher recognition indexes than the iron-vehicle group's index ($p < 0.0001$). However, this group was found to present statistically lower recognition index when compared to the control group ($p = 0.013$), indicating that acute cannabidiol at the highest dose used was able to partially reverse neonatal iron-induced memory deficits (Fig. 2).

The effects of chronic administration of cannabidiol on iron-induced memory deficits are shown in Figure 3. Statistical comparison of recognition indexes using Kruskal-Wallis analyses of variance showed significant difference among groups in long-term retention test ($H_{(5)}$ = 40.65, $p < 0.0001$), but not in training session ($H_{(5)}$ =

5.19, p = 0.39), or in the total time exploring both objects in the training session ($H_{(5)}$ = 4.08, p = 0.54). Further analyses with Mann-Whitney U tests showed that rats neonatally treated with iron that received vehicle in adulthood present significantly lower recognition indexes in long-term retention test than the control group veh-veh (p < 0.0001), indicating that iron given in the neonatal period induces severe recognition memory impairment (Fig. 3),as previously demonstrated.

Chronic administration of cannabidiol at the lowest dose improved memory in animals that received iron in the neonatal period, as the recognition index of the iron-cannabidiol 5 mg/kg group was significantly higher than the iron-veh group (p < 0.0001). The highest dose of cannabidiol was able to completely reverse iron-induced memory deficits as the recognition index of this group was significantly higher when compared to the iron-veh group (p < 0.0001), and this group presented no statistically significant differences when compared to the control group (p = 0.23). Moreover, a statistically significant difference was found in the recognition indexes of the group that received iron-cannabidiol 5 mg/kg compared to iron-cannabidiol 10 mg/kg, suggesting that cannabidiol displays a dose-dependent effect on recognition memory impairment (Fig. 3).

In addition, results showed that chronic cannabidiol by itself has no effect on recognition memory in adult control rats, revealed by comparisons between the control group (veh-veh) and the groups that were given vehicle in the neonatal period and cannabidiol at the doses of 5 and 10 mg/kg (p's = 0.40 and 0.65, respectively) (Fig.3).

Neonatal administration of iron or chronic adult cannabidiol did not affect open-field behavior (Table 1). There were no statistically significant differences

among the groups in the number of crossings ($F_{(5,72)}$ = 0.99; p = 0.43), number of rearings ($F_{(5,72)}$ = 1.64; p = 0.16), latency to start locomotion ($F_{(5,72)}$ = 0.64; p = 0.67) and defecation ($F_{(5,72)}$ = 0.80; p = 0.55). The results indicate that neonatal treatment with iron and chronic adult cannabidiol did not affect locomotion, exploration, or anxiety.

Discussion

Cannabidiol has been recently proposed as a neuroprotective agent in neurodegenerative diseases (Iuvone, *et al* 2009). However, its potential usefulness as a therapeutic tool to ameliorate cognitive decline that accompanies neurodegenerative and psychiatric disorders is less understood. Here we found that cannabidiol was able to rescue memory in iron-overloaded animals. Thus, the present findings provide evidence that cannabidiol might rescue memory impairments specifically associated with brain disorders. Importantly, memory of control animals was not significantly affected by both acute and chronic treatment with cannabidiol, neither were general parameters of behavior such as exploratory activity, locomotion and anxiety.

Here we tested the effects of acute systemic injections of cannabidiol in control rats using two different learning and memory paradigms known to assess different types of memory, i.e. recognition memory and an aversive contextual type of memory. Results suggest that cannabidiol by itself does not affect memory consolidation. In agreement, other studies found that acute cannabidiol does not affect spatial memory, assessed in the eight-arm radial maze in control animals as well (Lichtman, *et al* 1995). Additionally, we were able to demonstrate that a chronic

cannabidiol treatment has also not affected memory in control rats (vehicle-treated rats).

