Universidade Federal De Pelotas
Centro de Ciências Químicas, Farmacêuticas e de Alimentos
Programa de Pós-Graduação em Bioquímica e Bioprospecção

Tese de doutorado

Inosina como um agente neuroprotetor em modelos experimentais para a Doença de Alzheimer

Fernanda Cardoso Teixeira

Pelotas, outubro de 2020

Fernanda Cardoso Teixeira

Inosina como um agente neuroprotetor em modelos experimentais para a Doença de Alzheimer

Tese de doutorado apresentada ao Programa de Pós-Graduação em Bioquímica e Bioprospecção da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutora em Ciências (Bioquímica e Bioprospecção).

Orientadora: Prof.ª Dr.ª Roselia Maria Spanevello

Co-orientador: Dr. Jessié Martins Gutierres

Pelotas, outubro de 2020

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

T111i Teixeira, Fernanda Cardoso

Inosina como um agente neuroprotetor em modelos experimentais para a doença de alzheimer / Fernanda Cardoso Teixeira ; Roselia Maria Spanevello , orientador ; Jessie Gutierres , coorientador. — Pelotas, 2020.

153 f. : il.

Tese (Doutorado) — Programa de Pós-Graduação Bioquímica e Bioprospecção, Centro de Ciências Químicas Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, 2020.

1. Alzheimer. 2. Memória. 3. Inosina. 4. Ratos. 5. Hipocampo. I. , Roselia Maria Spanevello, orient. II. , Jessie Gutierres, coorient. III. Título.

CDD : 574.192

Elaborada por Dafne Silva de Freitas CRB: 10/2175

Fernanda Cardoso Teixeira

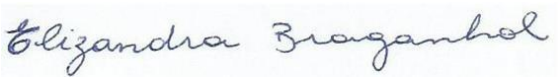
Inosina como um agente neuroprotetor em modelos experimentais para a Doença de Alzheimer

Tese aprovada, como requisito parcial, para obtenção do grau de Doutora em Ciências, Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas.

Data da Defesa: 30/10/2020

Banca examinadora:

Prof.ª Dr.ª Roselia Maria Spanevello (Orientadora) - Doutora em Ciências Biológicas-Bioquímica pela Universidade Federal do Rio Grande do Sul.



Prof.ª Dr.ª Elizandra Braganhol - Doutora em Ciências Biológicas - Bioquímica pela Universidade Federal do Rio Grande do Sul.

Prof.ª Dr.ª Maria Rosa Chitolina - Doutora em Ciências Biológicas - Bioquímica pela Universidade Federal do Paraná.

Prof.ª Dr.ª Margarete Dulce Bagatini - Doutora em Ciências Biológicas - Bioquímica Toxicológica pela Universidade Federal de Santa Maria.

Dedico este trabalho à minha avó Elci,
que me ensinou que o amor não se esquece.
In memoriam

Agradecimentos

Escrever esses agradecimentos é uma alegria! Agradecer a Deus e ao Universo pela oportunidade de realizar um sonho!

Agradeço aos meus pais, Marli e Teixeira, que sempre apoiaram nas minhas decisões mesmo não compreendendo muito o mundo da pesquisa. Nunca deixaram eu desistir do meu sonho, acreditaram em mim muito mais que eu mesma, e hoje de fato estamos aqui! Só tenho gratidão por ter vocês na minha vida, cada um com suas caracteristicas só agregaram na minha formação, sentimento e razão! Amo vocês!

À minha avó Elci que me motivou a estudar a doença de Alzheimer e me ensinou com muita doçura que o amor a gente nunca esquece. Ao meu avô Loni que sempre estimulou os netos a estudarem e serem profissionais exemplares em suas respectivas áreas (além de sempre perguntar quando eu iria me tornar doutora, - é hoje vô!).

À minha orientadora Roselia, agradeço por ter me recebido em seu grupo de pesquisa de forma tão carinhosa. Agradeço ainda, por ter me proporciando trabalhar na linha de pesquisa que tenho maior apreço e por ter me ‘’apresentado a inosina’’. Sempre com muito entusiasmo e positividade me orientou durante a realização deste trabalho. Obrigada por me desafiar e contribuir para meu crescimento científico e pessoal. Obrigada pela relação de amizade que construimos. Acertei na escolha que fiz!

Ao meu co-orientador Jessie um super agradecimento. Obrigada pela ajuda na elaboração do desenho experimental, pelo ensinamento de técnicas, pelas discussões científicas e pela parceria. És uma pessoa incrível, obrigada pela amizade. Te guardo no meu coração.

Ao grande colaborador desde trabalho, Fabiano Carvalho, que me recebeu na UFCSPA com carinho e me ensinou tanto com muita generosidade. Serei sempre muito grata por tudo, meu amigo. Tua colaboração em várias das etapas do meu trabalho foi essencial.

Ao grupo lindo NEUROBio, toda minha gratidão. Como eu sempre digo, ninguém faz um trabalho sozinho e eu certamente contei com a ajuda e o apoio de muita gente sensacional, pela responsabilidade e dedicação que tem pela pesquisa. Durante esses quatros anos aprendi muito sobre bioquímica e também relações humanas, gracas a vocês. Assim, aqui deixo registrado meu obrigada pela parceria,

boa vontade, disposição, companherismo, além do mate, café e bolo de cada final de experimento clássico do nosso lab! Sempre existem pessoas que se destacam pela alegria, energia, amizade, bom humor e disposição, assim gostaria de agradecer em especial à Bruna, à Julia, à Juliane, ao Lorenço e à Mayara.

Agradeço aos colegas do mestrado que seguiram comigo se tornando verdadeiros amigos: Gabi Debom, Carlus Augustu e Juliana. Cada um de vocês com suas particularidades são extraordinários e só acrescentam na minha vida!

Aos grupos dos pesquisadores Dr.ª Maria Rosa Chitolina, Dr.ª Marilda Cruz, Dr. Vinícius Campos e Dr. Relber Aguiar Gonçales que colaboraram para a realização deste trabalho.

À toda equipe do Biotério Central da UFPel, que sempre me atenderam de forma muito solícita e desempenham seu trabalho com muito comprometimento e responsabilidade. Em especial à responsável técnica veterinária Anelize Félix, pois sempre esteve presente em meus experimentos, prestanto um atendimento diferenciado com muito empenho em sanar todas minhas dúvidas e dificuldades encontradas pelo caminho, além de repassar seu conhecimento com muita tranquilidade. Obrigada Ane! Tua parceria foi fundamental durante meu trabalho, assim como teu mate e tua risada!

À CAPES pela bolsa concedida durante o período de 36 meses.

Ao PPGBBio e todo corpo docente pelos diversos aprendizados durante o doutorado.

Aos professores da banca por aceitarem o convite para avaliação deste trabalho.

À Universidade Federal de Pelotas pela formação acadêmica.

“A curiosidade é mais importante que o conhecimento.”

(Albert Einstein)

Resumo

TEIXEIRA, Cardoso Fernanda. **Inosina com um agente neuroprotetor em modelos experimentais para a Doença de Alzheimer.** 2020. 153f. Tese de Doutorado - Programa de Pós-Graduação em Bioquímica e Bioprospecção, Universidade Federal de Pelotas, Pelotas, 2020.

A doença de Alzheimer (DA) é uma patologia neurodegenerativa e a principal causa de demência em idosos. Acerca dos mecanismos patofisiológicos envolvidos na DA pode-se destacar o déficit colinérgico, o estresse oxidativo, a neuroinflamação e as alterações na sinalização purinérgica. Considerando que os fármacos disponíveis para a DA apenas aliviam os sintomas, a busca por terapias mais eficazes torna-se importante. A inosina é uma purina endógena que possui ações antioxidantes, anti-inflamatórias e neuroprotetoras. Assim, o objetivo desse trabalho foi investigar os efeitos dessa purina na memória e em parâmetros neuroquímicos em modelos experimentais de DA. Foram avaliados dois protocolos: no primeiro, os ratos foram divididos nos seguintes grupos: controle, escopolamina (SCO), SCO + inosina (50 mg/kg) e SCO + inosina (100 mg/kg). A inosina foi pré-administrada por 7 dias, por via intraperitoneal. No oitavo dia, a SCO foi administrada pré ou pós-treino da tarefa de esquiva inibitória. No segundo, os ratos foram divididos nos grupos: controle, estreptozotocina (STZ), STZ + inosina (50 mg/kg) e STZ + inosina (100 mg/kg). STZ foi administrada por injeção intracerebroventricular e os animais foram tratados com inosina durante 25 dias. Em ambos os protocolos, a inosina foi capaz de prevenir os déficits de memória, alterações em enzimas colinérgicas e estresse oxidativo em córtex cerebral e hipocampo. No modelo de DA induzido por STZ, a inosina também foi capaz de diminuir a imunorreatividade do receptor de adenosina $A2_A$, aumentar os níveis de citocinas anti-inflamatórias e a expressão do fator neurotrófico derivado do cérebro (BDNF) e seu receptor (TrkB). As alterações morfológicas induzidas pelo STZ no hipocampo também foram atenuadas pelo tratamento com inosina (50 e 100 mg/kg). Os resultados obtidos demonstraram que a inosina afeta múltiplos alvos cerebrais, sugerindo que este nucleosídeo pode ter um importante potencial terapêutico contra déficits cognitivos e danos teciduais associados à DA ou outras doenças neurodegerativas.

Palavras-chave: Alzheimer, memória, inosina, ratos, hipocampo, BDNF

Abstract

TEIXEIRA, Cardoso Fernanda. **Inosine as neuroprotective agent in experimental models for Alzheimer's disease.** 2020. 153f. Doctoral Thesis - Graduate Program in Biochemistry and Bioprospecting, Federal University of Pelotas, Pelotas, 2020.

Alzheimer's disease (AD) is a neurodegenerative disease and the main cause of dementia in the elderly. Regarding the pathophysiological mechanisms involved in AD, we can highlight the cholinergic deficit, oxidative stress, neuroinflammation and changes in purinergic signaling. Considering that the drugs available for AD only relieve symptoms, the search for more effective therapies becomes important. Inosine is an endogenous purine that has antioxidant, anti-inflammatory and neuroprotective actions. Thus, the objective of this work was to investigate the effects of this purine on memory and on neurochemical parameters in experimental models of AD. Two protocols were evaluated: in the first, the rats were divided into the following groups: control, scopolamine (SCO), SCO + inosine (50 mg / kg) and SCO + inosine (100 mg / kg). Inosine was pre-administered for 7 days, intraperitoneally. On the eighth day, SCO was administered pre- or post-training for the inhibitory avoidance task. In the second, the rats were divided into groups: control, streptozotocin (STZ), STZ + inosine (50 mg / kg) and STZ + inosine (100 mg / kg). STZ was administered by intracerebroventricular injection and the animals were treated with inosine for 25 days. In both protocols, inosine was able to prevent memory deficits, changes in cholinergic enzymes and oxidative stress in the cerebral cortex and hippocampus. In the STZ-induced AD model, inosine was also able to decrease the A2A adenosine receptor immunoreactivity, increase the levels of anti-inflammatory cytokines and the expression of the brain-derived neurotrophic factor (BDNF) and its receptor (TrkB). The morphological changes induced by STZ in the hippocampus were also mitigated by treatment with inosine (50 and 100 mg / kg). The results obtained demonstrated that inosine affects multiple brain targets, suggesting that this nucleoside may have an important therapeutic potential against cognitive deficits and tissue damage associated with AD or other neurodegenerative diseases.

Keywords: Alzheimer, memory, inosine, rats, hippocampus, BDNF.

Lista de abreviaturas, siglas e símbolos

A_1- Receptor de adenosina 1

A_{2A}- Receptor de adenosina 2A

A_{2B}- Receptor de adenosina 2B

A_3- Receptor de adenosina 3

ACh- Acetilcolina

AChE- Acetilcolinesterase

ADA- Adenosina desaminase

ADP- Adenosina difosfato

ADPR- Adenosina difosfato ribose

AMP- Adenosina monofosfato

AMPc- 3´5´-adenosina-monofosfato-cíclico

apoE- Apolipoproteína E

apoEω4- Apolipoproteína E alelo 4

APP- Proteína precursora amilóide

ATP- Adenosina trifosfato

ATPases- Adenosinatrifosfatases

Aβ- Peptídeo β amilóide

BDNF- Fator neurotrófico derivado do cérebro

BuChE- Butirilcolinesterase

C- Controle

Ca^{+2}- Cálcio

Cadpr- Adenosina difosfato ribose cíclico

CAT- Catalase

CD38- ADP-ribose hidrolase ciclica

CD73- Ecto-5'nucleotidase

ChAT- Colina acetiltransferase

CREB- Proteína de ligação responsiva ao AMPc

DA- Doença de Alzheimer

DAG- Diacilglicerol

DNA- Ácido desoxidoribonucleico

DP- Doença de Parkinson

DSM-5- Diagnostic and Statistical Manual of Mental Disorders, 5th Edition

EM- Esclerose múltipla

ERK ½- Quinase reguladora de sinal extracelular

ERN- Espécies reativas de nitrogênio

EROs- Espécies reativas de oxigênio

FDA- Food and drugs administration

GAP-43- Proteína associada ao crescimento 43

GFAP- Proteína ácida fibrilar glial

GLUT-2- Transportador de glicose 2

GPx- Glutationa peroxidase

GSK3 α/β- Glicogênio sintase quinase

H_2O_2- Peróxido de hidrogênio

HO•- Radical hidroxila

i.p.- Intraperitoneal

ICV- Intracerebroventricular

IL- Interleucinas

IL-10- Interleucina 10

IL-12- Interleucina 12

IL1-β- Interleucina 1β

IL-4- Interleucina 4

IL-6- Interleucina 6

IMP- Inosina monofosfato

IP_3- Inositol 1,4,5-fosfato

K^+- Potássio

LTP- Long Term Potentiation

mAChRs- Receptores muscarínicos

MAPK- Proteína quinase ativadora de mitógeno

MCI- Mild cognitive impairment

Mg^+- Magnésio

mRNA- Ácido ribonucleico mensageiro

MST3b- Mammalian sterile 20 like-quinase 3b

Na^+- Sódio

nAChRs- Receptores nicotínicos

NAD^+- Nicotinamida adenina dinucleotideo

NFkβ- Fator transcrição nuclear kβ

NMDA- N-metil D-aspartato

NO- Óxido nítrico

NPP1/CD203a- Pirofosfatase/fosfodiesterases 1

NTPDase- Ectonucleosídeo trifosfato difosfohidrolase

O_2 - Oxigênio singleto

$O2^{\bullet-}$ -Ânion superóxido

$ONOO^-$- Peroxinitrito

PARP- Enzima poli-ADP-ribose-polimerase

PKA- Proteína quinase A

PKC- Proteína quinase C

PNP- Purine nucleosideo fosforilase

Proteína Gi- Proteína G inibitória

Proteína Gq- Proteína G ativadora de fosfolipase C

Proteína Gs- Proteína estimulatória

REM- Rapid eye moviment

RI- Receptor de insulina

SCO- Escopolamina

SH- Sulfidrilas

SNC- Sistema nervoso central

SOD- Superóxido dismutase

STZ- Estreptozotocina

TBARS- Substâncias reativas ao ácido tiobarbitúrico

TCD4- Linfócitos T auxiliares CD4

TCD8- Linfócitos T citotóxicas CD8

TNF-α- Fator de necrose tumoral α

TrkBr- Receptor tirosina quinase B

UDP- Uracila difosfato

UTP- Uracila trifosfato

VAChT- Transportador vesicular de acetilcolina

Lista de Figuras

Sumário

1 Introdução

A doença de Alzheimer (DA) é caracterizada clinicamente por um declínio na função cognitiva, que afeta principalmente a memória de curto prazo, sendo observada majoritariamente na população idosa (BLENNOW, LEON e ZETTERBERG, 2006; DHULL e KUMAR, 2018; MOBLEY e CHEN, 2019; CHAKRABORT e DIWAN, 2020). A DA apresenta uma natureza progressiva, resultante de falha sináptica, dano neuronal e morte celular em áreas específicas do cérebro, como córtex cerebral e hipocampo, as quais são essenciais para o processamento da memória (PRESTON e EICHENBAUM, 2013; SCHELTENS et al., 2016; GHAI et al., 2020).

A caracterização clínica da DA inicia de forma sutil reconhecida pela falha de memória sendo denominada por comprometimento cognitivo leve (Mild Cognitive Impairment – MCI) que lentamente se torna mais grave e, em última fase denomina-se demência do tipo Alzheimer, que é a forma altamente incapacitante (MCKHANN et al.,1984; AMIDFAR et al., 2020; CHAKRABORT e DIWAN, 2020). Os principais achados neuropatológicos que caracterizam a DA são a presença de placas extracelulares compostas de agregados do peptídeo β-amiloide (Aβ) e emaranhados neurofibrilares intracelulares, os quais são formados pela hiperfosforilação da proteína Tau (CHEN, 2018). Além disso, tem sido demonstrado que outros mecanismos estão envolvidos na patofisiologia da DA tais como a neuroinflamação, estresse oxidativo, disfunção mitocondrial e alterações na sinalização colinérgica e purinérgica (FERRER, 2012; AMATO, TERZO e MULÈ, 2019; SONNTAG et al., 2017; HOYER, 2004; CELLAI et al., 2018; CIEŚLAK e WOJTCZAK, 2018; CHEN, 2018; MCLARNON et al., 2006; VARMA et al., 2009, LANGE et al., 2020; UDDIN et al., 2020).

A prevalência da DA é diretamente proporcional ao envelhecimento da população (PRINCE et al., 2013). Dados indicavam que em 2015 cerca de 46,85 milhões de pacientes com DA no mundo, para 2030 seriam 74,7 milhões e em 2050 131,5 milhões (INSTITUTO ALZHEIMER BRASIL, 2019; CHAKRABORT e DIWAN, 2020). A cada ano aproximadamente 7,7 milhões de novos casos são diagnosticados (ALZHEIMER'S ASSOCIATION, 2017). No Brasil, a estimativa é que em 2020 existem 1,6 milhões de pacientes portadores de DA (INSTITUTO ALZHEIMER BRASIL, 2019).

A epidemiologia é impactante e reflete a repercussão dessa patologia para a saúde pública, em função do grande número de acometidos e, ainda, em relação aos prejuízos acarretados no cotidiano do indivíduo portador e nas suas relações

interpessoais, bem como por haver intervenções terapêuticas limitadas (DHULL e KUMAR, 2018). O tratamento e o atendimento diário de pacientes com DA são considerados onerosos em aspectos tanto emocionais quanto econômicos (CRYSTAL e GLANZMAN, 2020). A terapêutica tem como objetivo maximizar a capacidade de funcionalidade na vida diária, manter a qualidade de vida e retardar a progressão dos sintomas, já que a cura ainda não é possível (KOUHESTANI, JAFARI e BABAEI, 2018; SCHELTENS et al., 2016; CUMMINGS et al., 2019). Compreendendo o impacto negativo que a DA possui e considerando que muitos pacientes se tornam refratários ao tratamento padrão com medicamentos inibidores das colinesterases e antagonistas dos receptores N-metil-D-aspartato (NMDA), torna-se imprescindível a busca por novas alternativas terapêuticas (ALZHEIMER'S ASSOCIATION, 2010; UNZETA et al., 2016; WOODS et al., 2016; DHULL e KUMAR, 2018; DOS SANTOS et al., 2018; HUANG, CHAO e HU, 2020).

A inosina é um metabólito proveniente da desaminação da adenosina em uma reação catalisada pela enzima adenosina desaminase (ADA) no meio intra e extracelular, ou ainda pela desfosforilação do monofosfato de inosina ou inosina monofosfato (IMP) pela enzima 5'-nucleotidase (CD73) (BARANKIEWICKZ e COHEN, 1985; RUHAL e DHINGRA, 2018). Dados da literatura têm demonstrado que a inosina possui importantes ações farmacológicas, tais como, atividade antioxidante, anti-inflamatória e neuroprotetora (GOMEZ e SITKOVSKY, 2003; HASKO, SITKOVSKYand SZABÓ, 2004; WU et al., 2008; MABLEY et al., 2009; DACHIR et al., 2014; KURICOVA et al., 2014; RHURAL e DHINGRA, 2019). Essas ações farmacológicas da inosina podem estar relacionadas com a interação dessa molécula com receptores de adenosina, especialmente A_1 e A_{2A} (JIM, 1998; WEI et al., 2011). Pode-se observar essas ações em diferentes estudos usando modelos experimentais de dor, inflamação, depressão e encefalomielite (NASCIMENTO et al., 2010; Da ROCHA-LAPA et al., 2013; MUTO et al., 2014; JUNQUEIRA et al., 2016; GONÇALVES et al., 2016).

Nesse contexto, o objetivo do presente trabalho foi investigar o potencial da inosina, em modelos experimentais para a DA, em prevenir déficits de memória, bem como investigar mecanismos bioquímicos, moleculares e morfológicos envolvidos no possível efeito neuroprotetor deste nucleosídeo.

2 Referencial teórico

2.1 Sistema Nervoso Central e memória

O sistema nervoso central (SNC) é composto por duas estruturas principais denominadas medula espinhal e encéfalo. O encéfalo por sua vez subdivide-se em cérebro, cerebelo e tronco encefálico. As células desse sistema são divididas em neurônios e células gliais (astrócitos, oligodendrócitos e micróglia) (NIEUWENHUYS, VOOGD e CHRISTIAAN, 2007).

Os neurônios são responsáveis pela sinalização elétrica e suas interconexões denominadas sinapses (FARHY-TSELNICKER e ALLEN, 2018). As células gliais participam da neurogênese, da migração e da sinaptogênese. Á medida que os circuitos neurais amadurecem, as células gliais cumprem papéis importantes na comunicação sináptica, plasticidade, homeostase e atividade no nível da rede por meio de monitoramento dinâmico e alteração da estrutura e função do SNC (ALLEN e LYONS, 2018). Destacam-se pela regulação da recuperação tecidual após lesão e sua disfunção é um possível fator contribuinte para doenças neurodegenerativas (GREENHALGH, DAVID e BENNETT, 2020).

Nesse complexo sistema são formadas as memórias, que são resultados das experiências vividas, onde o cérebro é capaz de converter a realidade em códigos ou esquemas (PRESTON e EICHENBAUM, 2013). Essa conversão pode ser em sinais elétricos ou bioquímicos, e os neurônios são as células responsáveis por essa tradução, que também pode ser denominada memória celular, ou seja, a conversão de um sinal transitório em uma resposta sustentada (INNISS e SILVER, 2013).

Não existe uma definição universal de memória, mas o termo pode ser entendido como um processo de aquisição, formação, conservação e evocação, o qual envolve a retenção de informações específicas (CRYSTAL e GLANZMAN, 2020). Primeiro, durante o aprendizado ou também chamado de aquisição, o cérebro deve formar rapidamente uma representação neural inicial da nova experiência. Segundo, o cérebro deve consolidar a nova representação em uma organização que é otimizada para recuperação quando alimentada por um estímulo, que pode estar apenas distante associado a uma característica da experiência inicial (PRESTON e EICHENBAUM, 2013). Uma simples classificação da memória pode ser feita de acordo com a função, o conteúdo e com o tempo de duração. Assim, é apresentada a memória de trabalho, as memórias declarativas e procedurais e, por fim, as memórias de curta e longa duração, respectivamente (IZQUIERDO et al., 1999).

A memória de trabalho é um tipo de memória que não produz arquivos e não é acompanhada de alterações bioquímicas ou comportamentais relevantes. Quanto à memória de conteúdo, existem as declarativas, que são registros de fatos, eventos ou conhecimentos e sua falha gera episódios de amnésia, e as procedurais, que são do tipo automático e implícito relacionadas com as capacidades, os hábitos e as habilidades do indivíduo (IZQUIERDO e MCGAUGH, 2000). Quanto à duração, há indícios que ambas utilizam as mesmas estruturas cerebrais, porém com mecanismos distintos, sendo consideradas fenômenos diferentes, mas relacionadas entre si (FURINI et al., 2013). Podemos observar a classificação básica das memórias na Figura 1 (IZQUIERDO et al., 1999).

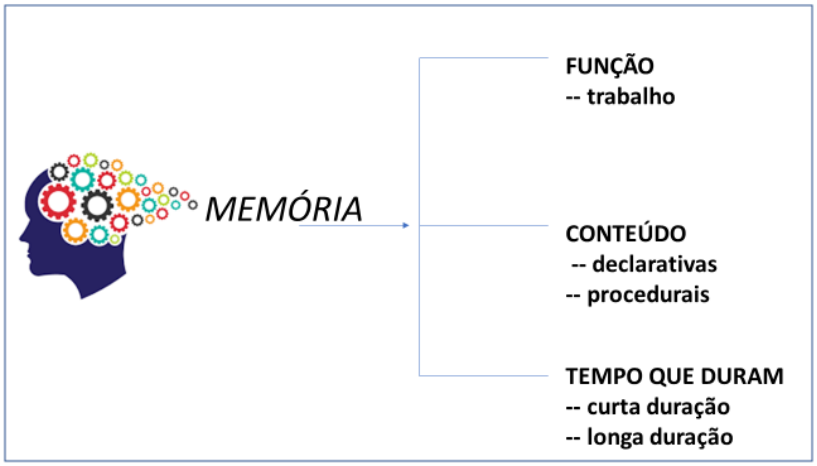


Figura 1. Tipos de mémoria. Adaptado de IZQUIERDO et al., (1999).

O mecanismo sináptico preferencial para aprendizagem e memória em mamíferos é o potencial de longo prazo (Long Term Potenciation- LTP), que é mediado pela ativação de receptores NMDA pós-sinápticos (CRYSTAL e GLANZMAN, 2020). Uma vez ativados, os receptores NMDA abrem os canais de cálcio e com o aumento da concentração desse íon há ativação de proteína quinase dependente de cálcio /calmodulina (CaMKII) e proteína quinase C (PKC), o que leva à fosforilação/atividade de p43, liberação de glutamato e favorece a persistência de LTP (IZQUIERDO, FURINI, e MYSKIW, 2016). Assim, a base biológica para a formação de memórias são as alterações estruturais das sinapses, e seu armazenamento se dá através de modificações da sua forma e sua função nas redes neurais em nível celular e sináptico (CRYSTAL e GLANZMAN, 2020), ou seja, a

memória está associada à remodelação e crescimento de sinapses preexistentes e à formação de novas sinapses (BAILEY, KANDEL, e HARRIS, 2015).

É relatado que os padrões eletrofisiológicos de neurônios induzidos pela aprendizagem na região do hipocampo são repetidos durante o sono sem movimento rápido dos olhos (Rapid Eye Moviment-REM) e essa reativação hipocampal tem um papel fundamental na consolidação da memória (ABEL et al., 2013). De acordo com Preston e Eichenbaum (2013), a interação pós-aprendizagem entre o hipocampo e o córtex é uma formação de esquemas de memória, que facilita a proteção das informações em caso de danos, e essas duas estruturas cerebrais suportam a codificação rápida de novas informações, a consolidação e organização das redes de memória. Observa-se que as memórias de conteúdo declarativas possuem como região cerebral moduladora mais específica o hipocampo, e vale ressaltar a relação direta das primeiras lesões características da DA nessa região (CRYSTAL e GLANZMAN, 2020).

No decorrer do envelhecimento dos indivíduos ocorre uma perda neuronal que irá refletir negativamente na função cognitiva, entretanto quando essa perda ocorre de forma muito intensa, além do que se poderia esperar em condições fisiológicas, caracteriza-se um quadro de demência (IZQUIERDO et al., 1999). O termo demência se refere à perturbação de múltiplas funções cerebrais incluindo memória, pensamento, orientação, compreensão, cálculo, capacidade de aprendizagem, linguagem e julgamento, que podem interferir nas atividades diárias do indivíduo (DESHMUKH et al., 2016). A definição atual para demência, como classificado pelo manual Diagnostic and Statistical Manual of Mental Disorders (DSM-5), é um transtorno neurocognitivo maior, caracterizado por declínio cognitivo significativo em um ou mais domínios cognitivos de forma evidente e suficiente para interferir no desempenho e independência do indivíduo nas suas atividades da vida diária (AMERICAN PSYCHIATRIC ASSOCIATION, 2013). Existem diversas causas para sua manifestação, entre elas podemos citar a demência vascular, a demência alcoólica, a demência com corpos de Lewy e a forma mais comum entre elas, que é a DA (IZQUIERDO et al., 2004).