It has been demonstrated that cannabidiol binds with low affinity to both CB1 and CB2 cannabinoid receptors (Scuderi, *et al* 2009), therefore, CB1- and CB2-independent modes of action for this phytocannabinoid have been investigated. Evidence indicates that cannabidiol binds to type-1 vanilloid (Bisogno, *et al* 2001) and to 5-HT1A receptors, which might explain some of its effects (Mishima, *et al* 2005; Campos and Guimarães, 2008). It is currently recognized that cannabidiol is a potent antioxidant, as it was first demonstrated in 1998 (Hampson, *et al* 1998). Subsequently, other studies have provided evidence that some of the protective effects of cannabidiol may be related to its antioxidant properties. Cannabidiol induced an up regulation of mRNA levels for Cu,Zn-superoxide dismutase, a key enzyme in endogenous defenses against oxidative stress in 6-OHDA lesioned rats (Garcia-Arencibia, *et al* 2007). Cannabidiol was also shown to inhibit NMDA-induced neurotoxicity by attenuating peroxynitrite formation in inner retinal neurons (El-Remessy, *et al* 2003). Those neuroprotective effects were attributed to cannabinoid receptor-independent properties. It is possible that in the present study the beneficial effects of cannabidiol on memory might be mediated by reversion/prevention of oxidative damage induced by iron in brain regions relevant to memory formation. Accordingly, we have demonstrated that iron treatment in the neonatal period increases lipid peroxidation, assessed by TBARS formation (Dal Pizzol, *et al* 2001; de Lima, *et al* 2005a; Budni, *et al* 2007), and protein carbonylation, an index of oxidative damage to proteins (Dal Pizzol, *et al* 2001) in the hippocampus, cortex and substantia nigra of adult rats. Moreover, we also have shown that pharmacological

treatments able to reduce oxidative stress in brain regions were also able to improve memory in rats (Pietá Dias, *et al* 2007; de Lima, *et al* 2005b; 2008b).

A recent study has demonstrated that cannabidiol was able to reverse cognitive deficits in an animal model of memory impairment induced by a global systemic insult, such as sepsis (Cassol-Jr, *et al* 2010). In this study the authors report that cannabidiol also reduced sepsis-induced oxidative stress. Interestingly, an acute single dose of cannabidiol 10 mg/kg was able to partially recover memory in iron-treated rats. In humans, in a recent naturalistic study with cannabis users, it was found that individuals who smoked cannabis high in cannabidiol showed no memory impairment (Morgan, *et al* 2010). In another human study, it was observed that higher THC and lower cannabidiol hair concentrations was associated with volume reduction in the right hippocampus in chronic cannabis users, further indicating neurotoxic effects of THC and neuroprotective effects of cannabidiol (Demirakca, *et al* 2010). These findings led us to speculate that cannabidiol, by acting through diverse mechanisms, yet to be revealed, may result in memory recover.

More recently we have shown that iron treatment increases apoptotic markers (Miwa, *et al* 2011), and reactive gliosis (Fernandez, *et al* 2011) in brain regions. It is possible that in addition to its antioxidant effects, cannabidiol was able to protect nervous tissue against apoptotic stimulus leading to a neurofunctional recover. A few studies reported that cannabidiol displays antiapoptotic effects. For instance, cannabidiol was able to reduce caspase-9 concentration in damaged tissue in an animal model of ischemic-hypoxic injury (Castillo, *et al* 2010). It also reduced beta-amyloid-induced caspase-3 appearance and DNA fragmentation in PC-12 cells (Iuovone, *et al* 2004).

In conclusion, the present study provides the first evidence that cannabidiol reverses memory impairment in an animal model of cognitive decline related to neurodegenerative disorders. These findings might be of great interest for the proposal of using cannabidiol for the treatment of cognitive impairment observed in patients suffering from neurodegenerative diseases.

Disclosure

The authors declare that they have no conflicts of interest to disclose.