2.2 Doença de Alzheimer

A DA é uma disfunção crônica e progressiva, dentre as mais prevalentes no mundo, manifestada clinicamente como uma insuficiência mental progressiva, funções

cognitivas desordenadas, alterações de personalidade e declínio das atividades diárias do indivíduo acometido (CHAKRABORTY e DIWAN, 2020). Ocorre devido a lesão neuronal, a perda sináptica e a morte celular que acomete principalmente as regiões do córtex cerebral e do hipocampo (PRESTON e EICHENBAUM, 2013; SCHELTENS et al., 2016; LUCHENA et al., 2018; GHAI et al., 2020).

A classificação da DA pode ser em: início precoce/familiar, que corresponde à 1-5% dos casos (resultado de mutações na presenilina 1 e 2), e de início tardio/esporádico, que representa a maior parte dos casos dos portadores desta patologia (WHO, 2015; CHAKRABORTY e DIWAN, 2020). Os fatores de risco identificados se referem ao avanço da idade, inatividade física ou doenças que potencialmente desempenham um papel importante no início da DA, como diabetes, hipertensão, tabagismo, obesidade e dislipidemia (GOEDERT e SPILLANTINI, 2006).

O primeiro relato de DA foi descrito pelo psiquiatra alemão Alois Alzheimer em 1906. Após mais de um século de sua descoberta, a etiologia da doença ainda não está completamente elucidada e nenhum tratamento modificador da doença está disponível (BERCHTOLD e COTMAN, 1998; HIPPIUS e NEUNDORFER, 2003; KROLAK-SALMON et al., 2018). Entretanto, está bem estabelecido na literatura que o acúmulo de Aβ e a hiperfosforilação da proteína Tau são mecanismos importantes envolvidos na fisiopatologia da DA (YIANNOPOULOU et al., 2013; SELKOE e HARDY, 2016; CHEN, 2018).

O Aβ é gerado a partir da proteína precursora amiloide (APP) por dois eventos de clivagem consecutivos através da atividade proteolítica realizada pela β- secretase e pelo complexo enzimático γ-secretase, o qual é constituído pelas proteínas presenilinas 1 e 2, dentre outras proteínas (GOEDERT e SPILLANTINI, 2006). Os Aβ, por sua vez, se depositam no meio extracelular em formações densas, de placas, prejudicando a morfologia e função das sinapses (AULD et al., 2002). De acordo com a “hipótese amilóide”, a produção de Aβ no cérebro inicia uma cascata de eventos que levam à síndrome clínica da DA, aos quais a neurotoxicidade é principalmente atribuída à inflamação local, oxidação e excitoxicidade (glutamato excessivo) (ANAND et al., 2017). Em pacientes com DA, também ocorre a hiperfosforilação da proteína Tau, a qual torna-se insolúvel e acumula-se como filamentos emaranhados que levam à degeneração neuronal e disfunção sináptica. A Tau é uma proteína que atua na estabilização dos microtúbulos no citoesqueleto, sendo fundamental no crescimento axonal e desenvolvimento neuronal (KAMAT et al., 2014). É provável que as vias do

Aβ e da proteína Tau hiperfosforilada atuem em paralelo causando a DA e, também, potencializando os efeitos tóxicos de ambas (YIANNOPOULOU e PAPAGEORGIOU, 2020).

Embora o mecanismo exato que desencadeia a formação de placas senis e emaranhados neurofibrilares ainda não seja totalmente conhecido, sabe-se que essas alterações provocam lesões no SNC com consequente morte neuronal, refletindo em perda de memória e alterações sintomáticas e comportamentais (ANSOLEAGA et al., 2015). Entretanto, outros mecanismos também estão envolvidos na patogênese da DA, tais como neuroinflamação, estresse oxidativo, disfunção mitocondrial, falha no metabolismo energético e alterações nas sinalizações colinérgica e purinérgica, alguns deles elucidados na Figura 2 (HOYER et al., 2004; MCLARNON et al., 2006; VARMA et al., 2009; FERRER, 2012; SONNTAG et al., 2017; CELLAI et al., 2018; CIEŚLAK e WOJTCZAK, 2018; CHEN, 2018; AMATO,TERZO e MULÈ, 2019; LANGE et al., 2020; UDDIN et al., 2020).

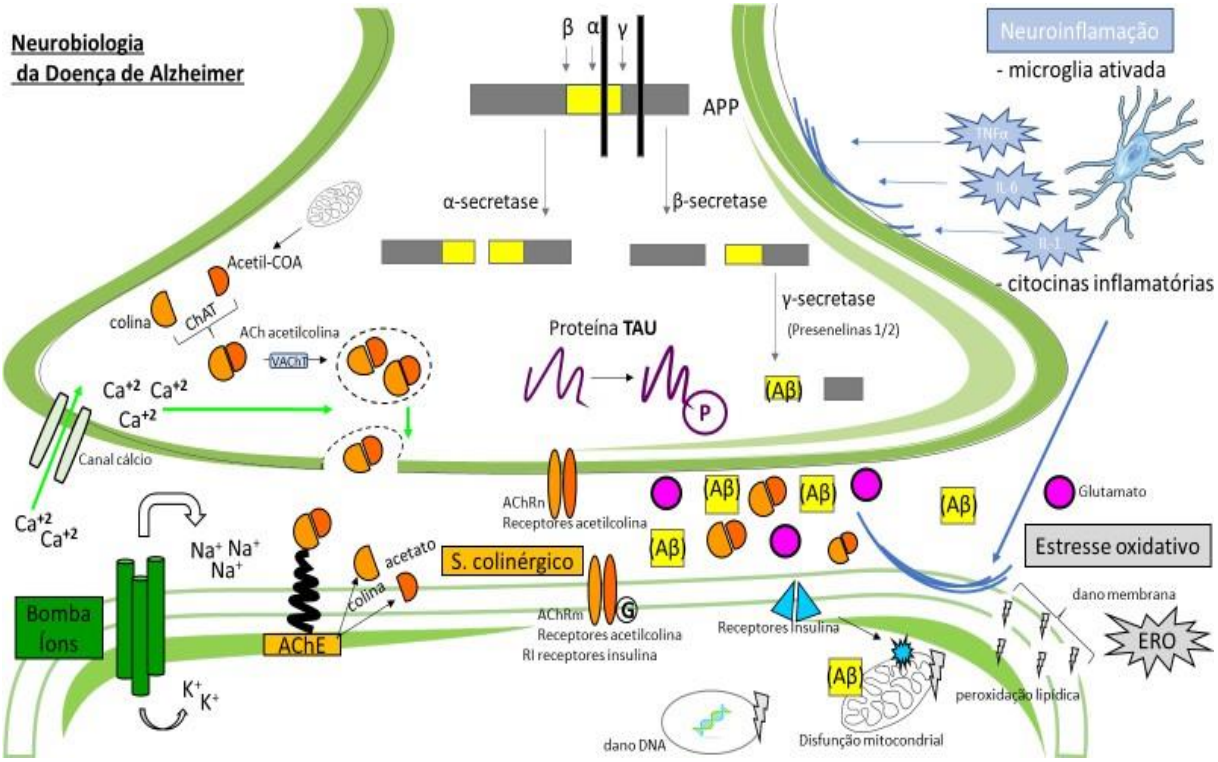


Figura 2. Mecanismos patofisiológicos envolvidos na doença de Alzheimer. Adaptado de DHULL e KUMAR, (2018).

Em paralelo, a identificação da variante e4 (apoE-ε4) do gene da apolipoproteína E (apoE), a principal apolipoproteína encontrada no cérebro, como o

fator genético de risco mais comum para a DA de início tardio, sugere que o colesterol pode ter um papel na patogênese da doença (LOWE, GASER e FRANKE, 2016; MOORE et al., 2019). Assim, esse fator também se encontra envolvido na patogênese da DA e está associado ao aumento da inflamação cerebral (OPHIR et al., 2005). Níveis elevados de colesterol foram correlacionados com uma maior incidência de comprometimento da memória e demência (SALKOVIC-PETRISIC et al., 2013). O mecanismo molecular pelo qual a apoE contribui para a fisiopatologia da DA ainda é debatido, no entanto um efeito bem caracterizado do apoE-ε4 inclui a integridade da barreira hematoencefálica prejudicada (HALLIDAY et al., 2015). Além da função clássica de transporte lipídico, a apoE pode participar do efluxo de partículas como o Aβ do cérebro para o sangue (BUTTERFIELD e MATTSON, 2020).

O diagnóstico atual da DA é clínico, baseando-se principalmente no histórico familiar, nas alterações cognitivas ou comportamentais, nos testes para avaliação física e neurológica, além dos exames sanguíneos e de imagens cerebrais para excluir outras causas potenciais de demência (ALZHEIMER'S ASSOCIATION, 2017). A confirmação do diagnóstico só pode ser obtida com a análise morfológica *post-mortem* do tecido encefálico (KALARIA et al., 2008; CHAKRABORTY e DIWAN, 2020).

O tratamento disponível tem como principal objetivo maximizar a capacidade do paciente de funcionalidade na vida diária, manter a qualidade de vida e retardar a progressão dos sintomas, já que a cura ainda não é possível (KOUHESTANI, JAFARI e BABAEI, 2018). Os medicamentos aprovados pelo Food and Drugs Association (FDA) são inibidores de colinesterase (donepezil, rivastigmina e galantamina), e um antagonista do receptor *N*-metil-D- aspartato (memantina) (SCHELTENS et al., 2016; YIANNOPOULOU e PAPAGEORGIOU, 2020). Esses fármacos não atuam diretamente na patologia da DA, mas permitem que o cérebro compense a perda de neurônios que se comunicam através da acetilcolina (ACh), e ajudam a gerenciar alguns dos sintomas da demência (dos SANTOS et al., 2018). No entanto, cabe ressaltar que estes fármacos causam efeitos colaterais e além disso, muitos pacientes podem tornar-se refratários ao tratamento padrão (ASHFORD et al., 2015; GRAHAM, BONITO-OLIVA e SAKMAR, 2017).

Além do impacto biopsicossocial gerado na vida do indivíduo portador e de sua família, a DA tem implicações econômicas significativas em termos de custos médicos (CRYSTAL e GLANZMAN, 2020). A pesquisa visando compreender os mecanismos patofisiológicos ainda pouco esclarecidos, bem como desenvolver novas ferramentas

terapêuticas, se tornam relevantes tanto pelo aumento da expectativa de vida populacional quanto pelo apelo de medicamentos inovadores. Assim, desenvolver alternativas que possibilitem melhorar a qualidade de vida dos pacientes e talvez encontrar a cura da DA tornam-se essenciais (FARLOW, MILLER e PEJOVIC, 2008; YIANNOPOULOU e PAPAGEORGIOU, 2020).

2.2 Mecanismos envolvidos na patofisiologia da Doença de Alzheimer

2.3.1 Déficit Colinérgico

O sistema colinérgico é composto pelo neurotransmissor ACh, que é sintetizado no citosol dos neurônios colinérgicos pré-sinápticos pela enzima colina acetiltransferase (ChAT), a partir dos substratos colina e acetilcoenzima A (acetil-CoA). Uma vez sintetizado, a ACh é transportada através do transportador vesicular de ACh (VAChT) e armazenado em vesículas sinápticas (PICCIOTTO, HIGLEY e MINEUR, 2012; FERREIRA-VIEIRA et al., 2016).

A liberação da ACh na fenda sináptica ocorre por exocitose, podendo interagir com os receptores específicos que irão efetivar e determinar seus efeitos biológicos. Esses receptores dividem-se em muscarínicos (mAChRs), os quais são acoplados à proteína G e 5 subtipos foram descritos, M1, M2, M3, M4 e M5 (LANGMEAD, WATSONA e REAVILLA, 2008), e nicotínicos (nAChRs), os quais são do tipo canal iônico, e podem ser compostos pela combinação de 17 diferentes subunidades (α1-10, β1-4, δ, ε e γ) (ALBUQUERQUE et al., 2009).

Na fenda sináptica, a ACh é rapidamente hidrolisada pela enzima acetilcolinesterase (AChE) ou ainda pela enzima butirilcolinesterase (BuChE) em acetato e colina que, pode ser recaptada pelo transportador de colina de alta afinidade para o neurônio pré-sináptico a fim de ser utilizada para síntese de novas moléculas de ACh (FERREIRA-VIEIRA et al., 2016). A AChE é a principal enzima responsável por essa hidrólise, já que está presente principalmente nas sinapses do SNC e alterações na sua atividade podem ser relacionadas à disponibilidade de ACh e consequentemente distúrbios no SNC (GUTIERRES et al., 2014). Os principais componentes do sistema colinérgico podem ser observados na Figura 3.

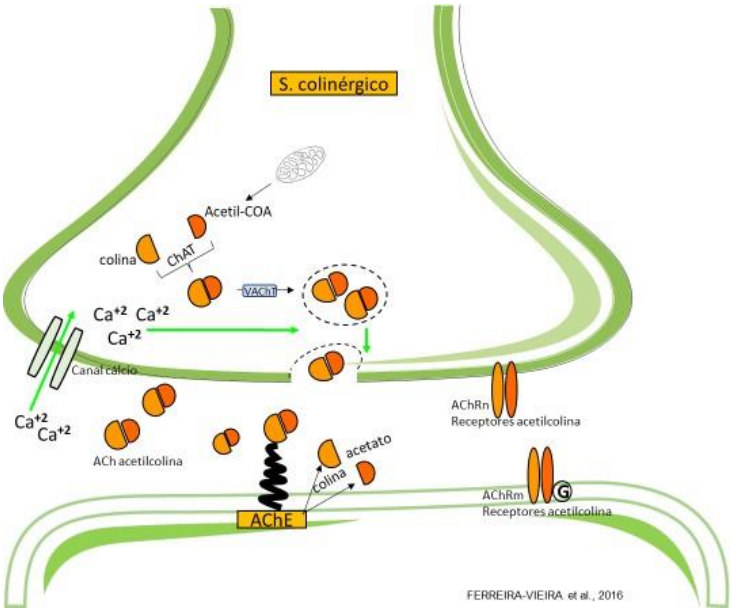


Figura 3. Representação do Sistema colinérgico. Adaptado de Ferreira- Vieira et., (2016).

As estratégias terapêuticas disponíveis para combater os déficits cognitivos baseiam-se no aumento dos níveis do neurotransmissor ACh, uma vez que estes estão reduzidos na DA, através dos inibidores das colinesterases (UNZETA et al., 2016; YIANNOPOULOU e PAPAGEORGIOU, 2020). Assim, se dá a validação do sistema colinérgico como um importante alvo terapêutico na DA (HAMPEL et al., 2018, CHAKRABORTY and DIWAN, 2020, STANCIU et al., 2020), uma vez que destaca-se a importância desses inibidores em atuar em ambas enzimas colinesterases: BuChE e AChE (GUO et al., 2020; WU et al., 2020; HA et al., 2020).

A hipótese mais antiga sobre a DA introduzida no início da década de 80 foi descrita como a hipótese colinérgica. Iniciou com achados sobre a diminuição da atividade da enzima ChAT em pacientes com DA no Reino Unido em 1976 (NESTOR et al., 2004; ASFORD, 2015). Desde então várias alterações nos componentes do sistema colinérgico têm sido documentados na patologia da DA, tais como: aumento na atividade da AChE em regiões específicas do SNC como a amígdala, hipocampo e córtex cerebral (IZQUIERDO et al.,2004; AGRAWAL et al., 2009; UNZETA et al., 2016; SORIAL e SAYED, 2017), redução do número de neurônios colinérgicos do núcleo basal de Meynert (GREENWALD e DAVIS, 1983), diminuição na expressão de VAChT (PRADO et al., 2006) e diminuição da atividade da ChAT (BLOKLAND e JOLLES, 1994; UNZETA et al., 2016).

O sistema colinérgico está envolvido em processos fisiológicos como atenção, memória, resposta ao estresse, sono e vigília, além de estar principalmente correlacionado com processos de aprendizagem (SCHLIEBS e ARENDT, 2006).

Considerando que a ACh tem um papel importante para os processos cognitivos, déficits na transmissão colinérgica podem potencialmente influenciar todos os aspectos da cognição e do comportamento (SCHLIEBS e ARENDT, 2011).

Além disso, uma ligação entre o sistema colinérgico e a inflamação foi estabelecida através da descoberta que a ACh suprimia a liberação de citocinas pró-inflamatórias de macrófagos ativados em tecidos periféricos via receptores nicotínicos (WANG et al., 2003). Assim observou-se também a presença de uma via semelhante no cérebro, uma vez que a ACh age nos mesmos receptores nicotínicos atenuando a liberação de citocinas pró-inflamatórias de células da micróglia. Portanto, essa via anti-inflamatória colinérgica fornece um mecanismo fisiológico que liga a ACh à inibição da inflamação (SHYTLE et al., 2004).

Neurônios colinérgicos parecem ter maior demanda de energia, e, portanto, devem responder de maneira mais sensível à deprivação de glicose relacionada ao evelhecimento (SCHLIEBS e ARENDT, 2011). Além disso, a ACh apresenta papéis adicionais na participação na homeostase cerebral geral e na plasticidade, destacando portanto que as alterações do sistema colinérgico são componentes importantes para o desenvolvimento da DA (HAMPEL et al., 2018).

2.3.2 Estresse Oxidativo

O estresse oxidativo é uma condição gerada pelo desequilíbrio entre a produção de espécies reativas e as defesas antioxidantes (HALLIWELL et al., 2006). Dentre as espécies reativas de oxigênio (EROs) pode-se citar o ânion superóxido ($O2^{\bullet-}$), radical hidroxila ($HO^{\bullet}$), peróxido de hidrogênio (H_2O_2), oxigênio singleto ($1O_2$) e sobre as espécies reativas de nitrogênio (ERN) podemos destacar o óxido nítrico (NO) e o peroxinitrito ($ONOO^-$). Em condições celulares normais, EROs e ERN funcionam como mediadores de processos celulares, que incluem resposta imune e sinalização celular (HALLIWELL, 2012). Entretanto, quando há o aumento de espécies reativas, sobrepondo as defesas antioxidantes, essas espécies podem gerar danos a componentes celulares como lipídios, proteínas e ácido desoxidoribonucleico (DNA) e contribuir para o desenvolvimento de várias patologias, dentre elas a DA (OLIVEIRA et al., 2018; DEBOM et al., 2016; PACHECO et al., 2018).

Para evitar efeitos deletérios das espécies reativas o organismo conta com uma proteção através de sistemas antioxidantes (SNEZHKINA et al., 2019). Antioxidante é

qualquer substância que, quando presente em baixas concentrações comparada com a de um substrato oxidável, atrasa ou inibe significativamente sua oxidação (BUTTERFIELD e HALLIWELL, 2019). Os sistemas antioxidantes encontrados no organismo podem ser classificados como enzimáticos ou não-enzimáticos. As defesas antioxidantes enzimáticas são compostas pelas enzimas catalase (CAT), superóxido dismutase (SOD) e glutationa peroxidase (GPx), que trabalham em conjunto afim de minimizar os danos oxidativos. Outro sistema antioxidante conhecido é o não-enzimático, que é composto pela glutationa (GSH), ácido ascórbico (Vitamina C), α-tocoferol (Vitamina E) e alguns polifenóis (HALLIWELL e GUTTERIDGE, 1995).

Durante o envelhecimento, a considerável formação de EROs pela cadeia transportadora de elétrons nas mitocôndrias associada a uma diminuição do sistema antioxidante constituem um fator de risco para o desenvolvimento da DA (HUANG, ZHANG e CHEN, 2016). Além disso, presença de Aβ interfere diretamente na cadeia transportadora de elétrons nas mitocôndrias e altera a produção de EROs, colaborando para o aumento do estresse oxidativo (MAO e REDDY, 2011). Ainda, o estresse oxidativo pode levar ao aumento dos níveis de cálcio intracelular, que gera disfunção sináptica, resultando em morte neuronal (Figura 4) (BUTTERFIELD e HALLIWELL, 2019).

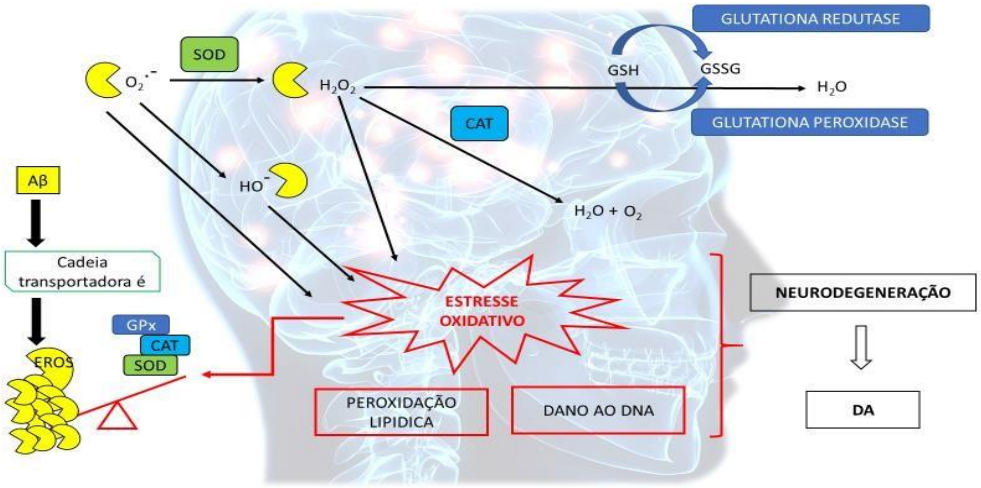


Figura 4. Envolvimento do estresse oxidativo na progressão DA. Fonte:autoria própria.

Outro fator relevante é a disfunção no metabolismo da glicose cerebral, em modelos animais e pacientes com DA, em decorrência do dano oxidativo e nitrosativo (SONTAG et al., 2017; DHULL e KUMAR, 2018). Alterações oxidativas em enzimas como aconitase (enzima ferro/enxofre do ciclo de kerebs), creatina quinase (enzima que ajuda os neurônios a manter os níveis de adenosina trifosfato (ATP) em equilíbrio)

e ATP sintase nas mitocôndrias cerebrais, possibilitam explicar a redução no metabolismo da glicose, bem como a diminuição da produção de ATP cerebral em pacientes com DA (SONTAG et al., 2017). A diminuição de ATP reduz a capacidade dos neurônios de manter os gradientes iônicos, dificultando a propagação do potencial de ação e consequentemente, a neurotransmissão e, por fim, gerando disfunção cognitiva (BUTTERFIELD e HALLIWELL, 2019). Assim, considerando o exposto, percebe-se que dados da literatura utilizando tanto estudos clínicos quanto modelos experimentais têm sido consistentes em demonstrar que o estresse oxidativo está associado à fisiopatologia da DA (MARKESBERY, 1999; GUTIERRES et al., 2014; HUANG, ZHANG e CHEN, 2016; GUMUSYAYLA et al., 2016; DESHMUKH et al., 2016; DHULL E KUMAR et al.,2018; PACHECO et al., 2018; KOUHESTANI, JAFARI e BABAEI, 2018; AMATO, TERZO e MULÈ, 2019).

2.3.3 Fator Neurotrófico Derivado do Cérebro (BDNF)

Dentre os importantes fatores neurotróficos secretados pelos neurônios e astrócitos podemos citar o fator neurotrófico derivado do cérebro (BDNF). O BDNF é o fator de crescimento neurotrófico mais amplamente distribuído no SNC e desempenha um importante papel nas áreas cerebrais envolvidas com a memória e funções cognitivas superiores, incluindo hipocampo, córtex e prosencéfalo basal (KORTE e SCHMITZ, 2016; LEAL et al., 2017). O papel neurobiológico do BDNF envolve promoção da sobrevivência e diferenciação neuronal e glial além da regulação da transmissão sináptica (CALDEIRA et al., 2007; JERONIMO-SANTOS et al., 2014). Posteriormente à sua liberação, ocorre a ativação do receptor da tirosina quinase B (TrkBR) e consequentemente as suas cascatas de sinalização, as quais contribuem para eventos de transcrição gênica críticos para plasticidade sináptica, LTP e função cognitiva (BEKINSCHTEIN et al., 2011; SONG et al., 2015; SASI et al., 2017).

Os níveis reduzidos de BDNF foram detectados em patologias neurodegenerativas, como a DA (CUNHA, BRAMBILLA e THOMAS, 2010). Em amostras cerebrais *post-mortem* de pacientes com DA foi observada uma diminuição nos níveis de mRNA do BDNF e do seu respectivo receptorTrkBR (CONNOR et al., 1997; HOLSINGER et al., 2000) e demostraram correlação negativa com a função cognitiva (PENG et al., 2005).

Segundo Espana e colaboradores, (2010) o Aβ foi responsável, no hipocampo, pela redução da expressão de BDNF e de seu receptor TrkBR, além disso, o Aβ interfere no transporte axonal de BDNF (RAMSER et al., 2013). O BDNF é capaz de aumentar a LTP na área do hipocampo CA1, a qual está diretamente relacionada à memória (JERONIMO-SANTO et al., 2014). Nesse sentido, reduções nos níveis de BDNF bem como na expressão de TrkbR são fortemente associadas com a fisiopatologia da DA (AMIDFAR et al., 2020). As alterações cognitivas observadas na DA podem ser resultado de plasticidade neuronal prejudicada através da via de sinalização interrompida dos fatores neurotróficos, contribuindo assim, para a disfunção cognitiva (TAPIA-ARANCIBIA et al., 2008; BUDNI et al., 2015).

Além disso, está bem estabelecido que a proteína de ligação responsiva ao 3´5´-adenosina-monofosfato-cíclico (cAMP) (CREB) é um fator de transcrição que desempenha um papel crítico na cognição. Na verdade, o CREB é um alvo molecular essencial subjacente ao aprendizado e à memória (LEE et al., 2009). A transcrição do CREB está diminuída no tecido hipocampal *post-mortem* de pacientes com DA (PUGAZHENTHI et al., 2011). A regulação negativa do BDNF e do CREB foi sugerida como dois importantes eventos das vias de sinalização pelas quais Aβ prejudica a cognição e a memória na DA, sendo então causa da disfunção sináptica (LEE et al., 2009). O efeito do Aβ na perda neuronal do hipocampo, na inibição da LTP e na neurodegeneração causa comprometimento da plasticidade sináptica e déficit de memória, mediado por alterações dependentes da sinalização BDNF/CREB via cAMP/proteína quinase A (PKA) (VITOLO et al., 2002; FAHNESTOCK, 2011; AMIDFAR et al., 2020). Os níveis de expressão alterada de BDNF ou uma quebra de sinalização BDNF-TrkB, pode levar à perda de sinapses e disfunção cognitiva, bem como a ocorrência precoce de regulação negativa do BDNF está associado com o curso da DA sugerindo gravidade do comprometimento cognitivo (SONG et al., 2015).

A diminuição do BDNF surge como possível motivo subjacente que promove disfunção neuronal na DA. Medidas para restaurar os níveis cerebrais desse fator aparecem como uma estratégia de tratamento, visando efeitos benéficos na aprendizagem e memória, pois modulando o aumento da expressão de BDNF/CREB poderia mesmo após o início clínico da DA, proteger os pacientes de déficits de memória e problemas cognitivos (ROSA e FAHNESTOCK, 2015). Porém, o próprio BDNF apresenta propriedades farmacocinéticas desfavoráveis para administrações sistêmicas devido à sua curta meia-vida plasmática e baixa penetração na barreira

sangue/cérebro, logo, tentativas de aumentar os níveis cerebrais, de forma indireta, seriam uma abordagem terapêutica válida (TANILA, 2017).

2.3.4 Na^+, K^+- ATPase

Em 1957 foi proposta a ideia de proteínas ligadas à membrana plasmática das células eucarióticas com propriedades catalíticas denominadas adenosinatrifosfatases (ATPases), ou seja, enzimas que hidrolisavam única e exclusivamente a molécula de ATP. Com o avanço das pesquisas, foi demonstrado que essas enzimas possuem várias funções fisiológicas importantes tais como a manutenção dos potenciais de membrana, a regulação do volume e do pH intracelular e o co-transporte de moléculas. As ATPases são classificadas em: F-ATPases, P-ATPases, V-ATPases, ABC-ATPases. Entre as P- ATPases, denominadas bomba de íons, temos as enzimas sódio (Na^+) potássio (K^+) ATPase (Na^+,K^+-ATPase) e cálcio (Ca^{+2}) ATPase (Ca^{+2}-ATPase) (SKOU e ESMANN, 1992).

Em condições fisiológicas, a Na^+,K^+- ATPase é responsável pela translocação de três íons Na^+ para o meio extracelular e dois íons K^+ para o meio intracelular, contra seus gradientes de concentração, caracterizando um transporte ativo e produzindo um gradiente eletroquímico na membrana (ZHANG et al., 2013). Assim, a Na^+,K^+-ATPase torna-se uma enzima fundamentalmente responsável pela manutenção do gradiente iônico necessário para a excitabilidade neuronal (MOSELEY et al., 2007).

A relação da Na^+,K^+-ATPase com o déficit de memória tem sido estabelecida, uma vez que a diminuição da sua atividade ou expressão resulta em redução de K^+ intracelular e acúmulo de Na^+, despolarizando a membrana e aumentando as concentrações de Ca^{+2} intracelular, prejudicando assim a sinalização neuronal e causando morte celular (LEES et al., 1990; GUTIERREZ et al., 2014; MOSELEY et al., 2007). A ruptura da homeostase de íons e do balanço osmótico interfere no processamento do sinal, ou seja da informação que será convertida em memória. Assim, o envolvimento da Na^+,K^+-ATPase em processos representativos para estudo de memória e aprendizado é relevante e sua modulação é fundamental (ZHANG et al., 2013).

Uma das hipóteses para a redução da atividade da Na^+,K^+-ATPase está relacionada com o estresse oxidativo (STRECK et al., 2001). Postula-se que as espécies reativas são responsáveis por perturbações na atividade da bomba de íons, que pode ocorrer devido à oxidação dos grupos sulfidrilas (SH) ou, ainda, dos lipídeos de membrana, desestabilizando dessa forma o transporte iônico (CARVALHO et al., 2015; CARVALHO et al., 2017). Outra explicação plausível para a diminuição da atividade e/ou expressão da Na^+,K^+-ATPase na DA é essa enzima ser diretamente inibida por altas concentrações do Aβ (DICKEY et al., 2005). Assim, sugerindo que as alterações enzimáticas encontradas na DA podem não ser puramente secundárias à perda neuronal, mas resultar de efeitos diretos do Aβ. Ainda, a atividade da enzima pode ser prejudicada pela redução na disponibilidade de ATP devido a disfunções mitocondriais (BENARROCH, 2011).

A enzima Na^+,K^+-ATPase, independente da sua função de bomba de íons, é capaz também de formar complexos com outras proteínas de membrana e desencadear sinais de transdução intracelular, que estão envolvidos na plasticidade sináptica (BENARROCH, 2011). Dessa forma, pode desencadear o crescimento de dendritos em neurônios corticais via ativação de quinases de proteínas ativadas por mitógeno (MAPK) e vias das proteínas quinases dependente de Ca2/calmodulina (DESFRERE et al., 2009).

Considerando a implicação da Na^+,K^+-ATPase tanto na função de bomba de íons quanto na plasticidade sináptica, observa-se que alterações na atividade da enzima podem ser geradas por distúrbios de estresse oxidativo, de neuroinflamação e pela presença de Aβ (STRECK et al., 2001; DICKEY et al., 2005; ZHANG et al., 2013; GUTIERRES et al., 2014; PACHECO et al., 2018). Sendo esses componentes patofisiológicos característicos e presentes na patologia DA, surge a necessidade de estudos visando compreender os mecanismos de relação entre eles, bem como, a avaliação de compostos com potencial de restaurar ou prevenir alterações nas funções da Na^+,K^+-ATPase associadas a distúrbios neurodegenerativos

.

2.3.5 Neuroinflamação

A inflamação é uma resposta do sistema imune projetada para proteção, e o termo neuroinflamação se refere à inflamação no SNC (HENEKA et al., 2015). A DA está fortemente associada à uma inflamação crônica no SNC, uma vez que estudos

têm demonstrado que o Aβ pode ligar-se a receptores específicos presentes nas células residentes, desencadeando a resposta imune inata e liberando mediadores inflamatórios, que contribuem para progressão e gravidade da doença (FEMMINELLA et al., 2019, GRAY, KINGHORN, e WOODLING, 2020).

O papel das respostas imunes inata e adaptativa na patogenia da DA está relacionado com a neuroinflamação (SCHETTERS et al., 2017). Na imunidade inata as células dendríticas são responsáveis pela produção de citocinas pró-inflamatórias. Os monócitos, se diferenciam em macrófagos ou micróglia sendo responsáveis pela fagocitose (BAJRAMOVIC, 2011). A micróglia atua principalmente regulando a homeostase do microambiente e ao ter seu fenótipo ativado por insultos ao SNC aumenta a liberação de citocinas pró-inflamatórias. Observa-se nesse contexto da neuroinflamação, que os astrócitos também são capazes de produzir citocinas, além de exercer o reconhecimento e depuração de Aβ (LI et al., 2011, TEJERA e HENEKA, 2016).

Em relação à resposta imune adaptativa já foi observada uma diminuição dos linfóitos T no sangue de alguns pacientes com DA (SCHETTERS et al., 2017), embora um aumento da infiltração de células T também foi mostrado em cérebros de pacientes (MCGEER et al., 1988), principalmente no hipocampo (TOGO et al., 2002). Assim, observa-se uma migração de linfócitos T auxiliares CD4 (TCD4) para o SNC que induz a diferenciação da micróglia e participa da minimização dos danos da DA, enquanto os linfócitos T citotóxicos CD8 (TCD8) atuam interagindo com a micróglia associada Aβ, modulando sua atividade. Por fim, o Aβ estimula os linfócitos B a produzirem anticorpos anti-Aβ e esses anticorpos ativam células da glia, desencadeando uma resposta imune (GUILLOT-SESTIER e TOWN, 2013; MARSH et al., 2016; ANDREASSON et al., 2016; UNGER et al., 2018).

A micróglia é reconhecida como um dos principais componentes da resposta imune inata cerebral (LATTA, BROTHERS e WILCOCK, 2014; CALSOLARO e EDISON, 2016). A ativação micróglial no cérebro de pacientes com DA suporta a chamada "hipótese inflamatória" (HENEKA et al., 2015). Nesse contexto, o fenótipo ativado da micróglia produz fatores pró-inflamatórios, como citocinas (IL-1 β, IL-6, IL-12 e fator de necrose tumoral α (TNF-α), radicais livres além de neurotoxinas que promovem disfunção no sistema imune (COUTURIER et al., 2010; KRAFT e HARRY, 2011).

No microambiente da DA, há perda de feedback microglial e o equilíbrio das ações pró e anti-inflamatórias é desviado do equilíbrio homeostático fisiológico (GRAY, KINGHORN, e WOODLING, 2020). Alterações neuroinflamatórias também podem ser denominadas circuitos, uma vez que em resposta a um estímulo (envelhecimento, estresse oxidativo, Aβ) há ativação microglial através da via dependente de fator nuclear kappa B (NFkB) e da via MAPK, com consequente liberação de fatores pró-inflamatórios (TNF-α, IL-1B e IL-6) de forma cíclica, resultando em dano neuronal. Dessa forma, sugere-se que as funções microgliais devem ser mantidas em equilíbrio para apoiar a função neuronal saudável (GRAY, KINGHORN e WOODLING, 2020).

Existe uma associação entre produção de citocinas pró-inflamatórias de células residentes no cérebro e a neurodegeneração (HASKO, SITKOVSKY e SZABO, 2004), tanto em modelos animais quanto em pacientes, tornando a inflamação uma característica crucial relacionada à DA (RIVERA-OLIVER e DIAZ-RIOS, 2014). Dentre as citocinas secretados no SNC a IL-10 possui ação anti-inflamatória e contribui para a neuroproteção (ZHOU et al., 2009, FOUDA et al., 2013), neurogênese (PEREZ-ASENSIO et al., 2013) e plasticidade sináptica do hipocampo relacionada ao aprendizado e à memória (KELLY et al., 2001; TALAAT et al., 2016). A IL-10 é regulada negativamente em ratos idosos submetidos a um modelo de DA (RAVELLI et al., 2017; MAJKUTEWICZ et al., 2018). Zhou e colaboradores (2009) constataram que, quando regulada positivamente após o dano, essa citocina apresenta um efeito neuroprotetor. Nesse âmbito, também destaca-se a citocina IL-4, por desempenhar um papel importante na memória e na aprendizagem, além de regulador a atividade do sistema imunológico. A IL-4 pode ser responsável por efeitos neuroprotetores nos astrócitos, através da produção de BDNF, fornecendo um mecanismo pelo qual essa citocina afeta a cognição de forma positiva. Em suma, parece que a ausência de IL-4 aumenta a vulnerabilidade à neuroinflamação (GAMBI et al., 2004; DERECKI et al., 2010; GADANI et al., 2012).

Dessa forma, assim como é relatado que a ativação microglial é um importante mecanismo que contribui para o declínio cognitivo e para a neurodegeneração, a literatura também sugere que os astrócitos desempenham um papel crucial para o processo de neuroinflamação e neurodegeneração (PHILLIPS et al., 2014; OSBORN et al., 2016). Juntamente com a ativação da micróglia, astrócitos hipertróficos reativos se reúnem em torno de Aβ, conforme relatado em estudos *post-mortem* em humanos

(MEDEIROS e LAFERLA, 2013), bem como em modelos animais (OLABARRIA et al., 2010). Os astrócitos representam entre 30 e 50% das células neurais e são reconhecidos por fornecer suporte aos neurônios. Essas células possuem múltiplos papeis como a secreção de importantes fatores neurotróficos, manutenção dos níveis iônicos no meio extracelular, captação e liberação de diversos neurotransmissores, participação na formação da barreira sangue/cérebro, além de auxiliar na defesa imune por meio da síntese e secreção de diversas citocinas inflamatórias (GOMES et al., 2013). Uma vez expostos ao Aβ, os astrócitos liberam várias moléculas pró-inflamatórias, como citocinas, interleucinas (IL), óxido nítrico e outros compostos citotóxicos, ampliando a resposta neuroinflamatória (MEDEIROS e LAFERLA, 2013).

É relatada que a depuração de Aβ é realizada pelas enzimas neprisilina e metaloproteases, que estão presentes nos astrócitos, e quando há desregulação nesse processo o resultado é o acúmulo patológico desse peptídeo, determinando uma correlação positiva entre essa alteração e o comprometimento cognitivo (MINTER, TAYLOR e CRACK, 2016). Considerando o papel dos astrócitos no SNC, alterações no funcionamento destas células, bem como a liberação exacerbada de mediadores inflamatórios, podem impactar na homeostase cerebral, contribuindo assim para a neurodegeneração (Figura 5). Os astrócitos respondem a insultos no SNC através de um processo chamado astrogliose reativa, o qual pode ser definido como uma resposta de reparo que se caracteriza por hipertrofia de astrócitos, alongamento protuberante, expressão de proteínas ácida fibrilar glial (GFAP) e proliferação de astrócitos após dano (SAWIKR et al., 2017).

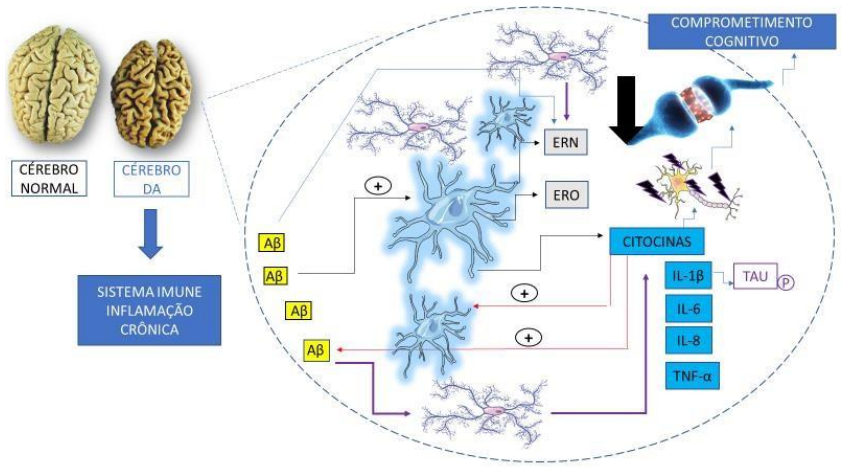


Figura 5. Processo de neuroinflamação contribuindo para fisiopatologia DA. Fonte:autoria própria.

Em geral, as células gliais desempenham um papel importante na transmissão neural e na remodelação das sinapses, pois facilitam a remoção de sinapses não essenciais pela erradicação de conexões inadequadas (COHEN e TORRES, 2019; ARRANZ e STROOPER, 2019). Entretanto, na fisiopatologia da DA, observa-se que essas células sustentam uma resposta inflamatória superexpressa que sinergiza com o acúmulo de Aβ e Tau hiperfosforilada e, impulsiona a sinaptotoxicidade e a neurodegeneração de uma maneira auto-reforçada. A partir dessa descoberta houve ampliação do panorama de pesquisa para prevenção do declínio cognitivo da DA (HAMPEL et al., 2020).

Em paralelo, pode-se relacionar a neuroinflamação com o sistema purinérgico, mais especificamente receptores adenosinérgicos pois, a ativação dos receptores de adenosina apresenta diferentes efeitos na inflamação. Como por exemplo, o receptor A_1 e o receptor A_3 têm efeitos inibitórios na gliose reativa, enquanto o receptor A_2 promove reações de ativação da glia (LIU et al., 2019). A ativação dos astrócitos pode levar ao aumento da secreção de mediadores inflamatórios como a liberação de óxido nítrico (NO), EROS e citocinas pró-inflamatórias (TNF-α, IL-1β e IL-6) (PHILLIPS et al., 2014). A astrogliose e a neuroinflamação parecem ser componentes integrais do início e progressão da DA, que juntamente com a perda neuronal colinérgica são achados comuns em análises cerebrais de pacientes *post-mortem* (BLUM e ZUO, 2013).

2.3.6 Sistema Purinérgico

O sistema purinérgico compreende os nucleotídeos e nucleosídeos de adenina como adenosina trifosfato (ATP), adenosina difosfato (ADP), adenosina monofosfato (AMP), adenosina e inosina, bem como enzimas e receptores específicos (BURNSTOCK, 2017). A sinalização induzida pelos nucleotídeos e nucleosídeos no meio extracelular está relacionada com a ação das enzimas como as ecto-nucleosídeo-trifosfo-difosfoidrolases (E-NTPDases), ecto-5´-nucleotidase (CD73) e a ADA (ZIMMERMANN, 2001). Essas enzimas atuam em conjunto formando uma cascata enzimática que tem início com a ação da NTPDase a qual hidrolisa ATP e ADP até AMP. O AMP gerado é hidrolisado pela CD73 formando a adenosina. A adenosina formada é degradada à inosina pela ação da ADA. No entanto, a adenosina também pode ser gerada de forma alternativa pela via não canônica, iniciada pela enzima ADP-ribose hidrolase ciclica (CD38) que hidroliza a nicotinamida dinucleotídeo de adenina (NAD+) como sustrato formando adenosina difosfato ribose (ADPR) ou

adenosina difosfato ribose ciclíco (cADPR), esse é hidrolisado pela pirofosfatase/fosfodiesterases 1 (NPP1/CD203a) promovendo a formação de AMP que é metabolizado pela CD73, originando adenosina. A CD203a também pode hidrolisar NAD+ diretamente gerando AMP que será hidrolisado pela CD73 originando adenosina, e consequentemente inosina pela ação da ADA (FERRETTI et al.,2019). A inosina gerada em ambas as vias, por sua vez, é hidrolizada a xantinas pela enzima purina nucleosideo fosforilase (PNP), e as essas xantinas através de enzimas oxidases dão origem ao ácido úrico, produto final da via (HASKÓ, SITKOVSKY e SZABÓ, 2004) (Figura 6).

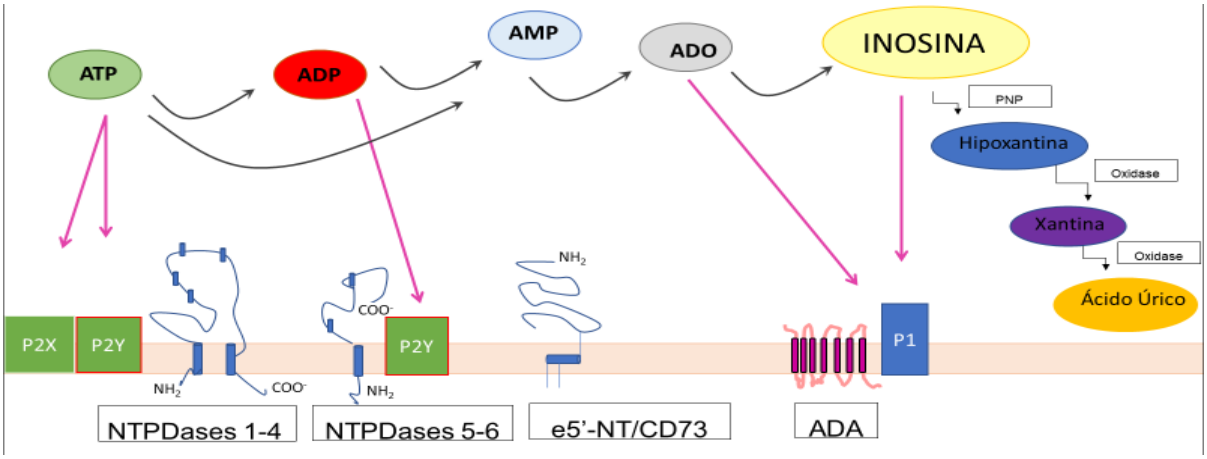


Figura 6. Componentes do Sistema Purinérgico. Fonte:autoria própria.

Os nucleotídeos e nucleosídeos de adenina podem mediar suas ações através de receptores purinérgicos localizados na superfície de vários tipos de células. Os receptores para o ATP e o ADP se dividem em dois grupos: P2X e P2Y (BURNSTOCK, 2009). A família dos receptores P2X é composta por sete membros denominados de P2X1-P2X7. Estes receptores estão ligados a canais iônicos seletivos para cátions monovalentes e bivalentes. Os receptores P2Y possuem oito subtipos: P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, e P2Y14 e são receptores acoplados à proteína G (YEGUTKIN, 2008).

Os receptores P2X são amplamente expressos no SNC tanto em neurônios quanto em células gliais (BURNSTOCK et al., 2011). O P2X7 se destaca por desempenhar um papel crucial na neuroinflamação e neurodegeneração, uma vez que esse receptor é capaz de regular a atividade de α-secretases, enzimas envolvidas na formação do Aβ, pois sua ativação afeta a atividade de PKC, MAPKs ou glicogênio

sintase quinase 3 (GSK3), que por sua vez modulam o processamento do APP e formação do Aβ (ORTEGA et al., 2010). Além disso, um aumento na expressão desse receptor também tem sido observado na micróglia em cérebro de pacientes com DA (MCLARNON et al., 2006). O P2X7 desempenha papel relevante na geração do ânion superóxido (O2•−), nas células da micróglia sugerindo que a ativação deste receptor está associado com a fisiopatologia da DA (PARVATHENANI et al., 2003; LEE et al., 2011). De acordo com o trabalho de Ryu e Mclarnon (2008) a inibição do P2X7 corresponde à uma ação neuroprotetora, tornando este receptor um alvo terapêutico atrativo para DA.

Os receptores P2Y também estão amplamente distribuídos no SNC e são ativados por diferentes moléculas, como por exemplo, ATP, UTP, adenosina 5'-difosfato (ADP), uridina 5'-difosfato (UDP). Esses receptores podem afetar a atividade neuronal e os processos neuroinflamatórios, exercendo efeitos neuroprotetores, como por exemplo, participando da degradação de Aβ neurotóxico e também das respostas imunes (IDZKO, FERRARI e ELTZSCHIG, 2014).

2.3.7 Sistema adenosinérgico

Adenosina é uma purina de extrema importância no SNC já que exerce função neuromodulatória das atividades neuronais, astrócitarias e afeta diretamente os processos cognitivos, como a memória e a aprendizagem, além de atuar de forma essencial na neuroproteção (FREDHOLM et al., 2005; STOCKWELL, JAKOVA e CAYABYAB, 2017). Os receptores para adenosina incluem quatro tipos: A_1, A_{2A}, A_{2B} e A_3 os quais são proteínas transmembranas acoplados a proteína G (JIN et al., 1998; YEGUTKIN, 2008; WEI et al., 2011). Dentre os quatro tipo de receptores de adenosina existentes, os receptores A_1 sao descritos como neuroprotetores, enquanto os receptores A_{2A} estão relacionados com processos neurodegenerativos (CUNHA, 2005).

O receptor A_1 é acoplado à proteína G inibitória (Gi) e está principalmente distribuído no SNC nas regiões do hipocampo, córtex cerebral, cerebelo, medula espinhal, tálamo e no tronco encefálico (SEBASTIAO e RIBEIRO, 2009). Esse receptor, quando ativado, é responsável por inibir a adenilil ciclase e reduzir a produção de AMPc, além de ativar a via da MAPK e as quinases reguladoras de sinais extracelulares, como a ERK1 e ERK2 (BOISON, CHEN e FREDHOLM, 2010).

Além disso, o receptor A_1 na membrana pré-sinaptica também pode ativar a fosfolipase C, aumentando o conteúdo de inositol 1,4,5-trifosfato (IP3) e diacilglicerol (DAG). O IP3 pode estimular a liberação de Ca^{2+} do armazenamento intracelular e inibir canais de cálcio, o que leva à redução do influxo deste íon, a inibição da liberação de glutamato e a redução da excitabilidade celular (FREDHOLM et al., 1994; LIU et al., 2019). Em modelo animal de DA foi observado redução dos niveis do receptor A_1 no hipocampo e estriado (RAHMAN et al., 2009).

No cérebro saudável o receptor A_{2A} têm baixa expressão, mas esse padrão de expressão e funcionalidade pode ser alterado em condições patológicas, pois observa-se expressão aumentada desse receptor em células micrógliais no hipocampo e córtex cerebral de pacientes com DA (ANGULO et al., 2003; RADMAN et al., 2009). Os receptores do tipo A_{2A} e A_{2B} quando ativados, se acoplam à proteína G estimulatória (Gs), possuindo como primeiro alvo a adenilil ciclase, com consequente aumento de AMPc e ativação da fosfolipase C (RALEVIC e BURNSTOCK, 1998). Os receptores A_{2A} distribuem-se regularmente no SNC e SNP, enquanto os receptores A_{2B} podem ser encontrados principalmente no intestino e bexiga, apresentando-se em baixas densidades no SNC (STONE, CERUTI e ABBRACCHIO, 2009).

O receptor A_3 está acoplado à proteína Gi ou à proteína ativadora de fosfolipase C (Gq). Aparentemente apresenta o mesmo tipo de sinalização que os receptores A_1, inibindo a adenil ciclase e reduzindo a produção de AMPc, entretanto possui uma baixa expressão em quase todo o encéfalo (SEBASTIAO e RIBEIRO, 2009).

Os receptores A_{2A} controlam fenômenos relacionados com a degeneração, ou seja dano e destruição neuronal irreversível, e o compartimento neuronal que é afetado de modo precoce é a sinapse (CUNHA e AGOSTINHO, 2010). Nas sinapses há uma suceptibilidade à disfunção mitocondrial porque há uma grande exigência de demanda metabólica. Assim, e o comprometimento mitocondrial é ocasionado pelo estresse oxidativo gerado nesse processo celular (NICHOLLS, 2003). Dessa forma, a função mitocondrial comprometida gera redução metabólica e consequentente deterioração da memória (WATSON e CRAFT, 2004) além de desregulação do cálcio intracelular pelo excesso de ativação do sistema glutamatérgico (LIPTON e ROSENBERG, 1994). Portanto, Pagnussat e cols. (2015) afirmam que os receptores A_{2A} são necessários e suficientes para desencadear o comprometimento da memória.

Nabbi-Schroeter (2018) sugeriu que a cafeína, um antagonista não seletivo dos receptores de adenosina, pode proteger contra degeneração neuronal e morte causada por Aβ, através do bloqueio do A_{2A}. Observou-se que o antagonista do receptor A_{2B} tem um efeito protetor na DA ao impedir o acúmulo de Aβ dentro e ao redor dos vasos sanguíneos cerebrais (CUPINO e ABEL, 2013; GAHR et al., 2013) além de diminuir os níveis Aβ cerebral no modelo de DA revertendo o comprometimento cognitivo (ARENDASH et al., 2009; CAO et al., 2009; CHU et al., 2012).

Outro relato importante foi que o bloqueio do receptor A_{2A} em cultivo de neurônios de cerebelo foi capaz de prevenir a neurotoxicidade induzida pelo Aβ, portanto, parece que a presença desse receptor é essencial para a toxicidade desse peptídeo (DALL'LGNA et al., 2003). Essa dado é confirmado uma vez que camundongos knockout para o receptor A_{2A} após a administração de Aβ não apresentaram déficits de aprendizado ou sinaptotoxicidade, relacionando e destacando a importância desse receptor na função cognitiva (CUNHA et al., 2005). Ainda, existem outros mecanismos envolvidos no controle de neurodegeneração por antagonozinar receptores A_{2A}, através de processos não-sinápticos conhecidos por impactar na função de memória e neurodegeneração, como a permeabilidade da barreira sangue/cérebro (CHEN et al., 2008) ,ação de fatores de crescimento (COSTA et al., 2008) e modulação do metabolismo do colesterol (REISS et al., 2004).

Em amostras cerebrais *post-mortem* de pacientes com DA foi observado uma diminuição de 40-60% na expressão do receptor A_1 em regiões como hipocampo (UŁAS et al., 1993). Em contraste, outro estudo mais recente que utilizou amostras *post-mortem* de córtex de pacientes com DA mostrou uma expressão aumentada de A_1 e A_{2A} tanto nos estágios iniciais quanto nos avançados da doença (ALBASANZ et al., 2008). Embora exista dados contraditórios, a perda de receptores A_1 parece estar entre os fatores responsáveis pela morte celular na região CA1 do hipocampo (RAHMAN et al., 2009).

Durante o processo patológico da DA, o equilíbrio dos receptores de adenosina é alterado ocorrendo uma diminuição da expressão do receptor A_1 e aumento da expressão do receptor A_{2A}, bem como perturbações nos processos de inibição e excitação celular, que acabam levando ao desenvolvimento da disfunção cognitiva (LIU et al., 2019). Contudo, o equilíbrio dos receptores de adenosina pode

desempenhar um papel neuroprotetor. A ativação de receptores A_1 e inibição de receptores A_{2A} poderia ser uma importante estratégia para prevenir a neurodegenração e o declínio cognitivo na DA (YAN et al., 2014).

Até o momento não foram realizados ensaios clínicos em humanos para investigar o potencial terapêutico de receptores purinérgicos e das ectoenzimas para o tratamento da DA, entretanto encontra-se em andamento nos Estados Unidos (EUA) um estudo clínico Fase II para determinar a eficácia terapêutica da infusão intravenosa de ATP no metabolismo cerebral e no estado mental em pacientes com DA moderada à grave (ClinicalTrials.gov: NCT02279511). Sendo assim, considerando a relevância das alterações encontradas nos componentes do sistema purinérgico relacionado a DA, a modulação desses receptores e enzimas torna-se uma via interessante para a pesquisa de novas alternativas terapêuticas para essa doença neurodegenerativa.

2.4 Inosina

A inosina é um metabólito proveniente da desaminação da adenosina em uma reação catalisada pela enzima ADA no meio intra e extracelular, ou ainda pela desfosforilação do IMP pela enzima 5'-nucleotidase (BARANKIEWICKZ e COHEN, 1985). A inosina pode ser degradada à hipoxantina e ribose 1-fosfato pela enzima PNP, e a enzima xantina oxidase é responsável pela conversão da hipoxantina, primeiro à xantina e por fim, gerando ácido úrico (HASKO, SITKOVSKY e SZABÓ, 2004). O mecanismo de ação da inosina, possívelmente, se dá através da sensibilização dos receptores de adenosina A_1, A_{2A} e A_3, com afinidades que normalmente são inferiores às da adenosina. Ainda não foi demonstrada na literatura evidências da ação da inosina nos receptores A_{2B} (JIN et al., 1998; WEI et al., 2011; WELIHINDA et al., 2016; WELIHINDA et al., 2018).

Em relação à estrutura química, a inosina apresenta fórmula molecular $C_{10}H_{12}N_4O_5$, possuindo uma ribose ligada à uma base nitrogenada (Figura 7). A adenosina e a inosina são similares em estrutura, diferindo apenas por um grupo amina, porém a estabilidade é diferente, com tempo de meia-vida para adenosina em 10 segundos, e para a inosina em torno de 15 horas (VIEGAS et al., 2000). A inosina quando atinge altas concentrações dentro da célula pode ser encaminhada para o meio extracelular através de transportadores específicos de nucleosídeos, concentrativos e equilibrativos (HASKO, SITKOVSKY e SZABÓ, 2004).