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Table 1 – Open Field Behavior in iron- treated rats submitted to chronic cannabidiol administration

Group		Latency (s)	Number of Crossings	Number of Rearings	Number of Fecal pellets
Veh-Veh	(Mean ± S.E.M.)	5.99 ± 1.3	61.31 ± 7.1	24.69 ± 3.26	2.69 ± 0.67
	N	13	13	13	13
Veh-CBD5	(Mean ± S.E.M.)	5.51± 0.95	72.0 ± 8.53	24.42 ± 5.11	2.08 ± 0.66
	N	12	12	12	12
Veh-CBD10	(Mean ± S.E.M.)	5.55 ± 0.46	58.08 ± 6.89	20.00 ± 2.74	2.17 ± 0.82
	N	12	12	12	12
Fe-Veh	(Mean ± S.E.M.)	4.36 ± 0.52	71.69 ± 6.90	27.92 ± 4.30	1.92 ± 0.58
	N	13	13	13	13
Fe-CBD5	(Mean ± S.E.M.)	4.47 ± 0.48	70.31 ± 7.62	25.69 ± 3.58	1.46 ± 0.63
	N	13	13	13	13
Fe-CBD10	(Mean ± S.E.M)	4.83 ± 0.83	76.47 ± 5.78	34.07 ± 3.21	1.13 ± 0.45
	N	15	15	15	15
Total	(Mean ± S.E.M)	5.10 ± 0.33	68.60 ± 2.9	26.44 ± 1.56	1.88 ± 0.26
	N	78	78	78	78

Open field behavior was analyzed during the habituation session for the object recognition task in iron treated rats after cannabidiol chronic administration. Data are expressed as mean ± S.E.M. No significant differences in latency to start locomotion, number of rearings, number of crossings, or defecation were found among groups.

Figure legends

Figure 1 - Effects of acute cannabidiol (CBD) on memory consolidation for the inhibitory avoidance task (A), and novel object recognition task (B) in naive rats. Vehicle or CBD (2.5, 5.0 or 10.0 mg/kg) were administered immediately after the training session. Long-term memory (LTM) retention test was performed 24 h after training. Behavioral testing was carried out when animals were 2 months old. *N* = 15 per group. Data are expressed as mean ± S.E.M. No significant differences were found among groups.

Figure 2 - Effects of a single acute injection of cannabidiol (CBD) on iron-induced recognition memory deficits. Vehicle or CBD (5.0 or 10.0 mg/kg) were administered immediately after the training session. Long-term memory (LTM) retention test was performed 24 h after training. Behavioral testing was carried out when animals were 2 months old. The proportion of the total exploration time that the animal spent investigating the novel object was the "Recognition Index" expressed by the ratio TN/(TF+TN), TF = time spent exploring the familiar object and TN = time spent exploring the novel object. Data expressed as mean ± S.E.M. *N* = 10 – 16 per group. Differences between vehicle-vehicle vs other groups are indicated as: ** $p < 0.001$ and * $p < 0.05$; difference between iron-vehicle vs iron-CBD is indicated as: ## $p < 0.001$.

Figure 3 - Effects of chronic cannabidiol (CBD) on iron-induced recognition memory deficits. A daily single injection of vehicle or CBD (5.0 or 10.0 mg/kg) were administered intraperitoneally for 14 consecutive days. Twenty-four hours after the

last injection animals were trained in the novel object recognition task. Long-term memory (LTM) retention test was performed 24 h after training. Behavioral testing was carried out when animals were 2-3 months old. The proportion of the total exploration time that the animal spent investigating the novel object was the "Recognition Index" expressed by the ratio TN/(TF+TN), TF = time spent exploring the familiar object and TN = time spent exploring the novel object. Data expressed as mean ± S.E.M. *N* = 12 – 15 per group. Differences between vehicle-vehicle vs other groups are indicated as: ** p <0.001; difference between iron-vehicle vs iron-CBD is indicated as: ## p <0.001, difference between iron-CBD 5mg/kg vs iron-CBD 10 mg/kg is indicated as: ++ p <0.001.