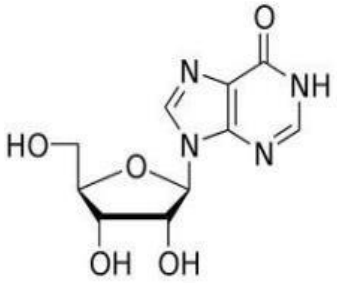


Figura 7. Estrutura química da inosina. Fonte:autoria própria.

Estudos têm relatado que o tratamento com inosina pode ser considerado seguro, sendo desprovido de efeitos colaterais significativos, mesmo após um período de tratamento prolongado (MARKOWITZ et al., 2009; KURICOVA et al., 2014; MUTO et al., 2014). Nenhum efeito adverso foi relatado, inclusive em indivíduos que consumiram inosina por até um ano (CHENG et al., 1990). Dessa forma, além da interação com receptores de adenosina tem sido postulado que a inosina é capaz de promover suas ações biológicas através do aumento da produção de ácido úrico, inibição da poli (adp-ribose) polimerase, geração da neuroproteção através do produto de degradação ribose-1-fosfato e regulação positiva da proteína associada ao crescimento 43 (GAP-43) em neurônios resultando em crescimento axonal (HASKÓ, SITKOVSKY e SZABÓ, 2004).

O papel imunomodulador da inosina foi descrito por Haskó e colaboradores (2004), além de achados que demonstram que a inosina é potencialmente um fator neurotrófico eficaz (MUTO et al., 2014) As atividades antioxidante, analgésica, antidepressiva e anti-inflamatória da inosina tem sido investigada em diversos modelos experimentais de dor, inflamação, depressão e encefalomielite (NASCIMENTO et al., 2010; da ROCHA-LAPA et al., 2013; MUTO et al., 2014; JUNQUEIRA et al., 2016; GONÇALVES et al., 2016), porém ainda não explorada em modelos de DA.

A administração oral de inosina na dose de 1,2 g/kg, duas horas após lesão medular experimental e depois continuamente por 28 dias, afetou positivamente a velocidade de recuperação da função locomotora dos membros posteriores em modelo de lesão medular (KURICOVA et al., 2014). A inosina também foi capaz de inibir as respostas pós-sinápticas do glutamato e reduzir a isquemia cerebral em animais. O efeito protetor contra insultos relacionados à isquemia/reperfusão parece

envolver a ativação de receptores de adenosina A_3 (FARTHING, FARTHING e XI, 2015). Esse estudo corrobora com o trabalho de Wu et al. (2008), no qual também demonstraram neuroproteção induzida pela inosina contra isquemia/hipóxia *in vivo.* Além disso, tem sido demonstrado que a inosina exerce um importante papel relacionado à plasticidade neuronal, através da regulação da expressão de genes envolvidos no crescimento, na reconexão e na reorganização axonal via regulação positiva de GAP-43 (BENOWITZ et al., 1998; CHEN et al., 2002; ZAI et al., 2009) e da ativação da proteína MST3b (Mammalian sterile 20 like-quinase 3b), uma quinase que participa da via de transdução de sinais e que regula o crescimento dos axônios (KIM et al., 2013; KURICOVA et al., 2014). Muto e colaboradores (2014), descreveram que a administração de inosina via oral (dose de 330 mg/kg de peso corporal do animal) é transportado para o cérebro, ativa os receptores de adenosina e desempenha seus efeitos neuromoduladores e neuroprotetores.

O tratamento com inosina já tem sido utilizado para evitar danos renais durante cirurgia renal isquêmica (FITZPATRICK et al., 1981), além de combinado com o dimepranol acedobeno, formando a isoprinosina (inosina pranobex) que é utilizado como imunoestimulante no tratamento de infecções virais respiratórias agudas (BERAN, SALAPOVÁ, SPAJDEL, 2016). Com base em sua eficácia em uma variedade de modelos pré-clínicos já citados, a inosina passou por avaliação em ensaios clínicos nos EUA para esclerose múltipla (EM) (https://clinicaltrials.gov/ct2/show/NCT00067327) e doença de Parkinson (DP) (http://clinicaltrials.gov/ct2/show/NCT00833690).

A administração oral de inosina (500 mg) foi avaliada em pacientes com EM em um estudo duplo-cego randomizado de um ano de duração. Os resultados demonstraram que o tratamento com inosina foi capaz de possibilitar uma melhora clínica dos pacientes com EM, bem como aumentar os niveis de urato, molécula com ação ‘’scavenger’’ de peroxinitrito ($ONOO^-$) que, acredita-se, ser responsável pelos danos oxidativos que contribuem para a patogênese das doenças neurogenerativas (MARKOWITZ et al., 2009).

A justificativa para ensaios clínicos que visam aumentar os níveis de urato é que estes estão inversamente correlacionados com o risco de DP (PAGANONI e SCHWARZSCHILD, 2017). Schwarzschild e colaboradores (2014) realizaram um estudo randomizado com adultos diagnosticados com DP inicial, tendo como tratamento cápsulas de 500 mg de inosina ingeridas três vezes ao dia, como uma

estratégia para retardar a progressão da incapacidade. Os resultados encontrados foram a ausência de efeitos adversos graves inaceitáveis (segurança), tratamento continuado sem evento adverso requerendo redução da dose (tolerabilidade) e elevação de urato no soro e líquido cefalorraquidiano. Esses resultados apoiam o avanço para desenvolvimento mais definitivo da inosina como uma terapia potencial de modificação da doença, auxiliando a retardar a progressão clínica da DP (SCHWARZSCHILD et al., 2014).

Estudos recentes tem sido realizados com o objetivo de entender como a modulação do sistema purinérgico, mais direcionado aos receptores adenosinérgico A_1 e A_{2A}, pode contribuir para a redução ou interrupção dos processos neurodegenerativos relacionados à DA. Diante do exposto acima, e considerando que o mecanismo de ação da inosina, possívelmente, se dá através da sensibilização desses receptores é plausível concluir que a utilização desse nucleosódeo torna-se uma alternativa terapêutica interessante para ser avaliada em modelos de DA, uma vez que esta já apresentou efeitos promissores em estudos clínicos em outras doenças neurodegenerativas como DP e EM.

2.5 Modelos experimentais

Os modelos experimentais utilizando animais na pesquisa pré-clínica são extremamente úteis para o melhor entendimento fisiopatológico de doenças, avaliação dos mecanismos de ação de fármacos e descoberta de alvos terapêuticos e biomarcadores. A DA constitui um grande desafio para a pesquisa básica, pois possui etiologia ainda não esclarecida. Nesse sentido, houve a necessidade do desenvolvimento de modelos experimentais no intuito de auxiliar o esclarecimento dos mecanismos patofisiológicos envolvidos (SJOBERG et al., 2017).

2.5.1 Modelo experimental de amnésia

Um dos modelos pré-clínicos para DA é o uso da escopolamina (SCO) como agente indutor de amnésia. A SCO é uma amina terciária da classe dos alcalóides da beladona. Esse fármaco é um antagonista competitivo não-seletivo de mAChRs. Em animais, a SCO é amplamente usada como modelo farmacológico experimental de dano cognitivo, pois quando administrada de forma sistêmica interfere na aquisição da memória (MORE et al., 2016).

Os efeitos mais pronunciados da SCO sobre a memória estão associados à

diminuição das funções colinérgicas centrais (GUTIERRES et al., 2012). Outros possíveis mecanismos envolvidos nas ações da SCO envolvem a diminuição da oxidação de glicose, um aumento da oxidação cerebral e uma diminuição dos níveis de ATP no cérebro (GUTIERRES et al., 2012). Além disso, Marisco e colaboradores (2013) demonstraram que administração intraperitoneal (i.p.) de SCO (1 mg/Kg) imediatamente após treino da esquiva inibitória foi capaz de reduzir a atividade das enzimas NTPDase, CD73 e ADA em hipocampo e córtex cerebral de ratos, sugerindo assim que o déficit cognitivo induzido pelo SCO também pode estar associado a alterações na sinalização purinérgica cerebral.

2.5.2 Modelo experimental de demência esporádica do tipo Alzheimer

A estreptozotocina (STZ) é um antibiótico nitrosamida metilnitrosureia, que uma vez metabolizada pela célula, gera nitrosureído, o qual causa fragmentação do DNA celular, seguido da ativação da enzima de reparo, a poli-ADP-ribose-polimerase (PARP), a qual consome NAD^+ e favorece a formação de radicais livres que acabam promovendo a morte celular (HOSOKAWA, DOLCI e THORENS, 2001).

Na pesquisa, a STZ geralmente é utilizada por via sistêmica para indução de modelos de diabetes (ZHUO et al., 2018). Quando administrada por via intracerebroventricular (ICV) em animais induz alterações comportamentais, neuroquímicas e patofisiológicas semelhantes às encontradas na demência do tipo Alzheimer (SALKOVIC-PETRISIC et al., 2013). O provável mecanismo envolvido nessas alterações está relacionado as disfunções no metabolismo de glicose pela STZ no SNC, uma vez que a administração ICV de STZ não causa comprometimento sistêmico na glicose, mas reduz o metabolismo deste monossacarídeo em regiões cerebrais como córtex e hipocampo (LANNERT e HOYER, 1998).

Os mecanismos envolvidos na demência causada pela STZ ainda não são totalmente esclarecidos, porém uma das hipóteses é que a STZ entra nas células pelo transportador de glicose 2 (GLUT-2) que está localizado em regiões específicas do cérebro, explicando as semelhanças nas alterações hipocampais entre esse modelo experimental e a DA (ARLUISON et al., 2004). A disfunção na sinalização da insulina, causada pela STZ sobre o receptor de insulina (RI) gera comprometimento da via enzimática GSK3, que está aumentada através da fosforilação de proteínas, gerando aumento da agregação dependendo de qual isoforma é modulada. Através da

isoforma GSK3α gera o aumento do Aβ, e pela isoforma GSK3β, o aumento da proteína Tau hiperfosforilada, ambos eventos críticos presentes na patogênese da DA (SALKOVIC-PETRISIC e HOYER, 2007; SALKOVIC-PETRISIC et al., 2013). Além disso, foi observado nesse modelo, que assim como no envelhecimento, ocorre uma diminuição da densidade dos RI e da insulina cerebral, eventos estes que podem ser associados ao declínio cognitivo (STOLK et al., 1997).

É bem estabelecido na literatura que esse modelo experimental causa déficit cognitivo nos animais (DUELLI et al., 1994; GUTIERREZ et al., 2014; NAZEM et al., 2015; PACHECO et al., 2018). Além do prejuízo na memória, foi observado que a administração de STZ foi capaz de induzir estresse oxidativo (PACHECO et al., 2018), desequilíbrio metabólico e neuroinflamação (KRASKA et al., 2012). Além disso, uma diminuição na atividade das enzimas NTPDase, 5'-nucleotidase e ADA em sinaptossomas do córtex cerebral e hipocampo também tem sido observada nesse modelo experimental de DA (DE OLIVEIRA et al., 2018).

Considerando que um modelo de doença necessita mimetizar a causa, as principais lesões e os sintomas presentes em uma ordem cronológica semelhante à doença em questão, entre os modelos disponíveis para DA, o modelo STZ-ICV é considerado, em termos de ordenação temporal dos eventos patológicos, um dos modelos mais compatíveis. Assim, esse modelo se torna uma alternativa aos modelos convencionais transgênicos, principalmente no que se refere à mimetizar os componentes patofisiológicos, como a neuroinflamação crônica (NAZEM et al., 2015), o estresse oxidativo (PACHECO et al., 2018) e as alterações no sistema purinérgico (DE OLIVEIRA et al., 2018), pois mesmo após décadas de pesquisa, a DA permanece um desafio, sendo necessária a utilização de todas as abordagens disponíveis para sua melhor compreensão.

3 Objetivos

3.1 Objetivo geral

Investigar os efeitos do tratamento com inosina em parâmetros comportamentais, bioquímicos e inflamatórios em modelos experimentais da DA.

3.2 Objetivos específicos

a) Em ratos tratados com inosina e submetidos a um modelo de déficit de memória induzido por SCO foram avaliados em córtex cerebral e hipocampo:

- O efeito da inosina nas fases de aquisição e consolidação da memória através do teste de esquiva inibitória;
- Parâmetros de estresse oxidativo como níveis de EROs, nitrito, conteúdo tiólico total, substâncias reativas ao ácido tiobarbitúrico (TBARS) e atividade das enzimas CAT, SOD e GPx;
- Atividade das enzimas AChE, BuChE e Na^{+},K^{+}- ATPase.

b) Em ratos submetidos a um modelo de demência esporádica do tipo Alzheimer induzido por STZ e tratados com inosina foram avaliados os seguintes parâmetros:

- Memória através dos testes de esquiva inibitória, reconhecimento de objetos e labirinto em Y e atividade Atividade locomotora através do teste do campo aberto;
- Parâmetros de estresse oxidativo como níveis de EROs, nitrito, conteúdo tiólico total, de TBARS e atividade das enzimas CAT, SOD e GPx em córtex cerebral e hipocampo;
- Expressão gênica e atividade das enzimas ChAT e AChE em córtex cerebral e hipocampo;
- Atividade das enzimas Na^{+},K^{+}-ATPase, Ca^{+2}- ATPase, magnésio (Mg^{+})- ATPase em córtex cerebral e hipocampo;
- Densidade dos receptores A_1 e A_{2A} em córtex cerebral e hipocampo;
- Expressão gênica do BDNF e do TrkB em em córtex cerebral e hipocampo;
- Níveis de citocinas IL-4 e IL-10 em córtex cerebral e hipocampo;
- Os níveis de glicose, colesterol, uréia e ácido úrico em soro;
- Imunoreativatidade para a proteína GFAP;
- Análise histológica do hipocampo.

4 Resultados

Os resultados que fazem parte desta tese estão apresentados sob a forma de um artigo e dois manuscritos. As seções materiais e métodos, resultados, discussão e referências encontram-se no próprio artigo e manuscritos, e representam a íntegra deste estudo.

Os itens discussão e conclusão que se encontram no final dessa tese apresentam interpretações e comentários gerais sobre os artigos contidos nesse trabalho.

As referências são apenas de citações que aparecem nos itens introdução, revisão de literatura e discussão da tese.

O artigo e os manuscritos estão estruturados de acordo com as revistas as quais foram publicados ou submetidos.

4.1 Manuscrito 1

Protective effects of inosine on memory acquisition and consolidation in a rat model of scopolamine-induced cognitive impairment: Involvement of cholinergic signaling, redox status, and ion pump activities

Submetido ao periódico Pharmacology, Biochemistry and Behavior

Protective effects of inosine on memory acquisition and consolidation in a rat model of scopolamine-induced cognitive impairment: Involvement of cholinergic signaling, redox status, and ion pump activities

Fernanda Cardoso **Teixeira**[a*], Bruna da Silveira de **Mattos**[a], Julia Eisenhart **de Mello**[a], Juliane de Souza **Cardoso**[a], Luiza **Spohr**[a], Karina Pereira **Luduvico**[a], Mayara Sandrielly Pereira **Soares**[a], Fabiano Barbosa **Carvalho**[b], Jessie Martins **Gutierres**[b], Anelize de Oliveira Campello **Felix**[c], Francieli Moro **Stefanello**[a], **Roselia** Maria **Spanevello**[a*]

[a]*Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Campus Universitário S/N, Pelotas, RS, Brazil.*

[b]*Programa de Pós-Graduação em Patologia - Laboratório de Pesquisa em Patologia, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil.*

[c]*Biotério Central, Universidade Federal de Pelotas, Pelotas, RS, Brazil.*

* Corresponding authors:

Roselia Maria Spanevello and Fernanda Cardoso Teixeira
Programa de Pós-Graduação em Bioquímica e Bioprospecção, Laboratório de Neuroquímica, Inflamação e Câncer, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Campus Universitário, Capão do Leão, 96010-900 Pelotas, RS, Brazil
Phone: +55 53 39217355
E-mail: roselia.spanevello@ufpel.edu.br and fe.t@hotmail.com

Abstract

This study investigated the effects of inosine on memory acquisition and consolidation, cholinesterases activities, redox status and Na^+, K^+-ATPase activity in a rat model of scopolamine-induced cognitive impairment. Adult male rats were divided into four groups: control (saline), scopolamine (1 mg/kg), scopolamine plus inosine (50 mg/kg), and scopolamine plus inosine (100 mg/kg). Inosine was pre-administered for 7 days, intraperitoneally. On day 8, scopolamine was administered pre (memory acquisition protocol) or post training (memory consolidation protocol) on inhibitory avoidance tasks. The animals were subjected to the step-down inhibitory avoidance task 24 hours after the training. Scopolamine induced impairment in the acquisition and consolidation phases; however, inosine was able to prevent only the impairment in memory consolidation. Also, scopolamine increased the activity of acetylcholinesterase and reduced the activity of Na^+, K^+-ATPase and the treatment with inosine protected against these alterations in consolidation protocol. In the animals treated with scopolamine, inosine improved the redox status by reducing the levels of reactive oxygen species and thiobarbituric acid reactive substances and restoring the activity of the antioxidant enzymes, superoxide dismutase and catalase. Our findings suggest that inosine may offer protection against scopolamine-induced memory consolidation impairment by modulating brain redox status, cholinergic signaling and ion pump activity. This compound may provide an interesting approach in pharmacotherapy and as a prophylactic against neurodegenerative mechanisms involved in Alzheimer's disease.

Keywords: memory, oxidative stress, acetylcholinesterase activity, Na^+, K^+-ATPase activity

1 Introduction

Memory is a process that has several stages, including acquisition and consolidation (Abel and Lattal, 2001). Memory acquisition can be understood as the entry of information in the system and depends on long-term potentiation (LTP) mechanisms. In contrast, the consolidation of information is the retention and conservation of the information obtained through the recurrent reactivation of LTP (Dudai, Karni and Borni, 2015). Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory loss with deficits in acquisition and consolidation (Moulin et al., 2004) and is recognized by the World Health Organization as a global public health priority. Until now, no curative therapies have been developed to treat patients with Alzheimer's disease (Lane et al., 2018). In order to evaluate new therapeutic strategies, given the current therapeutic limitations, research using experimental models in preclinical research is useful (Singh, 2020). Among experimental pharmacological models (induced by scopolamine or corticosterone), environmental (induced by aluminum and noise stress) and physiological (natural aging) the most significant results of cognitive impairment were observed in rats injected with scopolamine (Haider, Tabassum and Perveen, 2016).

It is well established that cognitive impairment in Alzheimer's disease is associated with neurochemical alterations in the cholinergic system, redox status, and ion pump activities (Teixeira et al., 2020). The involvement of the cholinergic system in pathology of AD is well established, with a reduction of cholinergic neurons in the brain coding regions of memory, hippocampus and cerebral cortex (Stanciu et al., 2020). It has been shown that oxidative stress is a factor associated with alterations in Na^+, K^+- ATPase as well in other mechanisms involved in the development and progression of AD (Tonnies and Trushing, 2017; Amato, Terzo and Mule, 2019; Teixeira et al., 2020). Besides, the Na^+, K^+-ATPase plays a crucial role in the maintenance of cellular ionic homeostasis and changes in the activity of this enzyme directly affect cellular signaling, neuronal activity, and memory process (Moseley et al., 2007; Pacheco et al., 2018, Teixeira et al., 2020). Thereby, alterations in Na^+, K^+-ATPase have been described in both animal models and patients with AD (Hatori et al., 1998; Gutierres et al., 2014; Ianiski et al., 2016; Ohnishi et al., 2015).

Inosine, a product of the breakdown of adenosine, is normally present in the body and has a good tolerability profile in humans even when taken at high doses. It

has been shown that inosine has potent antioxidant, anti-inflammatory, and neuroprotective properties in models of depression (Kaster et al., 2013) and Parkinson's disease (Cipriani et al., 2014). Besides, inosine has been found to have beneficial effects on improvement off memory in old female rats (Ruhal and Dhingra, 2018) and a model of sporadic dementia-like AD (Teixeira et al., 2020). This action has been associated in part to the activation of adenosine receptors or the production of metabolite uric acid (Haskó, Sitkovsky and Szabo, 2004; Welihinda et al., 2016; Doyle et al., 2018). Therefore, this study aimed to evaluate the effect of inosine on acquisition and consolidation memory in a rat model of scopolamine-induced cognitive impairment. In addition, we also evaluated some neurochemical markers such as redox status, cholinesterases, and Na^+, K^+-ATPase activities in the cerebral cortex and hippocampus, which are important brain regions involved in cognitive function.

2 Materials and Methods

2.1 Chemical

Inosine, scopolamine, acetylthiocholine iodide, Coomassie brilliant blue G, ouabain, dichloro-dihydro-fluorescein diacetate (DCFH-DA), 5,5'-dithiobis-(2-nitrobenzoic acid; DTNB), and adenosine triphosphate were purchased from Sigma Aldrich Chemical Co. (3300, S 2nd ST #3306, St. Louis, MO, 63118, USA). All other reagents used in the detailed experiments were of analytical grade and the highest purity.

2.2 Pretreatment with inosine and the memory acquisition and consolidation protocol

The animals were kept in cages under standard conditions of temperature (23 ± 1 °C), relative humidity (45-55%), and lighting (12-h light/dark cycle) and had free access to a standard rodent diet and water *ad libitum*. Eighty male Wistar rats weighing between 300 and 350 g were divided into two sets of experiments. Forty animals were used in the protocol of memory acquisition, and forty animals were used for the memory consolidation protocol. In both protocols, the animals were subdivided into four groups: control (C), scopolamine, scopolamine plus inosine 50 mg/kg (scopolamine + inosine 50 mg/kg) and scopolamine plus inosine 100 mg/kg (scopolamine + inosine 100 mg/kg).

The rats were treated for 7 days once daily (around 9 a.m.) with inosine at doses of 50 mg/kg or 100 mg/kg of their body weight. Scopolamine was dissolved in saline and injected intraperitoneally at a dose of 1 mg/kg according to the following protocols:

Memory acquisition protocol: to assess memory acquisition, on day 8, the animals received scopolamine 1 h pre-training. The test session occurred 24 h after training.

Memory consolidation protocol: to assess memory consolidation, on day 8, the animals received scopolamine 1 h post-training. The test session also occurred 24 h after training. More detail on the protocols can be seen in Figure 1.

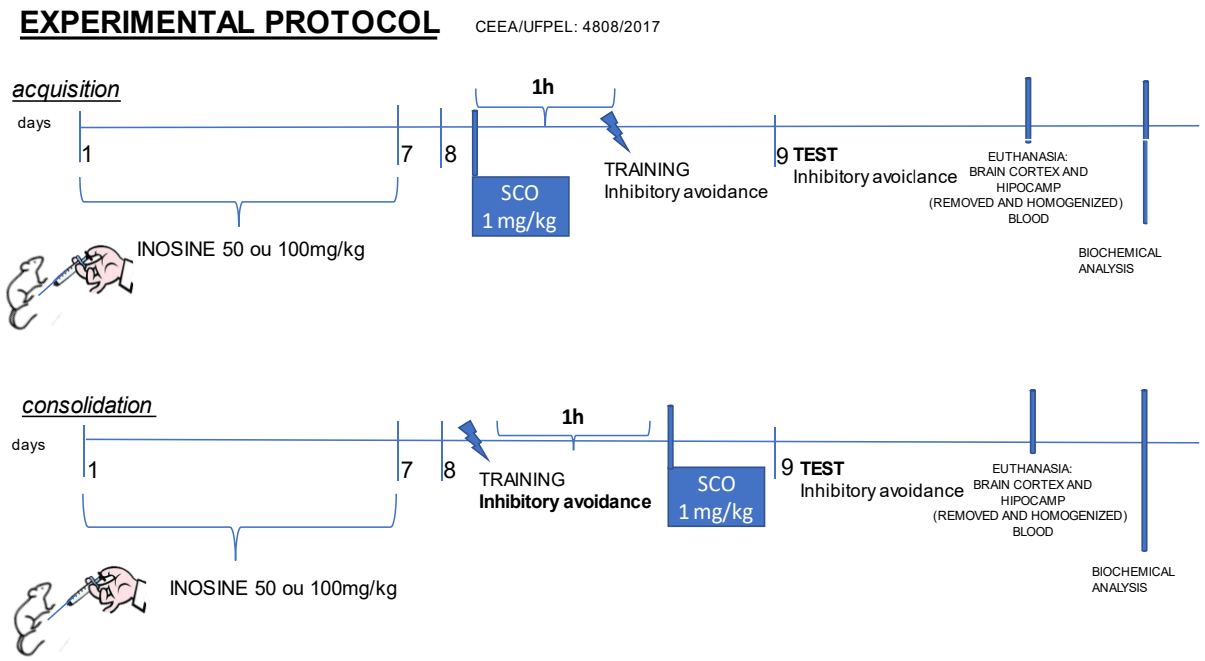


Figure 1. Protocol design. The animals were pretreated with inosine (50 or 100 mg/kg) for 7 days. On day 8, scopolamine (SCO) was administered before (memory acquisition protocol) and after training (memory consolidation protocol) on the inhibitory avoidance tasks. Then, 24 h after the training, the animals were subjected to the step-down inhibitory avoidance task.

These protocols were determined based on other studies that also used scopolamine to induce memory alterations (Gutierres et al., 2012; Wong-Guerra et al., 2019). It should be noted that the doses of inosine used in this study were equal to or lower than those shown in the literature, in which inosine showed antioxidant and anti-inflammatory activity with memory evaluation in traumatic brain injury and aging protocols (Dachir et al., 2014; Ruhal and Dhingra, 2018).

2.3 Inhibitory avoidance test

The rats were subjected to a single training session in a step-down inhibitory avoidance apparatus, which consisted of a 25 × 25 × 35-cm box with a grid floor whose right portion was covered by a 7 × 25-cm platform that was 2.5-cm high. The rats were placed gently on the platform facing the rear left corner, and when a rat stepped down with all four paws on the grid, a 3-s 0.5-mA electrical stimulation was applied to the grid. Then, 24 h after the training, the animals were subjected to the step-down inhibitory avoidance task. Test step-down latency was taken as a measure of retention (latency in seconds), and a cut-off time of 300 s was established (Gutierres et al., 2012). After the test, the animals were euthanized, and their brain and blood were collected for biochemical assays.

2.4 Cholinesterases activities in the brain and serum

The cerebral cortex and hippocampus were homogenized on ice in a glass potter with 10 mM Tris HCl (pH 7.4). The protein content was determined using the Coomassie blue method using bovine serum albumin as the standard. The acetylcholinesterase enzymatic assay was determined as previously described (Ellman et al., 1961). The reaction system was composed of 10 mM DTNB, 100 mM phosphate buffer (pH 7.5), and the enzyme (40-50 µg of protein) was pre-incubated for 2 min. The reaction was initiated by the addition of 0.8 mM acetylthiocholine iodide, and the absorbance was read in a spectrophotometer at 412 ηm. All samples were tested in duplicate, and the enzyme activity was expressed in µmol acetylthiocholine iodide/h/mg of protein.

Butyrylcholinesterase activity in serum was determined using a modified version of the method in Ellman et al. (1961). The method is based on the formation of 5,5′-dithiobis-acid nitrobenzoic measured at 412 ηm. The reaction was initiated by adding (0,8mM) butyrylthiocholine iodide. Butyrylcholinesterase activity was expressed in µmol butyrylthiocholine iodide/h/mg of protein.

2.5 Oxidative stress parameters in the brain

The hippocampus and cerebral cortex were homogenized in ten volumes (1:10 w/v) with sodium phosphate buffer (pH 7.4) containing KCl (10 mM). The homogenates were centrifuged at 3500 rpm for 10 min at 4°C. The pellet was discarded, and the supernatant was used for the measurements. The samples were stored at -80°C until

they were used. The protein content was determined using the Lowry method (1951) with bovine serum albumin as the standard solution.

2.5.1 Reactive oxygen species assay

Reactive oxygen species formation was determined in accordance with the method in Ali et al. (1992). In this assay, the oxidation of DCFH-DA to fluorescent 2',7'-dichlorofluorescein (DCF) was measured to detect intracellular reactive species. DCF fluorescence intensity emission was recorded at 525 and 488 ηm excitations 30 min after the addition of DCFH-DA to the medium. The results are expressed as μmol DCF/ mg of protein.

2.5.2 Nitrite levels

The nitrite content was determined using the Griess reaction, in accordance with the method in Huang et al. (2009). Briefly, 50 μL of the sample plus 50 μL of sulphanilamide in 5% phosphoric acid were incubated for 10 min at room temperature. Next, 50 μL of N-(1-naphthyl) ethylenediamine dihydrochloride (0.1 %) was added, and the mixture was incubated for 10 min at room temperature. Absorbance at 540 ηm was measured. A sodium nitrite solution was used as the reference standard, and the results are expressed as μmol NO_2^- /mg of protein.

2.5.3 Thiobarbituric acid reactive substance assay

The thiobarbituric acid reactive substance levels were determined by Esterbauer and Cheeseman (1990). The samples were mixed with trichloroacetic acid 15% and centrifuged. The supernatant was mixed with thiobarbituric acid (0.67%) and incubated in a dry block at 100°C for 30 min. The thiobarbituric acid reactive substance levels were determined by the absorbance at 535 ηm and reported as ηmol of thiobarbituric acid reactive substances/mg of protein.

2.5.4. Total sulfhydryl content assay

The total sulfhydryl content was determined in accordance with the method described by Aksenov and Markesbery (2001). Samples were added to the phosphate-buffered saline (pH 7.4) containing ethylenediaminetetraacetic acid (1 mM). The reaction was started by the addition of DTNB and incubated for 60 min in the dark. The reduction of DTNB by thiols became oxidized, generating a yellow derivative. The

result was measured spectrophotometrically at 412 ηm. The results were reported as ηmol DTNB/mg of protein.

2.5.5 Superoxide dismutase activity

The superoxide dismutase activity was measured in accordance with the method described by Misra and Fridovich (1972). This assay is based on the inhibition of superoxide dependent adrenaline auto-oxidation to adrenochrome. The intermediate in this reaction is superoxide, which is scavenged by superoxide dismutase and is measured with a spectrophotometer adjusted at 480 ηm. The specific activity of superoxide dismutase was reported as units/mg of protein.

2.5.6 Catalase activity

Catalase activity was measured in accordance with the method described by Aebi (1984). The decomposition of H_2O_2 was continuously monitored with a spectrophotometer at 240 nm for 180 s at 37°C. One unit of the enzyme was defined as 1 nmol of hydrogen peroxide consumed per min. The specific catalase activity was reported as units/mg of protein.

2.6 Total ATPase and Na^+, K^+-ATPase activity assays in the brain

The cerebral cortex and hippocampus were homogenized in Tris HCl (10 mM; pH 7.4) and centrifuged at 3500 rpm for 10 min at 4°C. The supernatant was used as previously described by Carvalho et al. (2012). Total ATPase activity was assayed in an incubation medium consisting of 30 mM of Tris HCl (pH 7.4), 50 mM of NaCl, 5 mM of KCl, 6 mM of $MgCl_2$, 3 mM of adenosine triphosphatase, and 50 µl of brain supernatant. The absorbency concentration range for proteins in the hippocampus and cortex was 0.8-1.0 in the final volume of 300 µl. Controls to correct for non-enzymatic substrate hydrolysis were performed by the addition of sample preparations after the reactions were stopped with trichloroacetic acid. The reaction was started by the addition of adenosine triphosphatase and was stopped after 30 min of incubation by the addition of 10% trichloroacetic acid.

Na^+, K^+-ATPase activity was assayed in the incubation reaction mixture containing 6 mM of $MgCl_2$, 50 mM of NaCl, 5 mM of KCl, 0.1 mM of ethylenediaminetetraacetic acid, and 30 mM of Tris HCl (pH 7.4). After 10 min of pre-incubation at 37°C, the reaction was initiated by the addition of adenosine triphosphate

to a final concentration of 3 mM and incubated for 30 min. The control experiments were conducted with the addition of 1 mM of oubain. The specific Na^+, K^+-ATPase activity was calculated by subtracting the ouabain-insensitive activity from the overall activity (in the absence of ouabain). The enzyme's activities are expressed in ηmol of Pi/min/mg of protein.

2.7 Statistical analysis

The inhibitory avoidance task results did not pass the normality test. Therefore, a non-parametric distribution was assumed. Thus, the data were analyzed using the non-parametric extension of a one-way analysis of variance and the Kruskal-Wallis test. The data are expressed as the median ± interquartile range.

The results of training in the inhibitory avoidance task and biochemical parameters were analyzed by a one-way analysis of variance, followed by Tukey's post-hoc test. These data are expressed as the mean ± standard error of the mean. GraphPad Prism version 5.0 (Intuitive Software for Science, San Diego, CA, USA) was used to perform the statistical analysis. A *P*-value of ≤ 0.05 was considered statistically significant.

3 Results

3.1 Inosine protects memory consolidation impaired by scopolamine

None of the treatments showed a significant alteration in training latency in both protocols (Figure 2A and 2C). Administration of scopolamine, both before and after training, induced impairment in the acquisition and consolidation of cognition (Figures 2B and 2D). Scopolamine reduced the latency time in both the acquisition (H=15.55; P=0.0014 Figure 2B) and consolidation (H=16.61; P=0.0009 Figure 2D) protocols, as evaluated by the inhibitory avoidance tasks. Pretreatment with inosine at doses of 50 mg/kg (H=16.61, P<0.05) and 100 mg/kg (H=16.61; P<0.001) was capable of preventing only memory deficit in the consolidation protocol (Figure 2D).

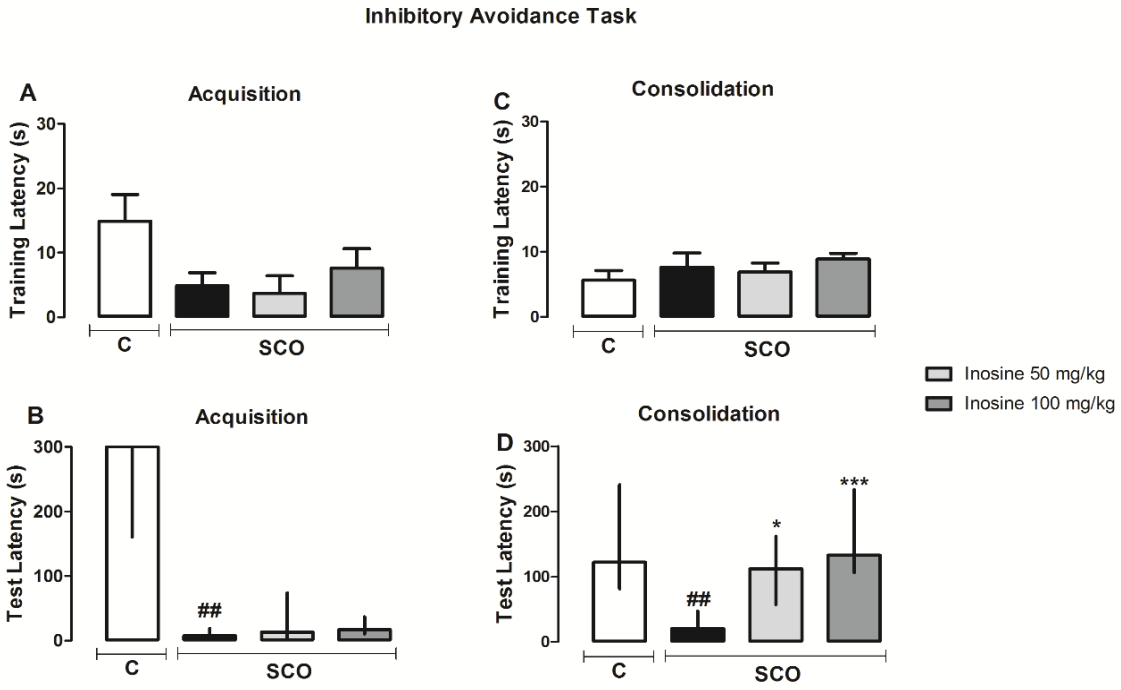


Figure 2. Effect of pretreatment with inosine (50 or 100 mg/kg, intraperitoneally) for 7 days on impairment of memory (acquisition and consolidation) induced by scopolamine (SCO) (1 mg/kg). The data represent the latency (in seconds) of all the animals in the inhibitory avoidance tasks. Bars represent the mean ± standard error of the mean. ## *P*<0.01 represents a significant difference compared to the control group (C) group. *P<0.05 and *** P<0.001 represents a significant difference compared to the scopolamine group (n=9-10).

3.2 Inosine prevents cholinergic enzyme alterations induced by scopolamine

No alterations were observed in the acetylcholinesterase activity in either the hippocampus ($F_{(3,16)}$=1.39 P>0.05) or cerebral cortex ($F_{(3,16)}$=1.35 P>0.05) in the memory acquisition protocol in any of the groups evaluated (Figure 3 A and B). In the consolidation memory protocol, scopolamine induced an increase in acetylcholinesterase activity in the hippocampus ($F_{(3,16)}$ = 26.2 P<0.05) and cerebral cortex ($F_{(3,16)}$= 9.8 P<0.05) when compared to the control group (Figures 3 C and D). Inosine 50 and 100 mg/kg treatment prevented this change in both the hippocampus and cerebral cortex (P<0.001).

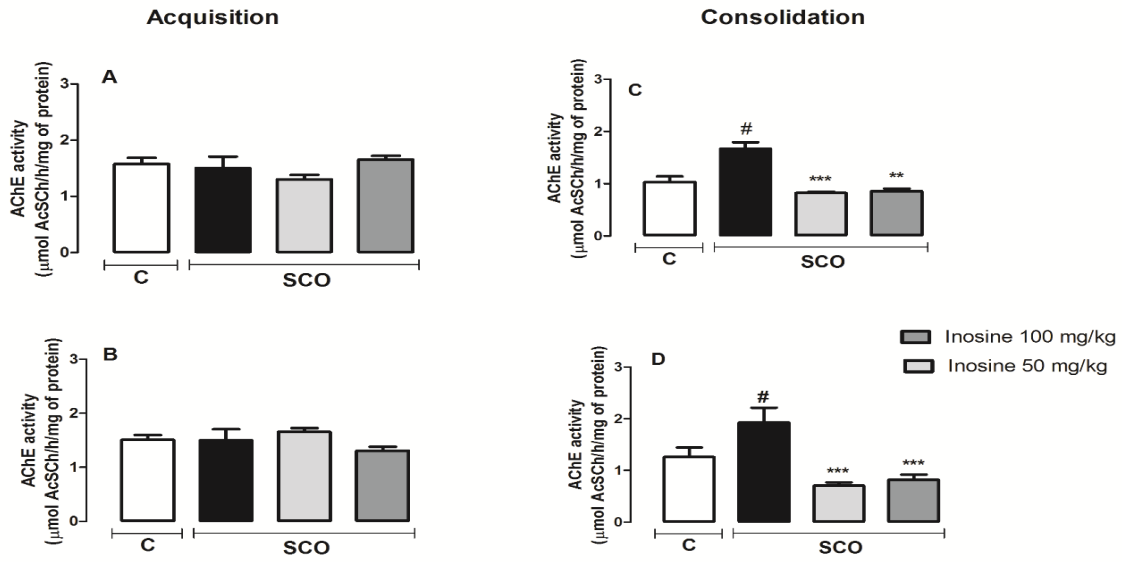


Figure 3. Effect of pretreatment with inosine (50 or 100 mg/kg, intraperitoneally) for 7 days on the acetylcholinesterase activity in the hippocampus (A) and cerebral cortex (B) in a protocol of memory acquisition. The hippocampus (C) and cerebral cortex (D) of the rats submitted to the memory protocol consolidation. Bars represent the mean ± standard error of the mean. # P<0.05 represents a significant difference compared to the control (C) group. **P<0.01 and *** P<0.001 represents a significant difference compared to the scopolamine group (n=5). One-way analysis of variance, Tukey's test.

Regarding the activity of the enzyme butyrylcholinesterase in serum, the results also showed that scopolamine induced an increase in this enzyme activity in both the acquisition ($F_{(3,16)=}$ 4.81 P<0.05) and consolidation ($F_{(3,16)=}$ 3.52 P<0.05) protocols compared to the control group. Pretreatment with inosine was capable of preventing the alterations induced by scopolamine in butyrylcholinesterase activity in only the animals submitted to the memory acquisition protocol (P<0.05; Figure 4).

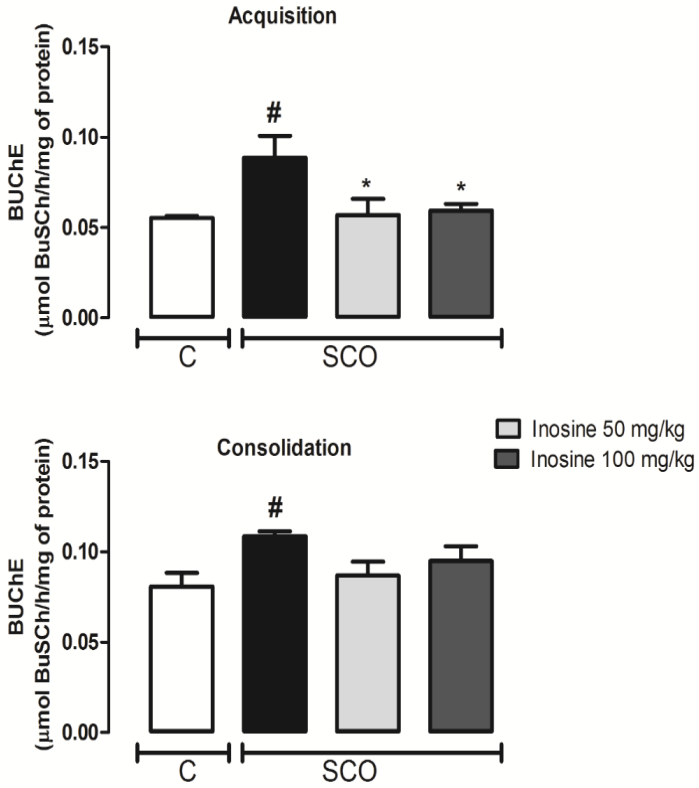


Figure 4. Effect of pretreatment with inosine (50 or 100 mg/kg, intraperitoneally) over 7 days on the butyrylcholinesterase activity in the serum of animals submitted to the memory acquisition or consolidation protocol. Bars represent the mean ± standard error of the mean. # A P-value of <0.05 represents a significant difference compared to the control (C) group. * A P-value of <0.05 represents a significant difference compared to the scopolamine group (n=5). One-way analysis of variance, Tukey's test.

3.3 Inosine prevents brain oxidative stress induced by scopolamine

In the memory acquisition protocol, scopolamine induced an increase in the levels of reactive oxygen species in the hippocampus ($F(3,15)= 14.75$ $P<0.01$) and cerebral cortex ($F(3,15)= 10.64$ $P<0.01$) compared to the control group. Inosine in both doses was used to prevent this effect (Figure 5 A and E). No significant changes were observed in the hippocampus regarding the level of sulfhydryl content ($F(3,15)= 0.6740$ $P=0.5240$), thiobarbituric acid reactive substance ($F(3,18)= 0.363$ $P=0.7802$), and nitrite ($F(3,17)= 0.880$ $P=0.4751$) in any of the groups (Figures 5 B, C and D).

In the cerebral cortex, inosine (100 mg/kg) increased the sulfhydryl content in animals that received scopolamine before training ($F(3,17)= 17.05$ $P<0.001$; Figure 5F). Inosine (50 and 100 mg/kg) also prevented the increase in thiobarbituric acid reactive substance levels ($F(3,16)=13.61$ $P<0.001$) in the cerebral cortex induced by

scopolamine in the memory acquisition protocol. However, no changes were observed in the nitrite levels ($F_{(3,15)=}$ 0.9933 =0.4290) in this brain structure in any of the evaluated groups (Figures 5 G and H)

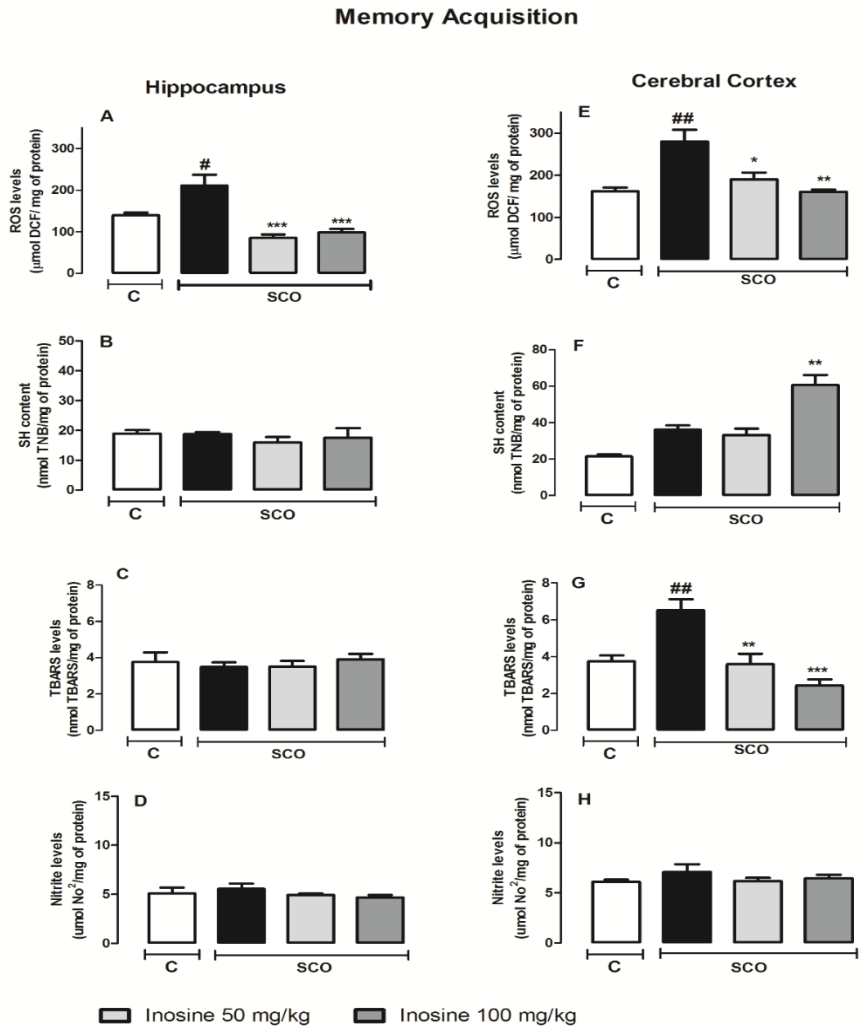


Figure 5. Effect of pretreatment with inosine (50 or 100 mg/kg, intraperitoneally) over 7 days on the reactive oxygen species levels (A and E), total sulfhydryl content (SH - B and F), thiobarbituric acid reactive substances (C and G), and nitrite levels (D-H) in the hippocampus and cerebral cortex of rats in the memory acquisition protocol. Bars represent the mean ± standard error of the mean. #P<0.05 and ## *P*<0.01 represents a significant difference compared to the control (C) group. *P<0.05, **P<0.01, and *** P<0.001 represents a significant difference compared to the scopolamine group (n=4-5). One-way analysis of variance, Tukey's test.

A decrease in superoxide dismutase activity was observed in the hippocampus ($F_{(3,15)}$= 7.43 P<0.001) and cerebral cortex ($F_{(3,15)=}$ 3.48 P<0.05) in a protocol of deficit memory acquisition induced by scopolamine compared to the control group (Figures 6

A and C). Only changes in superoxide dismutase activity in the hippocampus were prevented by inosine (Figure 6 A). Scopolamine induced a decrease in catalase activity only in the cerebral cortex ($F_{(3,17)=}$ 5.97 P<0.001) compared to the control group. This change was prevented only in the animals that received inosine (100 mg/kg). No catalase activity changes were observed ($F_{(3,17)=}$ 2.53 P= 0.098) in the hippocampus in any of the groups (Figure 6 B).

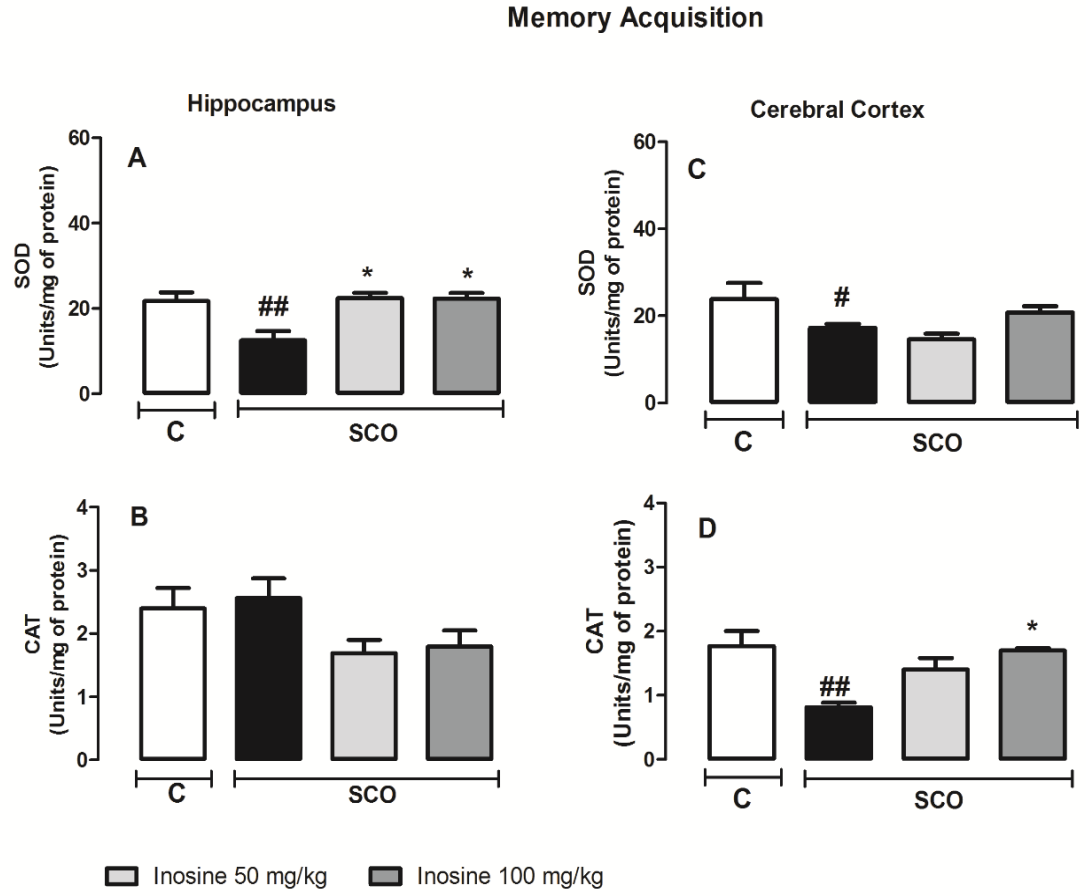


Figure 6. Effect of pretreatment with inosine (50 or 100 mg/kg, intraperitoneally) over 7 days on the superoxide dismutase and catalase activities in the hippocampus (A and B) and cerebral cortex (C and D) of rats in the memory acquisition protocol. Bars represent the mean ± standard error of the mean. #P<0.05 and ## *P*<0.01 represents a significant difference compared to the control (C) group. *P<0.05 represents a significant difference compared to the scopolamine group (n=4-5). One-way analysis of variance, Tukey's test.

Regarding the memory consolidation protocol in the hippocampus, an increase in the reactive oxygen species ($F_{(3,15)=}$ 16.5 P<0.001) and nitrite levels ($F_{(3,15)=}$ 19.60 P<0.0001), and a decrease in sulfhydryl content ($F_{(3,16)=}$ 19.60 P<0,001) in the

scopolamine group compared to the control group was observed (Figures 7 A, C, and D). Inosine treatment was capable of preventing these changes, particularly the 100 mg/kg dose. Scopolamine induced an increase in the levels of thiobarbituric acid reactive substances in the hippocampus ($F_{(3,15)=}$ 11.09 P<0,0001). Inosine was not capable of preventing this change (Figure 7C).

In the cerebral cortex, the administration of scopolamine after training increased the reactive oxygen species ($F_{(3,16)=}$ 6.36 P<0.001), thiobarbituric acid reactive substances ($F_{(3,19)=}$ 12.82 P<0.001), and nitrite levels ($F_{(3,15)=}$ 5.81 P<0.05) compared to the control group (Figures 7 E, G, H). Inosine in both doses prevented the alterations in reactive oxygen species and thiobarbituric acid reactive substance levels but not in the nitrate levels in the cerebral cortex. No alterations were observed in the sulfhydryl levels in the cerebral cortex ($F_{(3,18)=}$ 2.20 P=0.129) in any of the groups evaluated (Figure 7F).

The antioxidant enzyme results in the memory consolidation protocol showed that in the hippocampus, scopolamine induced a decrease in the superoxide dismutase (F(3,18)= 7.23 P<0.05) and catalase (F(3,16)=12.35 P<0.001) activities compared to the control group (Figures 8 A and B). Similar results were also observed in the cerebral cortex. Scopolamine administered after training decreased the superoxide dismutase (F(3,18)= 7.22 P<0.01) and catalase (F(3,17)= 4.27 P<0.05) (Figure 8 C and D) activities in this brain structure. Inosine prevented the alterations induced by scopolamine in antioxidant enzymes activities in both the hippocampus and cerebral cortex (Figure 8).

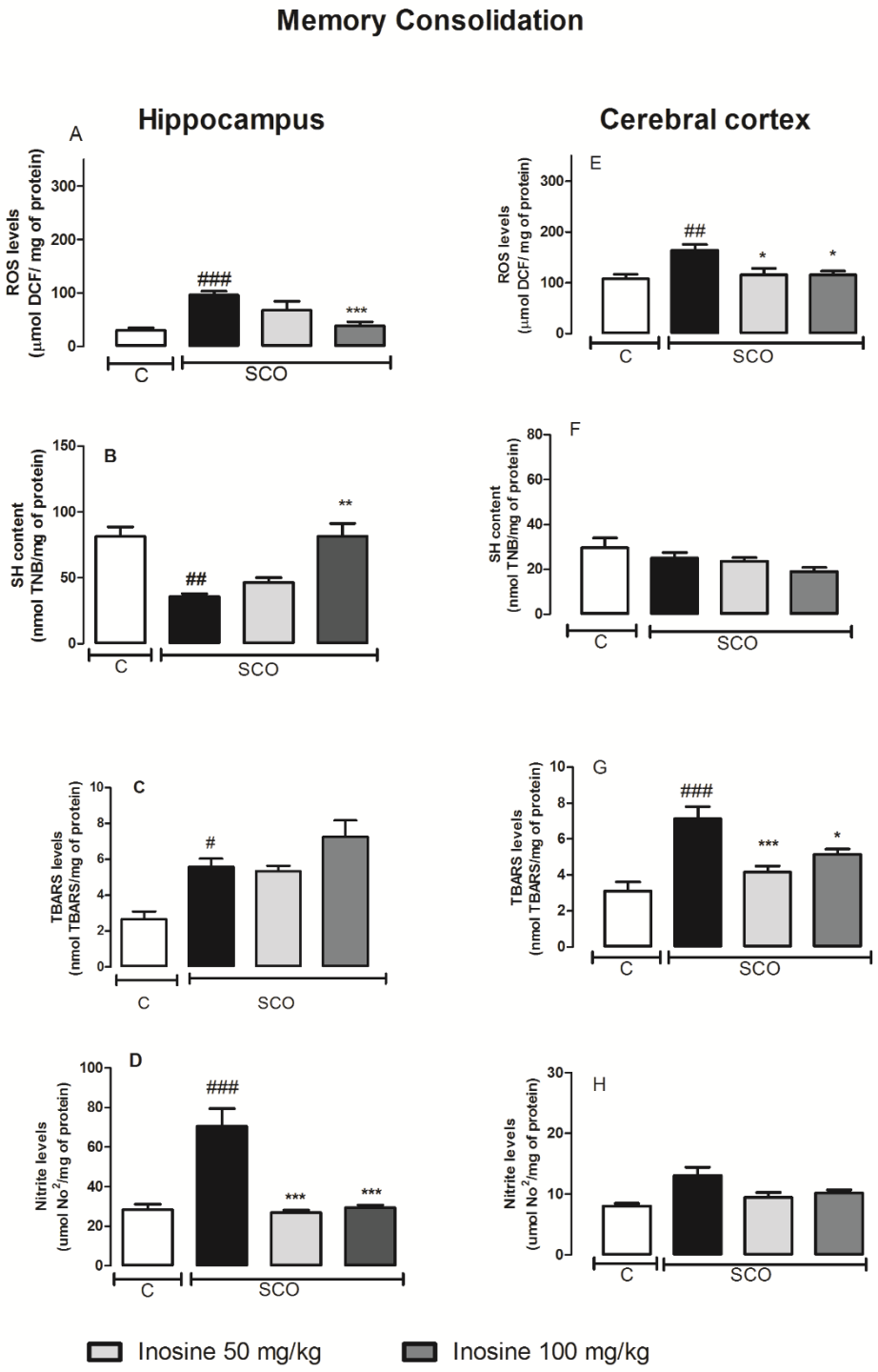


Figure 7. Effect of pretreatment with inosine (50 or 100 mg/kg, intraperitoneally) over 7 days on the reactive oxygen species levels (A and E), total sulfhydryl content (SH - B and F), thiobarbituric acid reactive substances (C and G), and nitrite levels (D-H) in the hippocampus and cerebral cortex of rats in the memory consolidation protocol. Bars represent the mean ± standard error of the mean. #P<0.05, ## *P*<0.01 and ### P< 0.001 represents a significant difference compared to control (C) group. *P<0.05, **P<0.01, and *** P<0.001 represents a significant difference compared to the scopolamine group (n=4-5). One-way analysis of variance, Tukey's test.