Figure 1

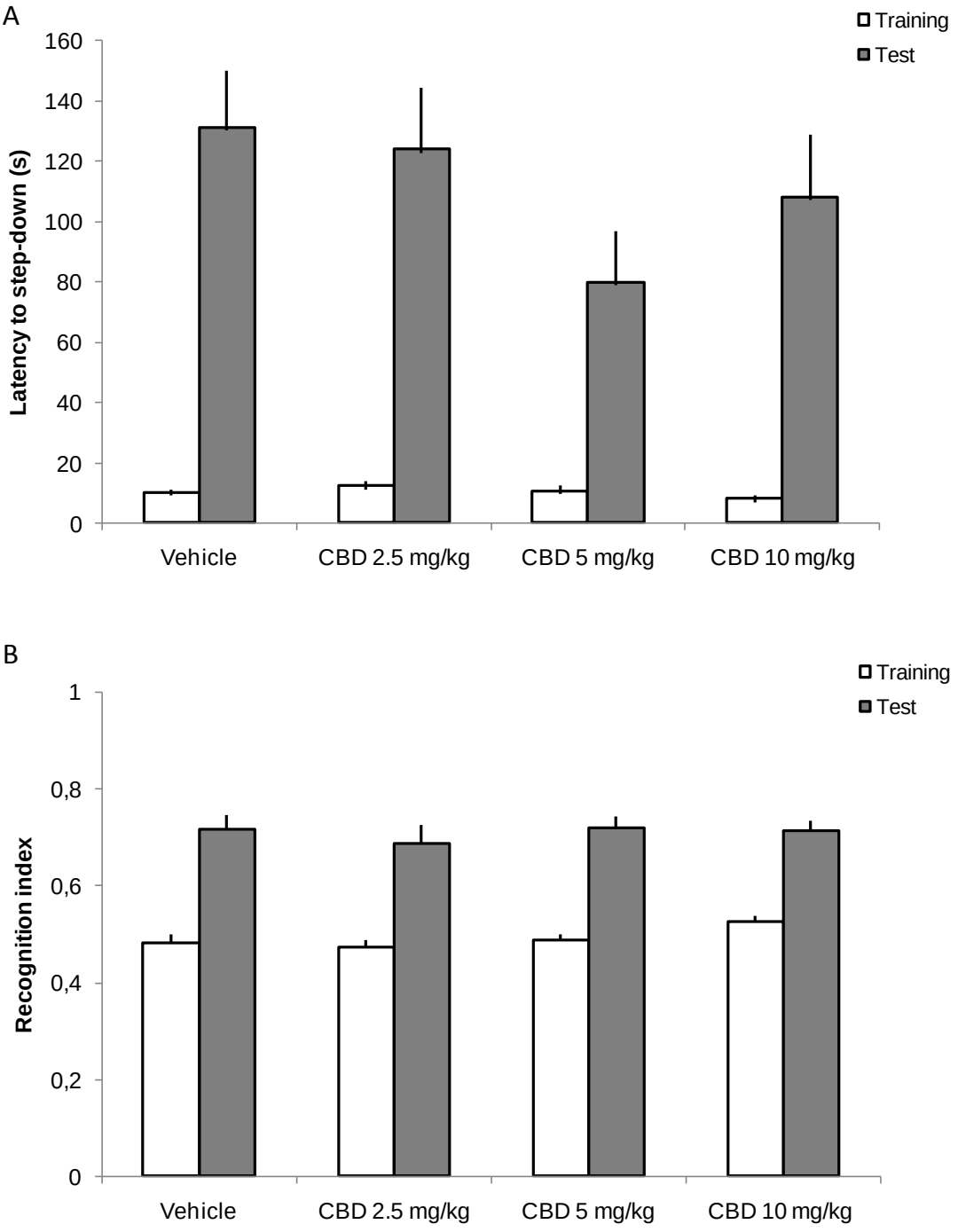

A
Training
Test
Latency to step-down (s)
160
140
120
100
80
60
40
20
0
Vehicle
CBD 2.5 mg/kg
CBD 5 mg/kg
CBD 10 mg/kg
B
Training
Test
Recognition index
1
0,8
0,6
0,4
0,2
0
Vehicle
CBD 2.5 mg/kg
CBD 5 mg/kg
CBD 10 mg/kg