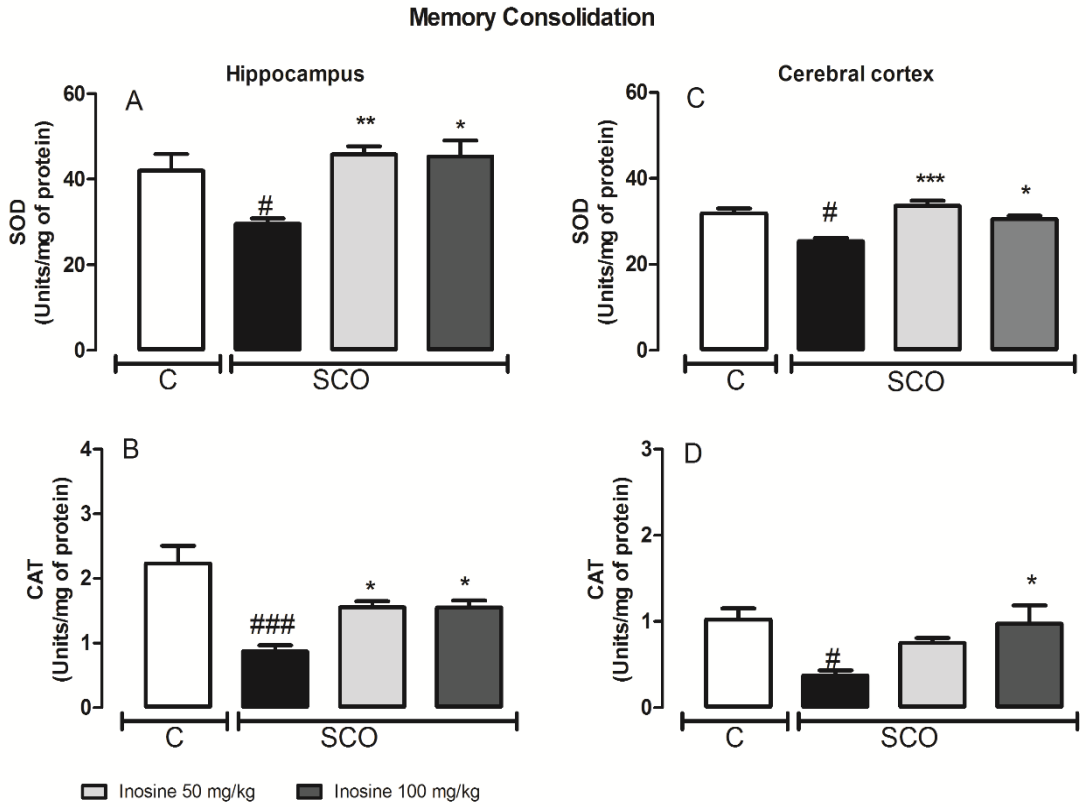


Figure 8. Effect of pretreatment with inosine (50 or 100 mg/kg, intraperitoneally) over 7 days on the superoxide dismutase and catalase activities in the hippocampus (A and B) and cerebral cortex (C and D) of rats in the memory consolidation protocol. Bars represent the mean ± standard error of the mean. #P<0.05 and ### P< 0.001 represents a significant difference compared to the control (C) group. *P<0.05, **P<0.01 and *** P<0.001 represents a significant difference compared to the scopolamine group (n=4-5). One-way analysis of variance, Tukey's test.

3.4 Inosine prevents an imbalance in ion pump activities in the memory consolidation protocol

We also observed that scopolamine reduced the total adenosine triphosphatase activity in the hippocampus ($F_{(3,17)}$=7.79 P<0.001) and cerebral cortex ($F_{(3,16)}$= 9.88 P<0.001) compared to the control group in the memory consolidation protocol (Figures 9 A and C). Inosine in both doses reversed these adenosine triphosphatase activity changes in the hippocampus and cerebral cortex (Figures 9 A and C). Scopolamine also induced a decrease in sodium-potassium adenosine triphosphatase activity in the hippocampus ($F_{(3,15)}$=17.22 P<0.001). Inosine pretreatment reversed these changes (Figure 9B). No alterations were observed in sodium-potassium adenosine triphosphatase activity ($F_{(3,19)}$ = 1.59 P=0.2557; Figure 9D).

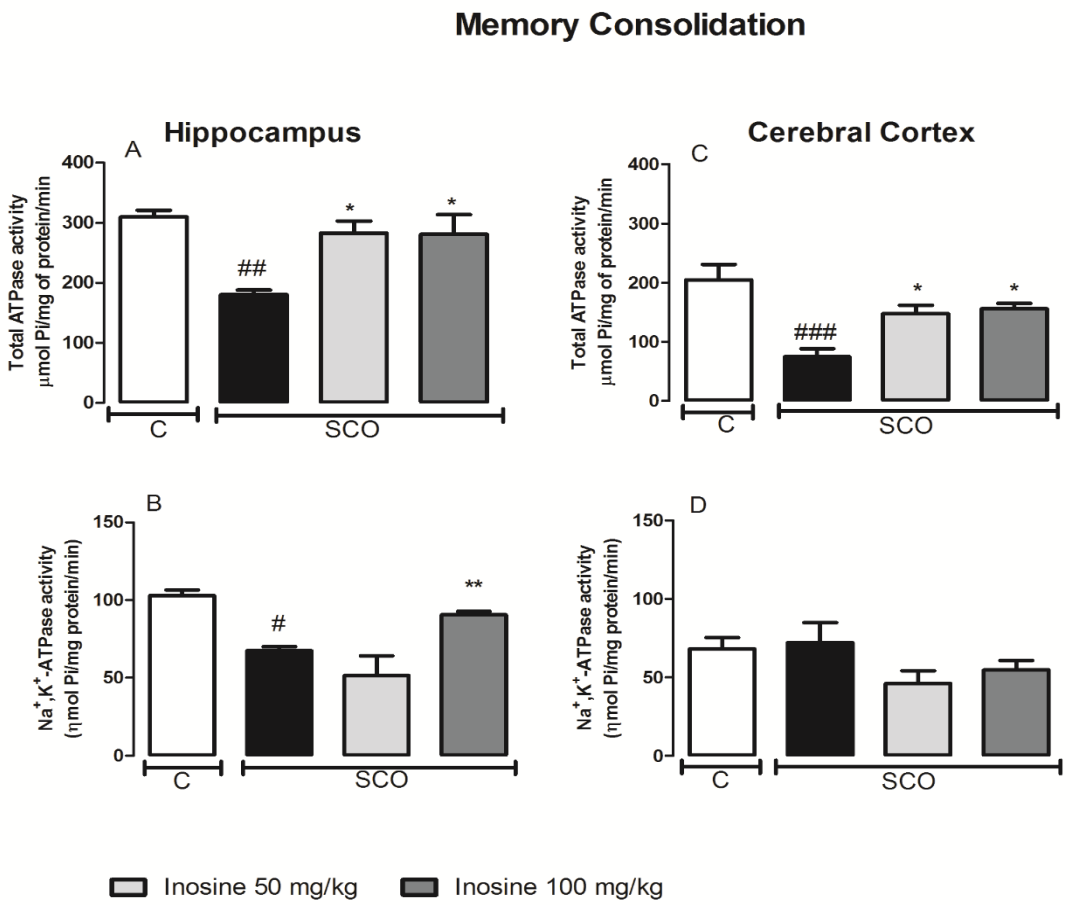


Figure 9. Effect of pretreatment with inosine (50 or 100 mg/kg, intraperitoneally) over 7 days on the total adenosine triphosphatase (A and B) and sodium-potassium adenosine triphosphatase (C and D) activities in the hippocampus and cerebral cortex of animals submitted to the memory consolidation protocol. Bars represent the mean ± standard error of the mean. $^{\#}P<0.05$ and $^{\#\#\#}P<0.001$ represents a significant difference compared to the control (C) group. *P<0.05 and **P<0.01 represents a significant difference compared to the scopolamine group (n=4-5). One-way analysis of variance, Tukey's test.

4 Discussion

This study investigated the protective effects of inosine at different stages of memory (acquisition and consolidation) in a rat model of cognitive deficit induced by scopolamine. Scopolamine is a common model used to study dementia, as it can cause memory and cognitive deficits. Although the cognitive changes induced by scopolamine do not mimic all that occur in AD, this model produces impaired cognition, which is considered the main symptom of this neurodegenerative disease (Gilles and Erthlé, 2000; Haider, Tabassum and Perveen, 2016). However, the scopolamine model is considered a reliable tool to study the effects of compounds or components involved in different phases of memory, having its validation as a pharmacological

model for cognitive impairment in animals recognized (Klinkenberg and Blokland, 2010; Haider, Tabassum and Perveen, 2016; Tang, 2019). Our findings demonstrate the protective effect of inosine only in the memory consolidation phase.

Scopolamine, a non-selective muscarinic receptor antagonist, impaired memory acquisition and consolidation in our study. These findings are in agreement with other studies (Gutierres et al., 2014; Da Silva et al., 2017; Ozawa et al., 2019; Ponne et al., 2019; Wong-Guerra et al., 2019). AD is known to be predominantly characterized by deficits in new memory acquisition. However, impairment in memory consolidation has also been described, and it has been reported that these memory steps depend on the same neuroanatomical regions, the hippocampus and cerebral cortex (Moulin et al., 2004).

Considering that the cholinergic neurotransmitter system is important in memory (Deutsch, 1971; Schneider, 2001) one of the first mechanisms evaluated in this study was the central and peripheral cholinesterase activities. Previous studies have related that the scopolamine model (1 mg/kg) decreased the acetylcholine level to <74% in the brain of male Wistar rats, significantly increased the activities of acetylcholinesterase and butyrylcholinesterase (Tang, 2019). We found an increase in the acetylcholinesterase activity in both the hippocampus and cerebral cortex only the memory consolidation protocol, which may be associated with the direct effect of scopolamine on the blockage on muscarinic receptors. Interestingly, inosine prevented this enzyme alteration in both brain regions. These findings are in accordance with previous studies from our research group, which showed that inosine treatment (50 and 100 mg/kg) over 25 days prevented the acetylcholinesterase alterations in the hippocampus and cerebral cortex of a rat model of sporadic dementia type AD (Teixeira et al., 2020). These findings suggest that inosine may contribute to increase in acetylcholine neurotransmitters in the synaptic cleft, which is a suitable mechanism for improving memory. The therapeutic strategies have been based on increasing the levels of this neurotransmitter because cholinesterase inhibition has continued to play a pivotal role in the management of symptoms and possibly slowing the rate of progression of AD (Schneider et al., 2001; Haake et al., 2020). Serum butyrylcholinesterase activity is high in patients with AD (Rao, Gumpeny and Das, 2007; Mushtaq et al., 2014). Note that the serum is a relatively non-invasive and reliable clinical sample, serum butyrylcholinesterase activity can be used as a biomarker to detect dementia and systemic inflammation associated with

neurodegenerative diseases (Mushtaq et al., 2014; Ha, Mathew and Yeong, 2020). In our study, scopolamine induced an increase in the serum butyrylcholinesterase activity in both the memory acquisition and consolidation protocols, however inosine prevented the alterations only the acquisition protocol. We emphasize that the dual inhibition of central and peripheral cholinesterase enzymes by inosine may be important for treating cholinergic deficit in neurodegenerative diseases associated with amnesic effects.

In both the memory acquisition and consolidation protocols, we observed scopolamine-induced changes in the redox status in the hippocampus and cerebral cortex, as demonstrated previously (Ishola et al., 2019; Wong-Guerra et al., 2019). In the acquisition protocol, the oxidative changes were more pronounced in the cerebral cortex, while in the consolidation protocol, both brain structures were similarly affected by scopolamine. According Qu et al., (2017) the scopolamine (1 mg / kg, ip) administered half an hour before the start of behavioral tests also reduced the levels and activities of glutathione (GSH), Glutathione peroxidase (GSH-Px) and superoxide dismutase in the hippocampus of male Wistar rats and the cognitive impairment induced in this animal model is associated with oxidative stress in the brain as it impairs the cellular antioxidant defense mechanism. In this sense, we observed that scopolamine caused a reduction in superoxide dismutase and catalase in both protocols and treatment with inosine was able to restore the enzymatic antioxidant defenses. Allied to scopolamine itself, suppression of antioxidant enzyme activities can lead to high levels of free radical accumulation in the cell, such as reactive oxygen species (ROS) (Tang, 2019), can lead to apoptosis and mitochondrial dysfunction, resulting in cognitive deficit (Haider, Tabassum and Perveen, 2016). The stress oxidative is an important factor associated with reticulum stress, nuclear DNA damage, membrane destabilization, calcium homeostasis imbalance, protein oxidation, and consequent neuronal damage with effects in memory mechanisms (Tonnies and Trushina, 2017; Da Silva et al., 2017; Amato, Terzo and Mule 2019). The increased of ROS was also observed in our study, in both protocols, and inosine was able to restore redox balance in hippocampus and cerebral cortex.

The effects of inosine in modulating the brain redox status have also been shown in another study from our research group in a model of AD (Teixeira et al., 2020). In addition, it is possible that the inosine degreasing product, urate (Venkatesan et al., 2016), activates the Nrf2 pathway, is a transcription factor that activates the endogenous antioxidant defense pathways and the production of antioxidant enzymes,

once that scopolamine treatment negatively regulates this pathway in the hippocampus (Zhang et al., 2013).

The antioxidant effect of inosine, reinforced in the results found in this work, can explain, as least in part, the prevention found in ATPase and Na^+, K^+-ATPase activities, and improvement in memory consolidation. Oxidative stress is an important factor contributing to changes in Na+, K+-ATPase, and reduction of this enzyme activity is related to memory decline (Zhang et al., 2013, Gutierres et al., 2014). The reduction in Na^+,K^+-ATPase activity impairs the ionic gradient, affecting many functions in the nervous system, such as the uptake of various neurotransmitters (Zhang et al., 2013). Thus, the modulation of Na^+, K^+-ATPase enzyme has become an important therapeutic tool to control intracellular ion homeostasis and maintain neuronal membrane potential in neurodegenerative disease protocols (Arnaiz and Ordieres, 2014; Da Silva et al., 2017).

Although inosine exerts protective effects in neurochemical parameters in both memory phases, mechanisms other than oxidative stress, cholinergic signaling, and ion pump activities may underlie the different actions of inosine in memory consolidation and acquisition. Other studies also showed the potential of inosine in memory improvement in old rats (Ruhal and Dhingra, 2018) and an experimental model of AD induced by streptozotocin (Teixeira et al., 2020). Although we did not explain the exact mechanism involved in the inosine effects on different memory phases, some hypotheses may be postulated such as the involvement of adenosine receptors. Some studies have reported that the effects of inosine in experimental brain diseases can be attributed to the activation of adenosine receptors such as A_1, A_{2A} (Kaster et al., 2013), and A_3 (Shen et al, 2005). Pagnussat et al. (2015) showed that the selective blockade of A_{2A} prevented the impairment of short-term memory induced by scopolamine. Kim and Riu (2008) investigated the effects of adenosine A_{2A} agonists on memory phases, using passive avoidance and a Morris water maze, and found that activation of this receptor impairs memory acquisition, but not consolidation or retrieval. Interestingly, preliminary results from our research group have demonstrated that inosine treatment for 25 days decreases the immunocontent of A_{2A} receptors in the brain in a model of AD (data not published). Thus, it is plausible that the effects of inosine on the adenosine receptors are involved in the differences observed between memory acquisition and consolidation.

5. Conclusion

Our study showed that inosine might offer protection from memory consolidation in a rat model of scopolamine-induced cognitive impairment. Inosine modulated neurochemical mechanisms in the hippocampus and cerebral cortex occur during both memory phases. Therefore, inosine may provide a novel approach for the treatment of diseases associated with cognition impairment.

Acknowledgments:

We thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa do Rio Grande do Sul. This study was also financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brazil (CAPES - Finance code 001). We would like to thank Editage (www.editage.com) for English language editing.

Conflicts of interest:

The authors declare that there are no conflicts of interest.

Funding:

This research was supported by Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS grant number- 19/2551-0001712-0).

Ethics approval:

The procedures used were previously approved by the Animal Research and Ethics Committee CEEA/UFPEL (4808/2017).

References

Abel, T., Lattal, K., 2001. Molecular mechanisms of memory acquisition, consolidation and retrieval. Curr. Opin. Neurobiol. 11, 180-187. doi: 10.1016/s0959-4388(00)00194-x

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121-126. doi: 10.1016/s0076-6879(84)05016-3

Aksenov, M.Y., Markesbery, W.R., 2001. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci. Lett. 302, 141-145. doi: 10.1016/s0304-3940(01)01636-6

Ali, S.F., Lebel, C.P., Bondy, S.C., 1992. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. Neurotoxicology. 13, 637-648.

Amato, A., Terzo, S., Mulè, F., 2019. Natural Compounds as Beneficial Antioxidant Agents in Neurodegenerative Disorders: A Focus on Alzheimer's Disease. Antioxidants. 8, 608. doi: 10.3390/antiox8120608

Arnaiz, G., Ordieres, M., 2014. Brain Na+, K+-ATPase Activity in Aging and Disease. Int. J. Biomed. Sci. 10, 85-112.

Carvalho, F.B., Mello, C.F., Marisco, P.C., Tonello, R., Girardi, B.A., Ferreira, J., Oliveira, M.S., Rubin, M.A., 2012. Spermidine decreases Na+, K+-ATPase activity through NMDA receptor and protein kinase G activation in the hippocampus of rats. Eur. J. Pharmacol. 684, 79-86. doi.org/10.1016/j.ejphar.2012.03.046

Cipriani, S., Bakshi, R., Schwarzschild, M., 2014. Protection by inosine in a cellular model of Parkinson's disease. Neurosc. 274, 242-249. doi: 10.1016/j.neuroscience.2014.05.038

Da Silva, F.D., Pinz, M.P., de Oliveira, R.L., Rodrigues, K.C., Ianiski, F.R., Bassaco, M.M., Silveira, C.C., Jesse, C.R., Roman, S.S., Wilhelm, E.A., Luchese, C., 2017. Organosulfur compound protects against memory decline induced by scopolamine through modulation of oxidative stress and Na+/K+ ATPase activity in mice. Metab. Brain Dis. 32, 1819-1828. doi: 10.1007/s11011-017-0067-4

Dachir, S., Shabashov, D., Trembovler, V., Alexandrovich, A.G., Benowitz, L.I., Shohami, E., 2014. Inosine improves functional recovery after experimental traumatic brain injury. Brain res. 1555, 78-88. doi.org/10.1016/j.brainres.2014.01.044

Deutsch, J.A., 1971. The cholinergic synapse and the site of memory. Sci. 174, 788-794. doi: 10.1126/science.174.4011.788

Doyle, C., Cristofaro, V., Sullivan, M.P., Adam, R., 2018. Inosine-a Multifunctional Treatment for Complications of Neurologic Injury. Cell Physiol. Biochem. 49, 2293-2303. doi: 10.1159/000493831

Dudai, Y., Karni, A., Born, J., 2015. The consolidation and transformation of memory. Neuron. 88, 20-32. doi.org/10.1016/j.neuron.2015.09.004

Ellman, G.L., Courtney, K.D., Andres Jr.V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7(2), 88-95. doi.org/10.1016/0006-2952(61)90145-9

Esterbauer, H., Cheeseman, K.H., 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol. 186, 407-421. doi: 10.1016/0076-6879(90)86134-h

Gilles, G., Ertlé, S., 2000. Pharmacological models of Alzheimer's disease research. Dialogues Clin. Neurosc. 3, 247-255. doi: 10.31887/DCNS.2000.2.3/cgilles

Gutierres, J.M., Carvalho, F.B., Schetinger, M.R., Rodrigues, M.V., Schmatz, R., Pimentel, V.C., Vieira, J.M., Rosa, M.M., Marisco, P., Ribeiro, D.A. and Leal, C., 2012. Protective effects of anthocyanins on the ectonucleotidase activity in the impairment of memory induced by scopolamine in adult rats. Life Sci. 91(23-24), 1221-1228. doi.org/10.1016/j.lfs.2012.09.013

Gutierres, J.M., Carvalho, F.B., Schetinger, M.R.C., Agostinho, P., Marisco, P.C., Vieira, J.M., Rosa, M.M., Bohnert, C., Rubin, M.A., Morsch, V.M., Spanevello, R., 2014. Neuroprotective effect of anthocyanins on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia in rats. Int. J. Develop. Neurosc. 33. 88-97. doi: 10.1016/j.ijdevneu.2013.12.006

Ha, Z., Mathew, S., Yeong, K., 2020. Butyrylcholinesterase: A Multifaceted Pharmacological Target and Tool. Curr. Protein Pept. Sci. 21, 99-109. doi: 10.2174/1389203720666191107094949

Haake, A., Nguyen, K., Friedman, L., Chakkamparambil, B., Grossberg, G.T., 2020. An update on the utility and safety of cholinesterase inhibitors for the treatment of Alzheimer's disease. Expert Opin. Drug Saf. 19, 147-157. doi: 10.1080/14740338.2020.1721456.

Haider, S., Tabassum, S., Perveen, T., 2016. Scopolamine-induced greater alterations in neurochemical profile and increased oxidative stress demonstrated a better model of dementia: A comparative study. Brain Res. Bull. 127, 234-247. doi: 10.1016/j.brainresbull.2016.10.002.

Haskó, G., Sitkovsky, M.V., Szabó, C., 2004. Immunomodulatory and neuroprotective effects of inosine. Trends Pharmacol. Sci. 25, 152-157. doi: 10.1016/j.tips.2004.01.006

Hattori, N., Kitagawa, K., Higashida, T., Yagyu, K., Shimohama, S., Wataya, T., Perry, G., Smith, M.A., Inagaki, C., 1998. Cl−-ATPase and Na+/K+-ATPase activities in Alzheimer's disease brains, Neurosci. Lett. 254, 141-144. doi: 10.1016/s0304-3940(98)00654-5

Huang, W.C., Lin, Y.S., Wang, C.Y., Tsai, C.C., Tseng, H.C., Chen, C.L., Lu, P.J., Chen, P.S., Qian, L., Hong, J.S., Lin, C.F., 2009. Glycogen synthase kinase-3 negatively regulates anti-inflammatory interleukin-10 for lipopolysaccharide-induced iNOS/NO biosynthesis and RANTES production in microglial cells. Immunology. 128, 275-286. doi: 10.1111/j.1365-2567.2008.02959.x

Ianiski, F.R., Alves, C.B., Ferreira, C.F., Rech, V.C., Savegnago, L., Wilhelm, E.A., Luchese, C., 2016. Meloxicam-loaded nanocapsules as an alternative to improve memory decline in an Alzheimer's disease model in mice: involvement of Na+, K+-ATPase. Met. Brain. Dis. 31, 793-802. doi: 10.1007/s11011-016-9812-3

Ishola, I., Akinyede, A., Eloke, J., Chatuverdi, J., Narender, T., 2019. Diastereomeric Mixture of Calophyllic and Isocalophyllic Acid Ameliorates Scopolamine-Induced Memory Impairment in Mice: Involvement of Antioxidant Defense and Cholinergic Systems. Neurotox. Res. 37, 58-66. doi: 10.1007/s12640-019-00117-8

Kaster, M.P., Budni, J., Gazal, M., Cunha, M.P., Santos, A.R., Rodrigues, A.L.S., 2013. The antidepressant-like effect of inosine in the FST is associated with both adenosine A 1 and A2A receptors. Purinergic Signal. 9, 481-486. doi: 10.1007/s11302-013-9361-8

Kim, D., Ryu, J., 2008. Activation of adenosine A2A receptor impairs memory acquisition but not consolidation or retrieval phases. Biomol. Therap. 16. 320-327. doi: 10.4062/biomolther.2008.16.4.320

Klinkenberg, I., Blokland, A., 2010. The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. Neurosci. Biobehav. Rev. 34, 1307-50. doi: 10.1016/j.neubiorev.2010.04.001

Lane, C., Hardy, J., Schott, J., 2018. Alzheimer's disease. Eur. J. Neurol. 25, 59-70. doi: 10.1111/ene.13439

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193, 265-275.

Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170-3175.

Moseley, A.E., Williams, M.T., Schaefer, T.L., Bohanan, C.S., Neumann, J.C., Behbehani, M.M., Vorhees, C.V., Lingrel, J.B., 2007. Deficiency in Na, K-ATPase α isoform genes alter spatial learning, motor activity, and anxiety in mice. J. Neurosci. 27, 616-626. doi: 10.1523/JNEUROSCI.4464-06.2007

Moulin, C., James, N., Jones, R., Freeman, J., 2004. Deficient acquisition and consolidation: intertrial free recall performance in Alzheimer's disease and mild cognitive impairment. J. Clin. Exp. Neuropsychol. 26, 1-10. doi:10.1076/jcen.26.1.1.23940

Mushtaq, G., Greig, N., Khan, J., Mohammad, K., 2014. Status of acetylcholinesterase and butyrylcholinesterase in Alzheimer´s diseases and type 2 diabetes mellitus. CNS Neurol. Dis. Drug Targets. 13, 1432-1439. doi.org/10.2174/1871527313666141023141545

Ohnishi, T., Yanazawa, M., Sasahara, T., Kitamura, Y., Hiroaki, H., Fukazawa, Y., Kii, I., Nishiyama, T., Kakita, A., Takeda, H., Takeuchi, A., 2015. Na, K-ATPase α3 is a death target of Alzheimer patient amyloid-β assembly. PNAS. 112(32), 4465-4474. doi: 10.1073/pnas.1421182112

Ozawa, T., Yamada, K., Ichitani, Y., 2019. d-Cycloserine reverses scopolamine-induced object and place memory deficits in a spontaneous recognition paradigm in rats. Pharmacol. Biochem. Behav. 187, 172798. doi: 10.1016/j.pbb.2019.172798

Pacheco, S.M., Soares, M.S.P., Gutierres, J.M., Gerzson, M.F.B., Carvalho, F.B., Azambuja, J.H., Schetinger, M.R.C., Stefanello, F.M., Spanevello, R.M. 2018. Anthocyanins as a potential pharmacological agent to manage memory deficit, oxidative stress and alterations in ion pump activity induced by experimental sporadic dementia of Alzheimer's type. J. Nutr Biochem. 56,193-204. doi: 10.1016/j.jnutbio.2018.02.014

Pagnussat, N., Almeida, A.S., Marques, D.M., Nunes, F., Chenet, G.C., Botton, P.H.S., Mioranzza, S., Loss, C.M., Cunha, R.A., Porciúncula, L.O., 2015. Adenosine A2A receptors are necessary and sufficient to trigger memory impairment in adult mice. Br. J. Pharmacol. 172, 3831-3845. doi: 10.1111/bph.13180

Ponne, S., Kumar, C., Boopathy, R., 2019. Verapamil attenuates scopolamine induced cognitive deficits by averting oxidative stress and mitochondrial injury, A potential therapeutic agent for Alzheimer's Disease, Metab. Brain Dis. 35, 503-515. doi: 10.1007/s11011-019-00498-x

Qu, Z., Zhang, J., Yang, H., Gao, J., Chen, H., Liu, C., Gao, W., 2017. Prunella vulgaris L., an edible and medicinal plant, attenuates scopolamine-induced memory impairment in rats. J. Agric. Food Chem. 65, 291-300. doi: 10.1021/acs.jafc.6b04597

Rao, A., Gumpeny, S., Das, U., 2007. Elevated butyrylcholinesterase and acetylcholinesterase may predict the development of type 2 diabetes mellitus and Alzheimer´s disease. Med. Hypoth. 69, 1272-1276. doi: 10.1016/j.mehy.2007.03.032

Ruhal, P., Dhingra, D., 2018. Inosine improves cognitive function and decreases aging-induced oxidative stress and neuroinflammation in aged female rats, Inflammopharmacol. 26, 1317-1329. doi: 10.1007/s10787-018-0476-y

Schneider, L., 2001. Treatment of Alzheimer's disease with cholinesterase inhibitors. Clin. Geriatr. Med. 17, 337-339. doi: 10.1016/s0749-0690(05)70072-0

Schwarzschild, M.A., Ascherio, A., Beal, M.F., Cudkowicz, M.E., Curhan, G.C., Hare, J.M., Hooper, D.C., Kieburtz, K.D., Macklin, E.A., Oakes, D., Rudolph, A., 2014. Inosine to increase serum and cerebrospinal fluid urate in Parkinson disease: a randomized clinical trial. JAMA neurology. 71(2), 141-150. doi: 10.1001/jamaneurol.2013.5528

Shen, H., Chen, G.J., Harvey, B.K., Bickford, P.C., Wang, Y., 2005. Inosine reduces ischemic brain injury in rats. Stroke. 36(3), 654-659. doi.org/10.1161/01.STR.0000155747.15679.04

Singh, R., 2020. Recent Trends in the Management of Alzheimer's Disease: Current Therapeutic Options and Drug Repurposing Approaches. Curr. neuropharmacol. 18(9),868-882. doi.org/10.2174/1570159X18666200128121920

Stanciu, G.D., Luca, A., Rusu, R.N., Bild, V., Beschea Chiriac, S.I., Solcan, C., Bild, W., Ababei, D.C., 2019. Alzheimer's Disease Pharmacotherapy in Relation to Cholinergic System Involvement. Biomolecules. 10(1), 40. doi.org/10.3390/biom10010040

Tang, S.K., 2019. The cellular and molecular processes associated with scopolamine-induced memory deficit: A model of Alzheimer's biomarkers. Life sci. 233, 116695. doi: 10.1016/j.lfs.2019.116695

Teixeira, F.C., Gutierres, J.M., Soares, M.S.P., da Siveira de Mattos, B., Spohr, L., do Couto, C.A.T., Bona, N.P., Assmann, C.E., Morsch, V.M., da Cruz, I.B.M., Stefanello, F.M., Spanevello, R.M. 2020. Inosine protects against impairment of memory induced by experimental model of Alzheimer disease: a nucleoside with multitarget brain actions. Psychopharmacol. 237, 811-823. doi:10.1007/s00213-019-05419-5.