Figure 2

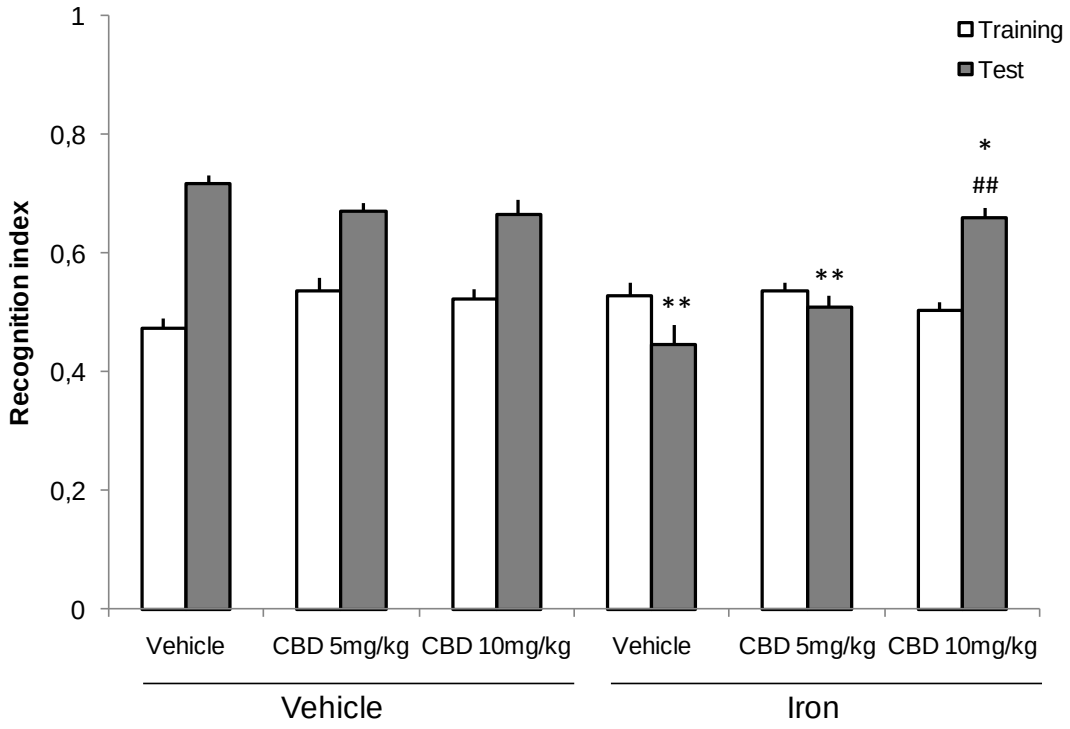
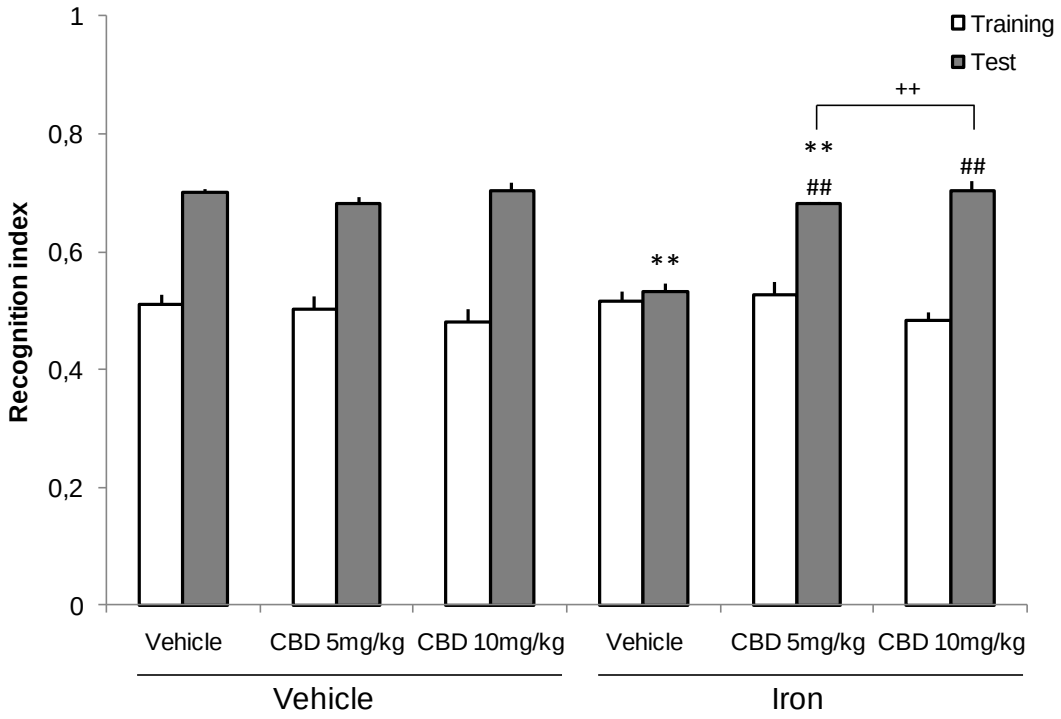