Tönnies, E., Trushina, E., 2017. Oxidative stress, synaptic dysfunction, and Alzheimer's disease. J. Alzheimers Dis. 57, 1105-1121. doi.org/10.3233/JAD-161088

Venkatesan, R., Subedi, L.E., Yeo, J., Kim, S.Y., 2016. Lactucopicrin ameliorates oxidative stress mediated by scopolamine-induced neurotoxicity through activation of the NRF2 pathway. Neurochem. Int. 99, 133-146. doi: 10.1016/j.neuint.2016.06.010

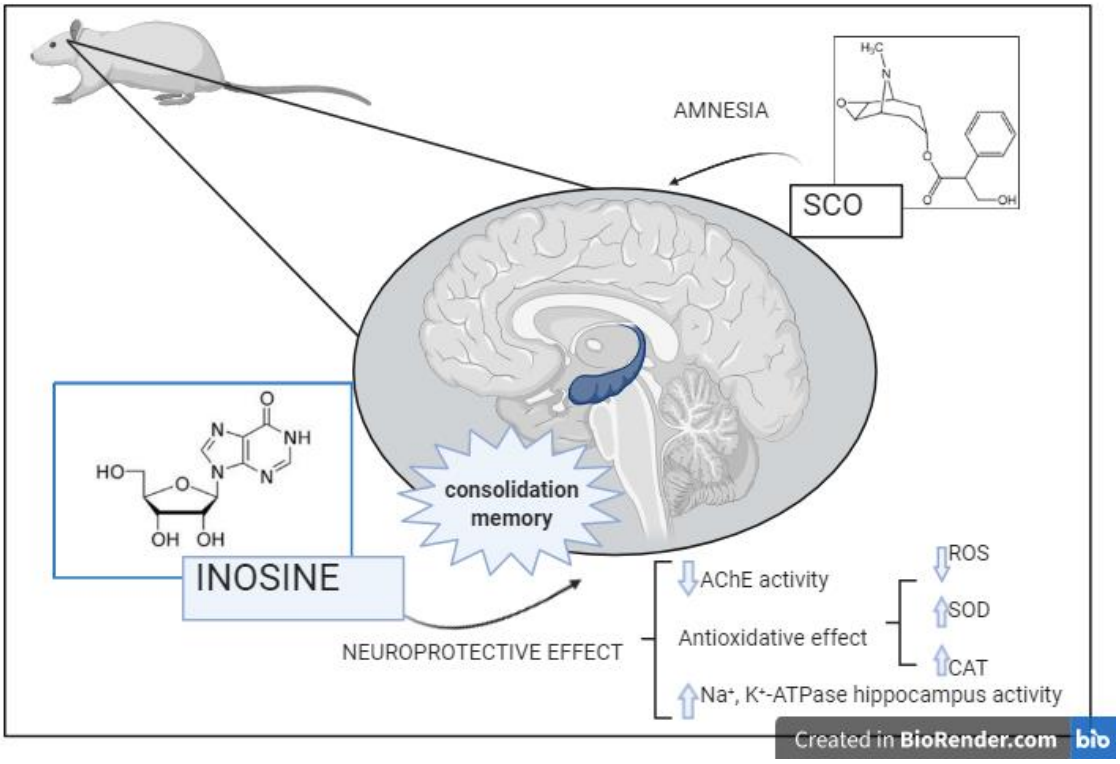
Wong-Guerra, M., Jiménez-Martin, J., Fonseca-Fonseca, L.A., Ramírez-Sánchez, J., Montano-Peguero, Y., Rocha, J.B., D´ Avila, F., De Assis, A.M., Souza, D.O., Pardo-Andreu, G.L., Del Valle, R.M.S., 2019. JM-20 protects memory acquisition and consolidation on scopolamine model of cognitive impairment. Neurol. res. 41(5), 385-398. doi:10.1080/01616412.2019.1573285

Zhang, L.N., Sun, Y.J., Pan, S., Li, J.X., Qu, Y.E., Li, Y., Wang, Y.L., Gao, Z.B., 2013. Na+-K+-ATPase, a potent neuroprotective modulator against Alzheimer disease. Fundam. and clin. pharmacol. 27. 96-103. doi: 10.1111/fcp.12000

Highlights

Inosine prevent the dysfunction in memory consolidation.
Inosine prevent against oxidative damage induced by scopolamine in brain.
Inosine prevent alterations in cholinesterase activities induced by scopolamine.
Inosine modulate the ion pump activity in a protocol of memory consolidation.

Graphical Abstract



4.2 Artigo 1

Inosine protects against impairment of memory induced by experimental model of Alzheimer disease: a nucleoside with multitarget brain actions

Publicado no periodico Psychopharmacology
DOI https://doi.org/10.1007/s00213-019-05419-5

Psychopharmacology
https://doi.org/10.1007/s00213-019-05419-5

ORIGINAL INVESTIGATION

Inosine protects against impairment of memory induced by experimental model of Alzheimer disease: a nucleoside with multitarget brain actions

Fernanda Cardoso Teixeira[1] · Jessié Martins Gutierres[2] · Mayara Sandrielly Pereira Soares[1] · Bruna da Siveira de Mattos[1] · Luiza Spohr[1] · Carlus Augustu Tavares do Couto[1] · Natália Pontes Bona[1] · Charles Elias Assmann[3] · Vera Maria Morsch[3] · Ivana Beatrice Mânica da Cruz[4] · Francieli Moro Stefanello[1] · Roselia Maria Spanevello[1,5]

Received: 1 May 2019 / Accepted: 27 November 2019

Abstract

Rationale Inosine is a naturally occurring purine nucleoside formed by adenosine breakdown. This nucleoside is reported to exert potent effects on memory and learning, possibly through its antioxidant and anti-inflammatory actions.

Objective The objective is to evaluate the effects of inosine on the behavioral and neurochemical parameters in a rat model of Alzheimer's disease (AD) induced by streptozotocin (STZ).

Methods Adult male rats were divided into four groups: control (saline), STZ, STZ plus inosine (50 mg/kg), and STZ plus inosine (100 mg/kg). STZ (3 mg/kg) was administered by bilateral intracerebroventricular injection. The animals were treated intraperitoneally with inosine for 25 days. Memory, oxidative stress, ion pump activities, acetylcholinesterase (AChE), and choline acetyltransferase (ChAT) activities and expression were evaluated in the cerebral cortex and hippocampus.

Results The memory impairment induced by STZ was prevented by inosine. An increase in the Na^+, K^+-ATPase, and Mg-ATPase activities and a decrease in the Ca^{2+}-ATPase activity were induced by STZ in the hippocampus and cerebral cortex, and inosine could prevent these alterations in ion pump activities. Inosine also prevented the increase in AChE activity and the alterations in AChE and ChAT expression induced by STZ. STZ increased the reactive oxygen species, nitrite levels, and superoxide dismutase activity and decreased the catalase and glutathione peroxidase activities. Inosine treatment conferred protection from these oxidative alterations in the brain.

Conclusions Our findings demonstrate that inosine affects brain multiple targets suggesting that this molecule may have therapeutic potential against cognitive deficit and tissue damage in AD.

Keywords Inosine · Oxidative stress · Brain · Alzheimer's disease · Acetylcholinesterase · Na^+, K^+-ATPase

✉ Roselia Maria Spanevello
rspanevello@gmail.com

[1] Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Campus Universitário S/N, Pelotas, RS, Brazil

[2] Programa de Pós-Graduação em Patologia, Laboratório de Pesquisa em Patologia, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil

[3] Programa de Pós-Graduação em Bioquímica Toxicológica, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

[4] Programa de Pós-Graduação em Farmacologia, Departamento de Fisiologia e Farmacologia, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

[5] Programa de Pós-Graduação em Bioquímica e Bioprospecção, Laboratório de Neuroquímica, Inflamação e Câncer, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Campus Universitário, Capão do Leão, Pelotas, RS 96010-900, Brazil

Published online: 13 December 2019

Introduction

Alzheimer's disease (AD) is the most frequently observed age-associated neurodegenerative disease that is clinically characterized by a progressive memory decline and cognitive capacity (Chen 2018). The pathological hallmarks of AD include β-amyloid deposition and neurofibrillary tangles containing hyperphosphorylated tau protein (Jeong 2017; Sonntag et al. 2017). The main risk factors for this disease include age, genetic aspects, lifestyle, and environmental aspects. The majority of cases manifest as a late onset sporadic form; however, a subgroup of patients suffers from the familial or early onset (< 65 years) form of AD. In the last few years, biochemical studies devoted to elucidating the mechanisms involved in AD pathophysiology have showed that chronic neuroinflammation, mitochondrial damage, brain energetic metabolism dysfunction, oxidative stress, and alterations in brain enzymes are directly related to neurodegeneration (Chen 2018).

Oxidative damage has been suggested as a possible mechanism involved in AD initiation and progression (Gumusyayla et al. 2016; Huang et al. 2016). An imbalance between the production and detoxification of reactive species could damage biological structures affecting crucial brain enzymes such as acetylcholinesterase (AChE) and Na^+, K^+-ATPase. AChE is responsible for hydrolyzing the neurotransmitter acetylcholine at cholinergic synapses (Soreq and Seidman 2001). Apart from its catalytic function, AChE also functions in postsynaptic differentiation, cellular adhesion, and neurite extension (Soreq and Seidman 2001). Na^+, K^+-ATPase is a ubiquitous plasma membrane enzyme responsible for generating action potentials by establishing Na^+ and K^+ concentration gradients across the plasma membrane, which are essential for cellular and body ion homeostasis (Lores Arnaiz and Ordieres 2014). Dysfunctions in both AChE and Na^+, K^+-ATPase have been associated with an impaired process of learning and memory (Zhang et al. 2013; Pacheco et al. 2018).

Inosine is a naturally occurring purine nucleoside formed by the breakdown of adenosine. Previous studies have indicated that inosine exerts potent effects on the neural system (Markowitz et al. 2009; Kuricova et al. 2014; Muto et al. 2015). Inosine improved the learning and memory of aged rats possibly through its antioxidant and anti-inflammatory effects (Ruhal and Dhingra 2018). In addition, the beneficial effects of inosine associated with plasticity, neuroprotection, and immunomodulation are also demonstrated in a variety of preclinical models (Haskó et al. 2004; Mabley et al. 2009; Kuricova et al. 2014; Muto et al. 2015). Considering the neuroprotective actions of inosine, we evaluated the effects of this compound against streptozotocin-induced memory deficits in rats. The modulatory role of inosine in oxidative stress parameters and brain enzyme activities and expression was also analyzed in the cerebral cortex and hippocampus of rats.

Material and methods

Chemicals

Inosine, streptozotocin (STZ), acetylthiocholine iodide (AcSCh), Coomassie Brilliant Blue G, ouabain, dichlorodihydro-fluorescein diacetate (DCFH-DA), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), and adenosine triphosphate (ATP) were purchased from Sigma Chemical Co. (St. Luis, MO, USA). All other reagents used in the detailed experiments were of analytical grade and the highest purity.

Animals

Forty adult male Wistar rats (2-month old) weighing 300–350 g were provided by the Central Animal House of the Federal University of Pelotas. Animals were kept in cages under standard temperature (23 ± 1 °C), relative humidity (45–55%), and lighting (12-h light/dark cycle) conditions. The animals had ad libitum access to standard rodent pelleted diet and water. All animal procedures were approved by the ethical committee of the Federal University of Pelotas (4808/2017).

Intracerebroventricular injection of streptozotocin

The animals were anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg). The head was positioned in the stereotaxic apparatus, and a midline sagittal incision was made in the scalp. The stereotaxic coordinates for the lateral ventricle were measured accurately as anterio-posterior − 0.8 mm, lateral 1.5 mm, and dorso-ventral − 4.0 mm relative to the bregma and ventral from the dura with the tooth bar set at 0 mm (Herman and Watson 1987). Through the skull hole, a 28-gauge Hamilton syringe of 10 μL attached to a stereotaxic apparatus and piston of a syringe was lowered manually into each lateral ventricle. STZ was dissolved in a citrate buffer (pH 4.5) and administered by bilateral intracerebroventricular (icv) injection (5 μL). Control rats group received an icv injection with the same volume of citrate buffer.

Inosine treatment

The animals were divided in four experimental groups ($n = 10$ each): (I) control (C), (II) STZ, (III) STZ + inosine 50 mg/kg, and (IV) STZ + inosine 100 mg/kg. The animals of groups II, III, and IV received bilateral icv injection of STZ whereas those of group I received only citrate buffer. After 3 days of the surgical procedure, the animals of groups III and IV were treated with inosine (50 mg/kg or 100 mg/kg) by the intraperitoneal route (ip). Inosine was dissolved in saline solution and administered to the animals for 25 days (Fig. 1). The animals of the groups I and II received only saline solution by the ip

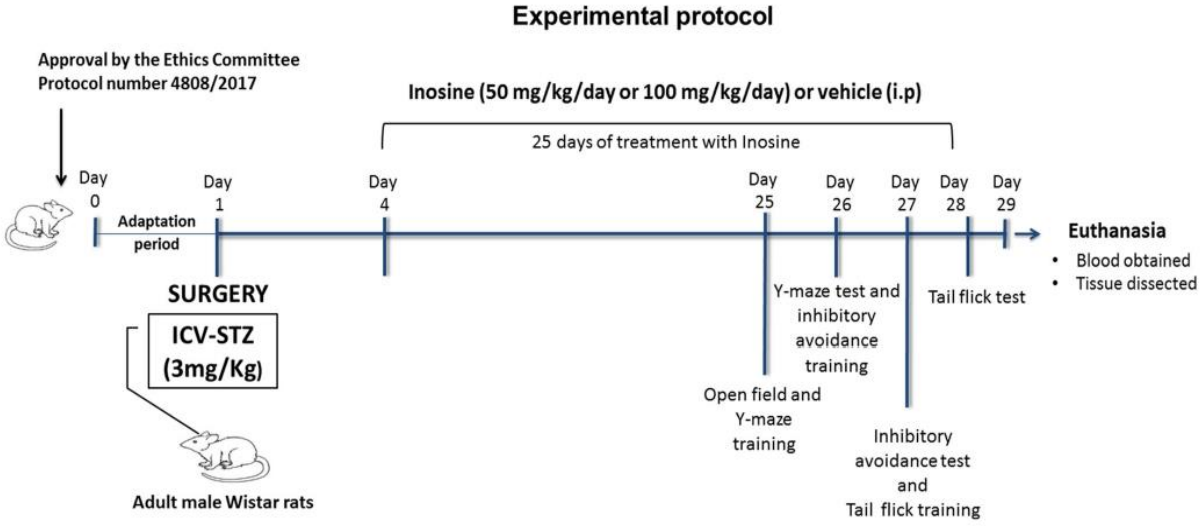


Fig. 1 Scheme of the protocol used to induce the experimental model of sporadic dementia of Alzheimer's type in rats and treatment with inosine (50 or 100 mg/kg)

route for 25 days. The inosine dose was chosen based on previous studies indicating neuroprotection (Dachir et al. 2014; Ruhal and Dhingra 2018).

Behavioral evaluation

Open-field test

Twenty-five days after surgical procedure, the locomotor behavioral tests were performed using an open-field apparatus as described previously by Pacheco et al. (2018). The open-field test was realized in an apparatus consisting of a box with the floor of the arena divided into 16 equal squares (18 × 18 cm) and placed in a sound-free room. Animals were placed in the rear left square and were allowed to freely explore during 5 min. The number of squares crossed with all paws (crossing) was counted manually. The apparatus was cleaned up with a 40% alcohol solution and dried after each individual rat session. This test was performed to identify motor disabilities that might influence the other behavioral tests performed.

Y-maze test

Spatial recognition memory was evaluated in the apparatus that had three arms: start arm, in which rats were placed to start to explore (always open); novel arm, which was blocked during training session, but open during the test session; and other arm (always open). In the training session, the animal was placed in the apparatus on the start arm and was free to explore only the start arm and other arm for 5 min. The novel arm remained blocked throughout the training session. After 24 h, the test session was performed with an open novel arm and the animal could freely explore all three arms over a 5-min period. The apparatus was cleaned with 40% ethanol after each session. The time spent in each arm was determined, and the results were expressed as the number of entries and time spent on the arms (Dellu et al. 1997; Pacheco et al. 2018).

Inhibitory avoidance task

The animals were submitted to the task of inhibitory avoidance as described by Gutierres et al. (2014). Briefly, the rats were subjected to a single training session in a step-down inhibitory avoidance apparatus, which consisted of a 25 × 25 × 35 cm box with a grid floor whose right portion was covered with a 7 × 25 cm platform, 2.5 cm high. The rats were placed gently on the platform facing the rear left corner, and when the rat stepped down with all four paws on the grid, a 0.5-mA shock was applied to the grid for 3 s. Twenty-four hours after the training, the animals were tested in a step-down inhibitory avoidance task. The test step-down latency was taken as a measure of retention, and a cutoff time of 300 s was established.

Tail flick

The tail flick test was performed to evaluate the nociceptive threshold of rats, using an automatic analgesiometer. On the first day, the rats were familiarized with the tail flick apparatus. On the second day, the animals were subjected to the tail flick test. Rats were placed on the apparatus, and the light source was positioned below the tail. This test determines the latency of the animal in withdrawing its tail when it reaches the nociceptive threshold. The tail flick test was performed in triplicate not exceeding the time of 10 s in the apparatus to avoid tissue damage (Gamaro et al. 2011). After

the behavioral tests, the animals were subjected to euthanasia and their brain and blood were collected (Fig. 1). The hippocampus, cerebral cortex, and serum were obtained and used for biochemical determinations.

Acetylcholinesterase activity

The brain structures were homogenized on ice in a glass potter with 10 mM Tris-HCl solution (pH 7.4). The protein content was determined using the Coomassie Blue method with bovine serum albumin as the standard. The AChE enzymatic assay was performed as previously described by Ellman et al. (1961). The reaction system comprising 10 mM DTNB, 100 mM phosphate buffer (pH 7.5), and the enzyme (40–50 μg of protein) was pre-incubated for 2 min. The reaction was then initiated by adding 0.8 mM AcSCh, and the absorbance was read on a spectrophotometer at 412 nm. All samples were tested in duplicate, and enzyme activity was expressed in μmol AcSCh/h/mg of protein.

mRNA expression of AChE and choline acetyltransferase enzyme genes

mRNA expression of AChE and choline acetyltransferase (ChAT) enzyme genes of hippocampus and cerebral cortex samples of rats was performed by quantitative real-time polymerase chain reaction (qRT-PCR). Briefly, RNA was isolated and extracted by adding TRIzol reagent to samples, and RNA content was measured for later steps. RNA was further treated with DNAse and then submitted to reverse transcription to cDNA by using the iSCript cDNA synthesis kit, containing the iScript reverse transcriptase enzyme. At last, gene expression was performed by using the QuantiFast SYBR Green PCR Kit and cDNA of each sample. To normalize the analysis, the β-actin gene was used as the housekeeping control as previously described (Assmann et al. 2018). Primer sequences (5′-3′) used for qRT-PCR: β-actin, forward (TGTGACGT TGACATCCGTAAAG), and reverse (GGCAGTAA TCTCCTTCTGCATC) AChE, forward (GAATCTTT GCTCAGCGACTTATG) and reverse (AGGTTCAG GCTCACGTATTG; ChAT, forward (GAGACCTCATCTGT GGAGTTTG), and reverse (GGCCTCTAGCTCTTTCCTTT G).

Na^+, K^+-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase activity assays

The cerebral cortex and hippocampus were homogenized in Tris-HCl buffer (pH 7.4) and centrifuged at 3500 rpm for 10 min at 4 °C. Na^+, K^+-ATPase activity was measured in the supernatant as previously described by Gutierres et al. (2014). The reaction mixture contained 6 mM $MgCl_2$, 50 mM NaCl, 5 mM KCl, 0.1 mM EDTA, and 30 mM Tris-HCl, pH 7.4. After 10 min of pre-incubation at 37 °C, the reaction was initiated by addition of ATP to a final concentration of 3 mM and incubated for 30 min. Control experiments were performed with the addition of 1 mM ouabain. The specific Na^+, K^+-ATPase activity was calculated by subtracting the ouabain-insensitive activity from the overall activity (in the absence of ouabain).

Total ATPase activity was assayed in an incubation medium comprising 30 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 3 mM ATP, and 50 μL of brain samples in a final volume of 300 μL. Controls to correct for non-enzymatic substrate hydrolysis were prepared by adding sample preparations after the reactions were stopped with TCA. To determine the Mg^{2+}-ATPase activity, ouabain (1 mM) was added to the reaction medium. The reaction was initiated by adding ATP and was stopped after 30 min of incubation by the addition of 10% TCA. The Ca^{2+}-ATPase activity was determined by subtracting the activity measured in the presence of Ca^{2+} from that was determined in the absence of Ca^{2+} (no added Ca^{2+} plus 0.1 mM EDTA). The enzyme activities were expressed in nmol of Pi/min/mg of protein.

Oxidative stress parameters

The hippocampus and cerebral cortex were homogenized in 10 volumes (1:10 *w*/*v*) of sodium phosphate buffer, pH 7.4 containing KCl. The homogenates were centrifuged at 3500 rpm for 10 min at 4 °C. The pellet was discarded, and the supernatant was used for the measurements. The samples were stored at − 80 °C until utilization. Protein content was determined using the method by Lowry et al. (1951) with bovine serum albumin as the standard solution.

Reactive oxygen species assay

Reactive oxygen species (ROS) formation was determined according to Ali et al. (1992). In this assay, the oxidation of DCFH-DA to DCF (fluorescent 2′,7′-dichlorofluorescein) was measured for the detection of intracellular reactive species. DCF fluorescence intensity emission was recorded at 525 and 488-nm excitation 30 min after the addition of DCFH-DA to the medium. The results were expressed as μmol DCF per mg of protein.

Nitrite levels

Nitrite content was measured using the Griess reaction as described by Huang et al. (2009). Briefly, 50 μL of sample plus 50 μL of sulfanilamide in 5% phosphoric acid was incubated for 10 min at room temperature. Next, 50 μL of *N*-(1-naphthyl) ethylenediamine dihydrochloride was added, and the mixture was incubated for 10 min at room temperature.

Absorbance at 540 nm was measured. A sodium nitrite solution was used as the reference standard, and the results were expressed as μmol NO_2/mg of protein.

Thiobarbituric acid reactive substance assay

Thiobarbituric acid reactive substance (TBARS) levels were determined as described by Esterbauer and Cheeseman (1990). The samples were mixed with 10% TCA and centrifuged. The supernatant was mixed with TBA (0.67%) and incubated in a dry block at 100 °C for 30 min. TBARS levels were determined by absorbance at 535 nm and reported as nmol of TBARS per mg protein.

Total sulfhydryl content assay

The total sulfhydryl content was determined according to Aksenov and Markesbery (2001). Samples were added to PBS buffer (pH 7.4) containing EDTA. The reaction was initiated by the addition of DTNB and incubated for 60 min in the dark. Reduction of DTNB by thiols generates a yellow derivative. The result is measured spectrophotometrically at 412 nm. The results were reported in nmol TNB/mg of protein.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was measured using the method described by Misra and Fridovich (1972). This assay is based on the inhibition of superoxide dependent adrenaline auto-oxidation to adenochrome. The intermediate in this reaction is superoxide, which is scavenged by SOD and is measured in a spectrophotometer adjusted at 480 nm. The specific activity of SOD was reported as units per mg of protein.

Catalase activity

Catalase (CAT) activity was evaluated as described by Aebi (1984). The decomposition of 30 mM H_2O_2 in 50 mM potassium phosphate buffer (pH 7.0) was continuously monitored with a spectrophotometer at 240 nm for 180 s at 37 °C. One unit of the enzyme is defined as the amount required to convert 1 nmol of hydrogen peroxide per minute. Specific CAT activity was reported as units/mg protein.

Glutathione peroxidase activity

For glutathione peroxidase (GPx) activity determination, a commercial kit (RANSEL; Randox Lab, Antrim, UK) was used. Absorbance was measured at 340 nm, and the GPx activity was reported as units per mg of protein.

Biochemical parameters

Serum glucose, uric acid, and urea levels were determined using commercially available diagnostic kits supplied by Labtest (Labtest, MG, Brazil).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for means comparison using GraphPad Prism version 5.0 program (Intuitive Software for Science, São Diego, CA, USA). Differences with $P \leq 0.05$ were considered statistically significant in the analysis. All data were expressed as mean ± standard error (SEM).

Results

Inosine prevents memory deficits induced by STZ

In the inhibitory avoidance task, the latency time in the STZ group was significantly decreased when compared with the control group ($F_{(3–35)} = 10.78$, $P < 0.01$) indicating memory impairment. Treatment with inosine 50 and 100 mg/kg increased the latency time when compared with that in the STZ group, suggesting that inosine could restore the memory deficits induced by this experimental model (Fig. 2). In Y-maze test, STZ also decreased both the time spent ($F_{(3–32)} = 6.12$, $P < 0.01$; Fig. 2a) and number of entries ($F_{(3–32)} = 7.36$, $P < 0.001$; Fig. 2b) into the novel arm. Inosine treatment in both doses (50 and 100 mg/kg) effectively attenuated the spatial memory deficits. As shown in Fig. 2, neither STZ nor STZ plus inosine treatment altered the locomotor activity in the open-field test ($F_{(3–36)} = 0.24$, $P > 0.05$) or the nociceptive responses on the tail flick test ($F_{(3–19)} = 2.40$, $P > 0.05$) excluding the possibility that the results of the inhibitory avoidance task or Y-maze test are associated with nociception or locomotor alterations.