Recognition index
1
0,8
0,6
0,4
0,2
0
Training
Test
**
**
*
##
Vehicle
CBD 5mg/kg
CBD 10mg/kg
Vehicle
CBD 5mg/kg
CBD 10mg/kg
Vehicle
Iron

Figure 3


Training
Test
Recognition index
1
0,8
0,6
0,4
0,2
0
++
**
##
**
##
Vehicle
CBD 5mg/kg
CBD 10mg/kg
Vehicle
CBD 5mg/kg
CBD 10mg/kg
Vehicle
Iron

3. CAPÍTULO 3

Considerações Finais

3.1 CONSIDERAÇÕES FINAIS

Nos últimos 45 anos estudos tem comprovado que o CBD possui uma ampla variedade de efeitos farmacológicos e muitos destes de grande interesse terapêutico. Essa substância não psicotrópica mostra-se promissora em estudos clínicos e pré-clínicos relacionados à cognição, ansiedade, desordens psiquiátricas e doenças neurodegenerativas (Zuardi, 2008; Scuderi et al., 2009).

Embora muitos trabalhos já tenham demonstrado que a neuroproteção e o efeito anti-oxidativo observado com o CBD não são afetados por antagonistas de receptores de canabinóides (Zuardi, 2008), a pesquisa sobre o completo mecanismo de ação do CBD em cada patologia ainda persiste. Baseado nisso, usamos um modelo animal de déficit de memória para avaliar seu possível uso em doenças neurodegenerativas.

Os resultados apresentados nesse trabalho evidenciaram que o CBD não afeta a memória de ratos adultos normais. No entanto, os animais que receberam o tratamento neonatal com ferro durante o 12º ao 14º dia de vida apresentaram uma melhora na memória de reconhecimento de objeto de uma forma dose dependente.

De acordo com estudos realizados anteriormente, podemos afirmar que o tratamento com ferro neonatal resulta no processo de estresse oxidativo em regiões cerebrais como córtex, hipocampo e *substantia nigra* (de Lima et al., 2005a). O ferro participa dessa reação catalisando a formação de radicais hidroxilas (•OH) que são altamente reativos. Essa reação é denominada Reação de Fenton, representada pela equação: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$, na qual o Fe^{2+} é estequiometricamente oxidado pelo peróxido de hidrogênio (H_2O_2) à Fe^{3+} originando o radical •OH. A produção exacerbada desse radical livre gera danos em componentes celulares e extra-celulares, incluindo alterações protéicas, lipídicas, no DNA e na mitocôndria. Essas modificações levam ao mecanismo de morte celular, que vem sendo estudado como uma possível causa das doenças neurodegenerativas (Polla at al., 2003).

Tem sido proposto que o estresse oxidativo é o principal mecanismo responsável pelos efeitos tóxicos do ferro observados em doenças neurodegenerativas como DA e DP (Bishop et al., 2002; Sian- Hülsmann et al., 2010).

Trabalhos recentes realizados em nosso grupo de pesquisa afirmam que esse mesmo protocolo de tratamento utilizando ferro neonatal resulta em um aumento de marcadores apotóticos como Par-4 e caspase-3 no córtex e região CA1 e CA3 do hipocampo de ratos adultos tratados com o metal no 12º, 13º e 14º dia de vida (Miwa et al., 2011). Da mesma forma, o estudo realizado por Fernandez e colaboradores que visava investigar mudanças neuropatológicas em ratos adultos e idosos demonstrou um aumento de astrocitose no hipocampo, estriado e substância negra dos ratos adultos que receberam ferro quando recém nascidos. Assim como uma considerável diminuição da densidade neuronal nos animais idosos (Fernandez et al., 2011).

Como já foi mencionado, há poucos estudos que descrevem os possíveis mecanismos de ação do CBD, mas são crescentes as evidências de que essa substância apresenta ação antioxidante e antiapoptótica (Scuderi et al., 2008; Castillo et al., 2010; Cassol-Jr et al., 2010).

Em resumo, este trabalho sugere que o CBD pode vir a ser usado no tratamento de distúrbios cognitivos tão evidentes em pacientes com doenças neurodegenerativas. No entanto, é necessário que mais estudos pré-clínicos e clínicos sejam realizados para melhor analisar as propriedades farmacológicas desse fitocanabinóide.

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ANEXO

Comprovante de submissão do artigo científico

Detailed Status Information

Manuscript #	NPP-11-0275
Current Revision #	0
Submission Date	23rd Mar 11
Current Stage	Submission
Title	Memory rescuing properties of cannabidiol in an animal model of cognitive impairment relevant to neurodegenerative disorders
Running Title	Cannabidiol in memory impairment
Manuscript Type	Original Article
Category	Behavioral Neuropsychopharmacology
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Abstract	Cannabidiol, the main non-psychotropic constituent of Cannabis sativa, possesses a large number of pharmacological effects including anticonvulsive, sedative, hypnotic, anxiolytic, antipsychotic, anti-inflammatory, and neuroprotective, as demonstrated in clinical and pre-clinical studies. Many neurodegenerative disorders involve cognitive deficits, and this has led to interest in whether cannabidiol could be useful in the treatment of memory impairment associated to these diseases. Here, we used an animal model of cognitive impairment, induced by iron overload in order to test the effects of cannabidiol in memory-impaired rats. Rats received vehicle or iron at postnatal days 12-14. At the age of 2 months, they received an acute intraperitoneal injection of vehicle or cannabidiol (5.0 or 10.0 mg/kg) immediately after the training session of the novel object recognition task. In order to investigate the effects of chronic cannabidiol, iron-treated rats received daily intraperitoneal injections of cannabidiol (5.0 or 10.0 mg/kg) for 14 days. Twenty-four hours after the last injection, they were submitted to object recognition training. Retention tests were performed 24 hours after training. A single acute injection of cannabidiol at the highest dose was able to partially recover memory in iron-treated rats. Chronic cannabidiol improved recognition memory in iron-treated rats in a dose-dependent manner. Acute or chronic cannabidiol does not affect memory in control rats. The present findings provide evidence suggesting the potential use of cannabidiol for the treatment of cognitive decline associated with neurodegenerative disorders. Further studies, including clinical trials, are warranted to determine the usefulness of cannabidiol in humans suffering from neurodegenerative disorders.
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Yours sincerely,

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