Inosine restores the changes induced by STZ on the activities and expression of enzymes of the cholinergic system

In the cerebral cortex ($F_{(3–16)} = 9.69$, $P < 0.05$) and hippocampus ($F_{(3–16)} = 14.02$, $P < 0.05$), STZ caused an increase in the AChE activity when compared with the control group. Inosine treatment at both doses (50 mg/kg and 100 mg/kg) was capable of preventing this alteration in the hippocampus. However, in the cerebral cortex, only the dose of 100 mg/kg was effective against STZ-induced increase in the AChE activity (Fig. 3). Figure 4 shows that STZ induced a decrease in the AChE expression in the hippocampus and only treatment with

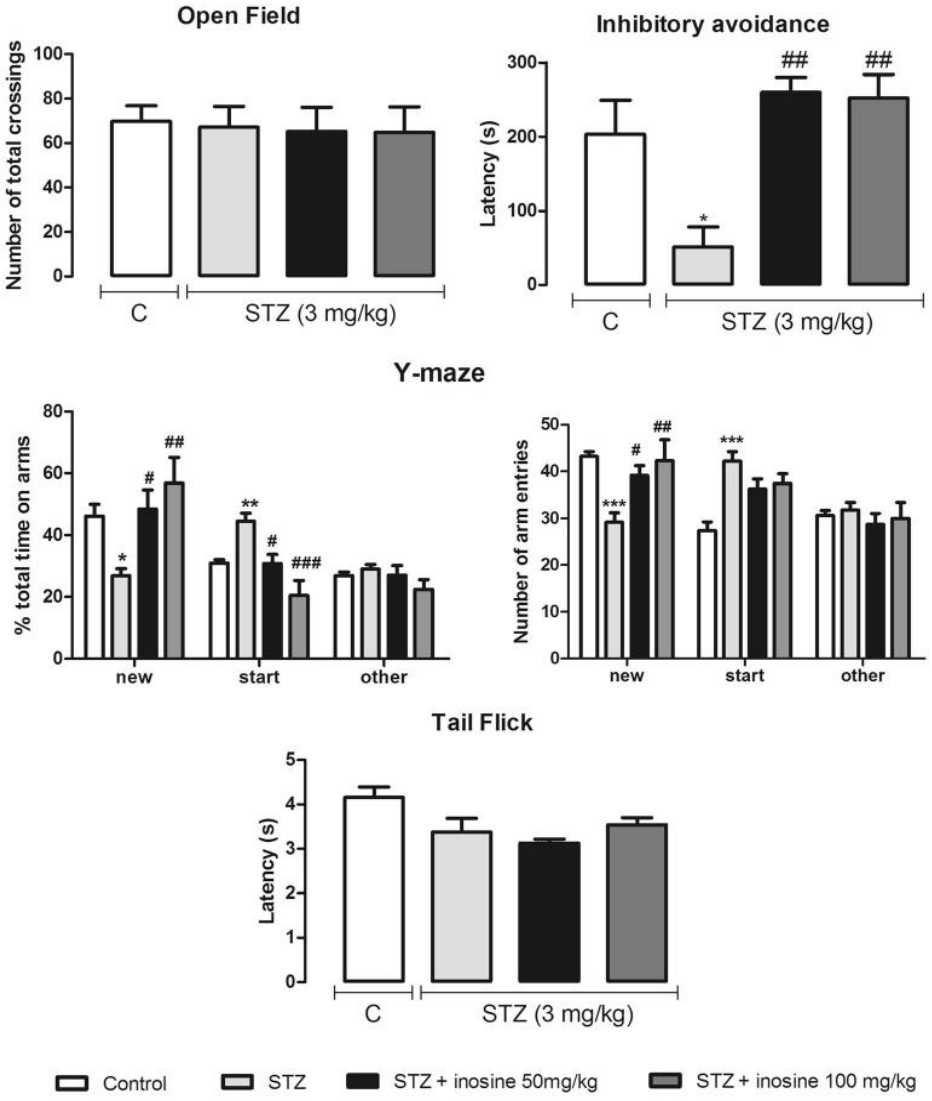


Fig. 2 Effects of treatment with inosine (50 or 100 mg/kg) on locomotor activity (evaluated by open-field task), memory (evaluated by inhibitory avoidance task and Y-maze test), and nociceptive response (evaluated by tail flick test) in rats subjected to intracerebroventricular injection with streptozotocin (STZ—3 mg/kg).). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ when compared with the control group and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ when compared with the STZ group ($n = 6$–10 animals per group)

50 mg/kg inosine could prevent this effect ($F_{(3–11)} = 105.0$, $P < 0.001$). In the cerebral cortex, a decrease in AChE expression was observed only in animals that received inosine (50 or 100 mg/kg) ($F_{(3–11)} = 20.38$, $P < 0.001$) (Fig. 4). STZ decreased ChAT expression in the hippocampus ($F_{(3–15)} = 31.20$, $P < 0.001$) and cerebral cortex ($F_{(3–15)} = 30.93$, $P < 0.001$) when compared with the control group. Inosine treatment prevented this alteration in both brain structures (Fig. 4).

Inosine restores ion pump activities

Total ATPase activity was significantly increased in the STZ group in both the cerebral cortex ($F_{(3–16)} = 14.00$, $P < 0.001$) and hippocampus ($F_{(3–16)} = 8.42$, $P < 0.05$) in relation to the control group. Treatment with inosine at both doses (50 and 100 mg/kg) prevented this alteration (Fig. 5). STZ also increased the Na^+, K^+-ATPase activity in cerebral cortex ($F_{(3–16)} = 11.59$, $P < 0.001$) and hippocampus ($F_{(3–16)} = 12.96$, $P < 0.001$) compared with the control group. Inosine could prevent this enzyme alteration (Fig. 5). On the other hand, Ca^{2+}-ATPase activity was decreased in the cerebral cortex ($F_{(3–16)} = 9.21$, $P < 0.001$) and hippocampus ($F_{(3–16)} = 8.16$, $P < 0.001$) of animals treated only with STZ, and inosine 100 mg/kg was capable of preventing this change only in the hippocampus. Finally, STZ also induced an increase in the Mg-ATPase activity in the cerebral cortex ($F_{(3–16)} = 21.32$, $P < 0.001$), which was prevented by inosine treatment (50 and 100 mg/kg). No changes were observed in the Mg-ATPase activity in the hippocampus in any experimental group evaluated ($F_{(3–16)} = 0.96$, $P = 0.4663$) (Fig. 5).

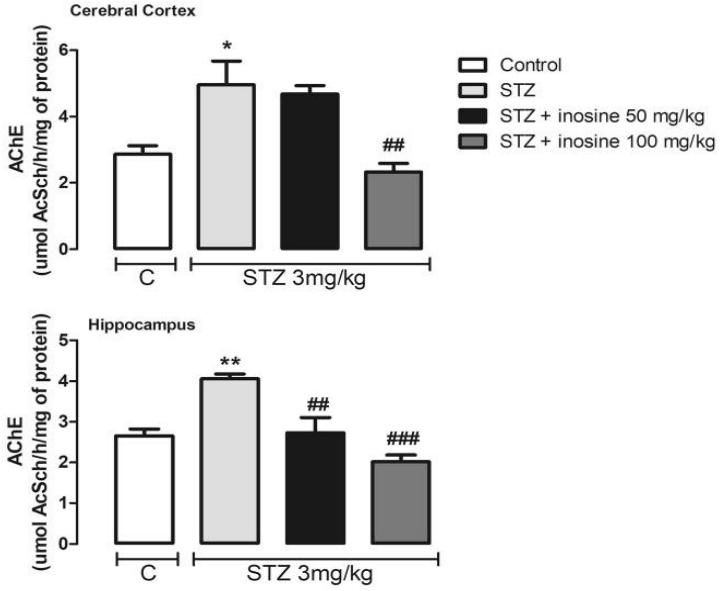


Fig. 3 Effect of inosine treatment (50 or 100 mg/kg) on acetylcholinesterase (AChE) activity of the cerebral cortex and hippocampus in rats subjected to intracerebroventricular injection with streptozotocin (STZ—3 mg/kg). $^{*}P < 0.05$ and $^{**}P < 0.01$ when compared with the control group. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ when compared with the STZ group ($n = 5$ animals per group)

Inosine prevents oxidative damage induced by STZ administration

The results of oxidative stress are showed in Figs. 6 and 7. ROS levels were increased in the cerebral cortex ($F_{(3–20)} = 8.39$, $P < 0.001$) and hippocampus ($F_{(3–16)} = 224.90$, $P < 0.001$) in the STZ group and inosine at both doses prevented this alteration. Inosine also prevented the STZ-induced increase in nitrite levels in the cerebral cortex ($F_{(3–18)} = 31.19$, $P < 0.001$) and hippocampus ($F_{(3–19)} = 19.61$, $P < 0.001$)

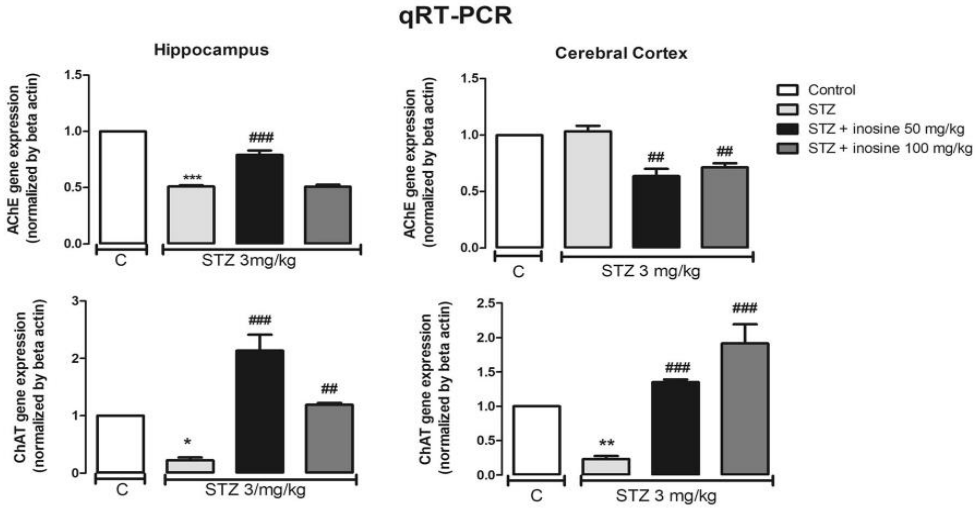


Fig. 4 Effect of inosine treatment (50 or 100 mg/kg) on the expression of acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) in the cerebral cortex and hippocampus of rats subjected to intracerebroventricular injection with streptozotocin (STZ—3 mg/kg). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ when compared with the control group. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ when compared with STZ group ($n = 3$–5 animals per group)

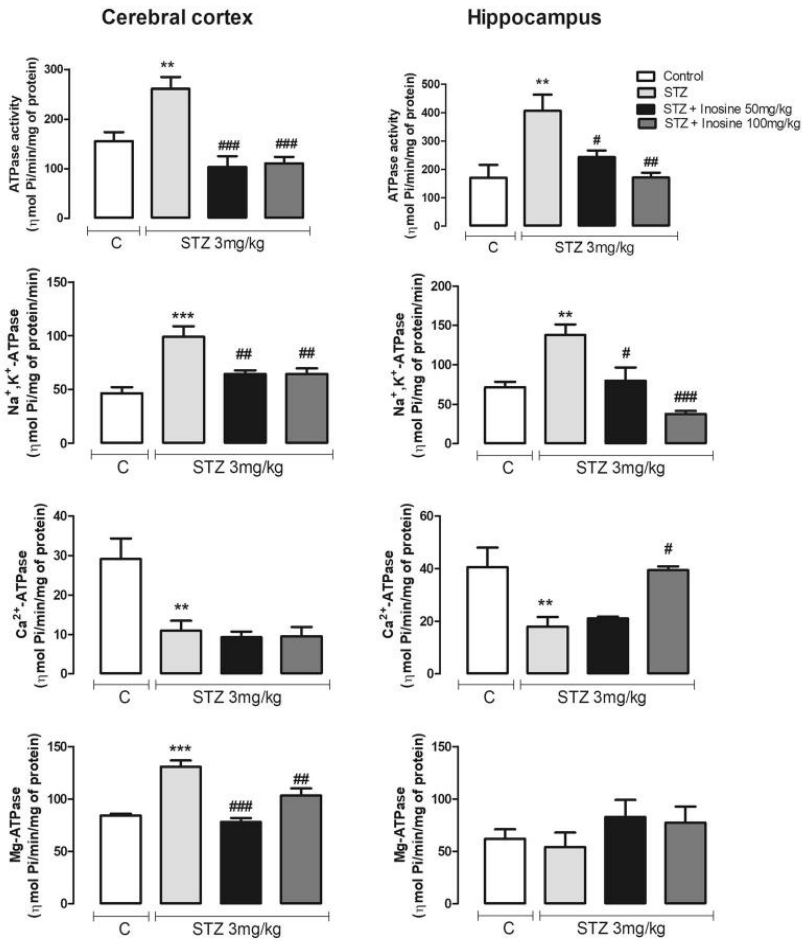


Fig. 5 Effect of inosine treatment (50 or 100 mg/kg) on total ATPase, Na^+, K^+-ATPase, Ca^{2+}-ATPase, and Mg-ATPase activities in the cerebral cortex and hippocampus of rats subjected to intracerebroventricular injection with streptozotocin (STZ—3 mg/kg). $^{**}P < 0.01$ and $^{***}P < 0.001$ when compared with the control group. $^{\#}P < 0.0\,5$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ when compared with the STZ group ($n = 5$ animals per group)

(Fig. 6). STZ administration increased the TBARS levels in the hippocampus ($F_{(3–16)} = 11.72$, $P < 0.001$) and cerebral cortex ($F_{(3–20)} = 11.60$, $P < 0.001$) when compared with the control group. Inosine was not able to protect against this increase in TBARS levels. No significant changes were observed in SH levels in the hippocampus ($F_{(3–20)} = 0.76$, $P = 0.76$) or cerebral cortex ($F_{(3–20)} = 3.24$, $P = 0.081$) in any experimental group evaluated.

In relation to antioxidant enzymes, the results showed an increase in SOD ($F_{(3–20)} = 20.14$) and a decrease in CAT ($F_{(3–20)} = 32.60$) and GPX ($F_{(3–20)} = 25.80$) activities in the hippocampus of rats that were treated only with STZ when compared with the control group ($P < 0.001$). These changes in the hippocampus were prevented by inosine treatment (Fig. 7). In the cerebral cortex, SOD activity was decreased only in the animals of the group STZ plus inosine (100 mg/kg) ($F_{(3–18)} = 3.70$, $P < 0.05$) when compared with other groups. STZ induced a decrease in CAT ($F_{(3–20)} = 15.90$) and GPX ($F_{(3–20)} = 7.94$) activities in the cerebral cortex compared with those in the control group ($P < 0.05$). Inosine at both doses prevented the alterations in CAT activity whereas only the dose of 50 mg/kg was effective at reverting the changes in GPX enzyme activity (Fig. 7).

Inosine increase the uric acid levels in serum

The levels of uric acid in the serum were decreased in animals that received STZ when compared with control group ($F_{(3–20)} = 44.94$, $P < 0.0001$). Treatment with inosine at 100 mg/kg could increase the levels of uric acid in serum (Fig. 8). No significant changes were observed in glucose and urea levels in any experimental group evaluated (Fig. 8).

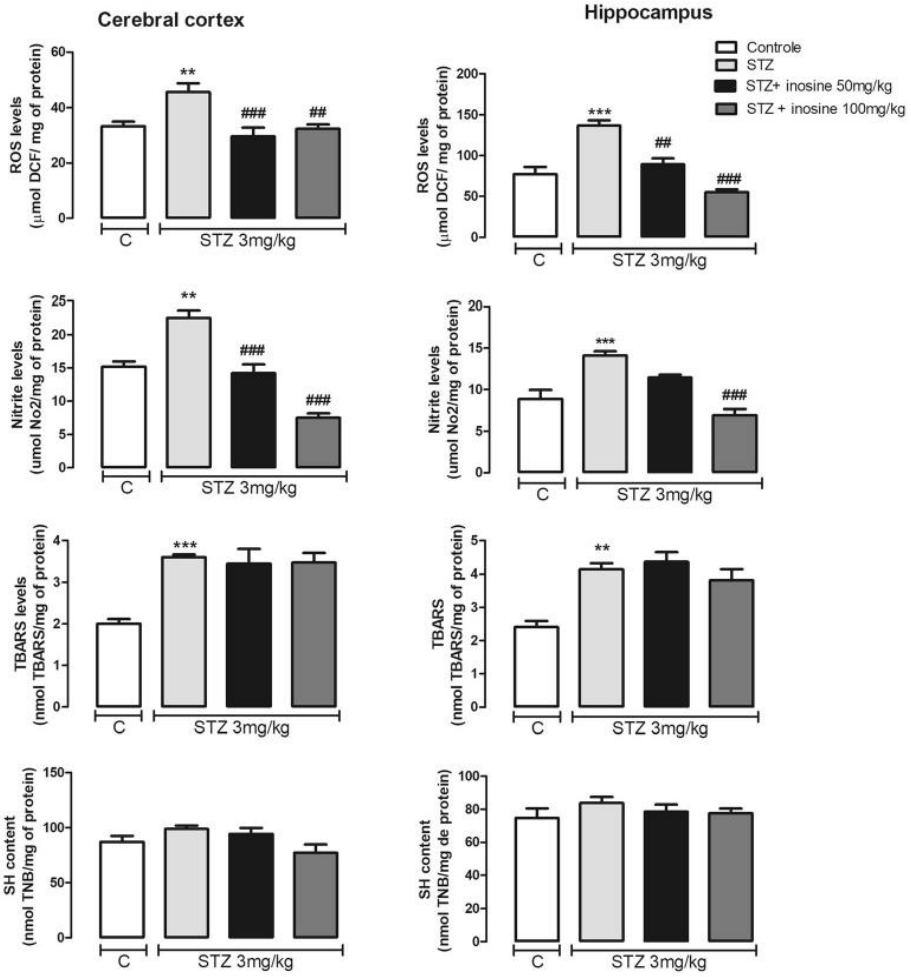


Fig. 6 Effect of inosine treatment (50 or 100 mg/kg) on reactive oxygen species (ROS), nitrite, reactive species of thiobarbituric acid (TBARS), and total sulfhydryl content (SH) levels in the cerebral cortex and hippocampus of rats subjected to intracerebroventricular injection with streptozotocin (STZ—3 mg/kg). $^{**}P < 0.01$ and $^{***}P < 0.001$ when compared with the control group. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ when compared with the STZ group ($n = 5$–6 animals per group)

Discussion

In the present study, we evaluated the potential effects of different doses of inosine in preventing the memory deficits and neurochemical alterations in an STZ-induced animal model of AD.

Our results showed that STZ induced memory impairment in rats corroborating the results of other studies (Gutierres et al. 2014). It is well established that administration of STZ in the brain generates alterations similar to those found in AD such as mitochondrial abnormalities, oxidative stress, neuronal cell damage, and learning and memory dysfunction (Grieb 2016; Gulyaeva et al. 2017). In addition, STZ alters the brain glucose metabolism and induces desensitization of cerebral insulin receptors (Grünblatt et al. 2007; Grieb 2016). Several abnormalities also were observed in the brain glucose metabolism of patients with AD suggesting that a substantial decline on energetic metabolism occurs before cognitive dysfunction becomes evident (Ibanez et al. 1998; Mosconi 2005). Thus, this model has been extensively used to investigate the therapeutic potential of compounds against the pathophysiological aspects of AD (Gutierres et al. 2014; Deshmukh et al. 2016; Pacheco et al. 2018; Martini et al. 2019).

Inosine at both doses used (50 and 100 mg/kg) was capable of preventing the memory deficits induced by STZ evaluated by inhibitory avoidance task and Y-maze test. The aversive memory deficit and spatial memory impairment can be associated with neurochemical changes observed in hippocampus and cerebral cortex. In fact, alterations in these brain structures have been well documented in both AD patients and experimental models (Dekosky and Scheff 1990; Yao et al. 2010; Gutierres et al. 2014; Pacheco et al. 2018).

The potential of inosine in improving memory has also been described in other experimental conditions such as the model of closed head injury (Dachir et al. 2014) and in older female rats (Ruhal and Dhingra 2018). Data from literature support that the beneficial effects of inosine in brain disorders can be associated to the activation of adenosine receptors or the production of its metabolite uric acid (Haskó et al. 2004; Welihinda et al. 2016; Doyle et al. 2018). Although we did not

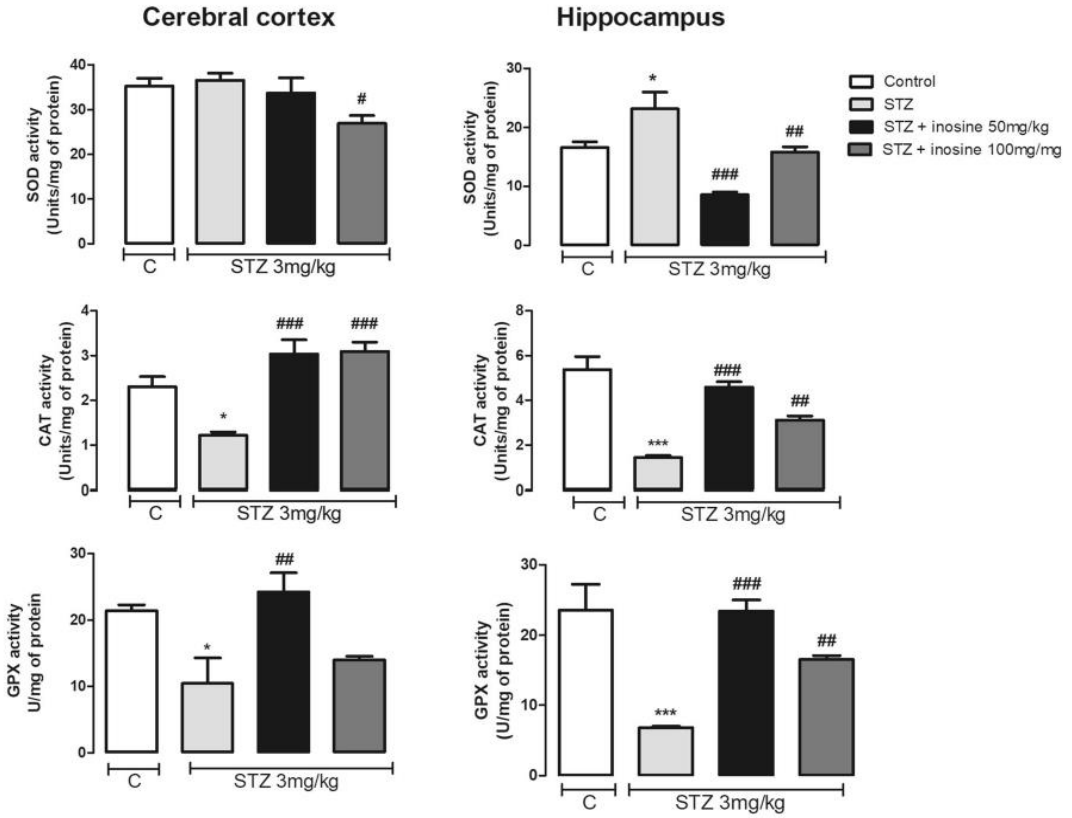


Fig. 7 Effect of inosine treatment (50 mg/kg or 100 mg/kg) on superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the hippocampus and cerebral cortex of rats subjected to intracerebroventricular injection with streptozotocin (STZ—3 mg/kg). $^{*}P < 0.05$ and $^{***}P < 0.001$ compared with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with the STZ group ($n = 5$–6 animals per group)

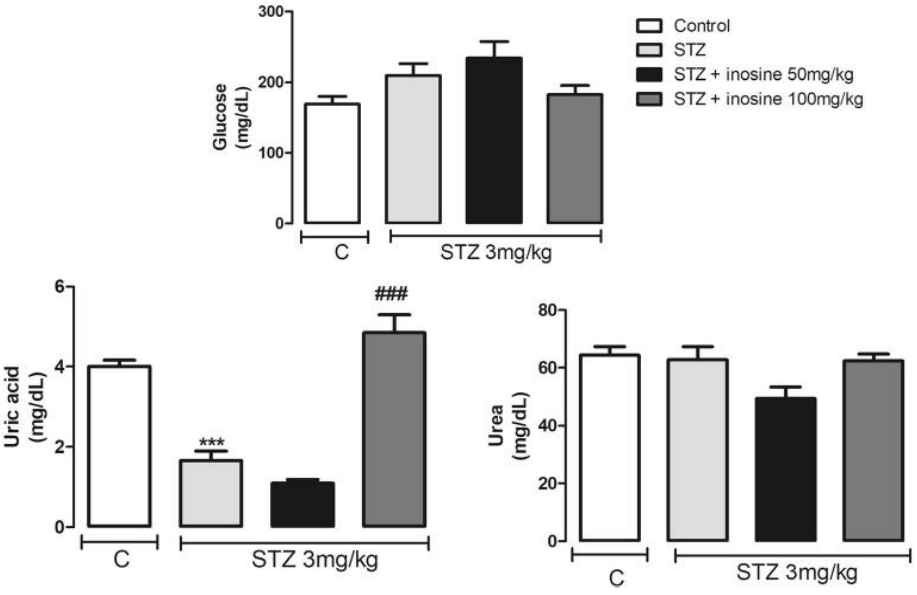


Fig. 8 Effect of inosine treatment (50 or 100 mg/kg) on glucose, uric acid, and urea levels in the serum of rats subjected to intracerebroventricular injection with streptozotocin (STZ—3 mg/kg). $^{***}P < 0.001$ compared with the control group. $^{\#\#\#}P < 0.001$ compared with the STZ group ($n = 5$–6 animals per group)

define the precise mechanism of action for inosine in memory improvement in the AD model, our results indicate that this compound modulates pathways crucial for cognitive functions such as cholinergic signaling, ion pump activities, and redox status.

The neurotransmitter acetylcholine is associated with cognitive, learning, and memory processes. Acetylcholine is synthesized by the enzyme ChAT from choline and acetyl-CoA and is posteriorly degraded by AChE at cholinergic synapses (Deiana et al. 2011). Changes in ChAT and AChE have been correlated with cognitive decline in patients and in experimental models of AD (Gutierres et al. 2014; Deshmukh et al. 2016; Sorial and Sayed 2017; Pacheco et al. 2018).

Our results suggest that the increase in AChE activity induced by STZ in the cerebral cortex and hippocampus could result in rapid acetylcholine degradation causing alterations in cholinergic signaling. In addition, a decrease in ChAT expression could lead to decreased synthesis of acetylcholine and low levels of this neurotransmitter in the brain. Besides, AChE also has effects on neurogenesis, cell adhesion, and synaptogenesis (Soreq and Seidman 2001); we cannot exclude that a decrease in AChE expression in the hippocampus by STZ can affect these crucial process in the brain, thus contributing to memory deficits observed in the STZ group.

Data from literature show that although inosine improved neuronal survival in the hippocampal CA1 region, this compound did not prevent the decrease in AChE activity in the hippocampus and frontal cortex of old female rats (Ruhal and Dhingra 2018). On the contrary, we observed that inosine prevented the alterations in AChE activity and the expression of cholinergic enzymes in an experimental model of AD. Thus, our findings suggest that memory improved by inosine can be explained, at least in part, by modulation of cholinergic signaling in this pathological condition.

Our results showed that in hippocampus, the lower dose of inosine used was more effective in increasing the AChE and ChAT expression. Although we cannot yet explain the exact mechanism responsible for this difference, it has been demonstrated that inosine is capable of sensitizing adenosine receptor A3 (Jin et al. 1997). Cinalli et al. (2013) demonstrated that the interaction of inosine with receptor A3 was able to modulate acetylcholine release at the neuromuscular junction. Thus, we can suggest that one of the mechanisms involved in the differences in relation to inosine dose and effects in brain structures may be associated with A3 receptors. In addition, in the rat brain, the hippocampus and the cerebellum show high levels of A3 mRNA while lower levels of these receptors were observed in the cortex and amygdala (Dixon et al. 1996).

Na^+, K^+-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase are enzymes responsible for generating and maintaining the intracellular ion gradients (Benarroch 2011). Na^+, K^+-ATPase maintains the Na^+ and K^+ gradients that are fundamental for neuronal excitability, membrane potential, and signal transduction (Aperia 2007; Benarroch 2011). In the present study, we observed an increase in the Na^+, K^+-ATPase activity in the cerebral cortex and hippocampus of rats that received only STZ. Previous studies from our laboratory also related an increase in total Na^+, K^+-ATPase activity and $\alpha1$ and $\alpha2/\alpha3$ isoforms in the brain after 28 days of STZ injection (Pacheco et al. 2018). Although the exact mechanism responsible for this increase has not been fully clarified, some studies have associated alterations in Na^+, K^+-ATPase activity with oxidative stress (Hitschke et al. 1994; Carvalho et al. 2015).

The Ca^{2+}-ATPase is essential for the control of calcium intracellular signaling thus contributing to modulate to a variety of functions such as cellular differentiation and neurotransmitter release (Mata and Sepulveda 2010). Our results suggest that inhibition of Ca^{2+}-ATPase by STZ administration could lead to dysregulation of cellular Ca^{2+} homeostasis affecting many intracellular signaling pathways. In fact, a decrease in Ca^{2+}-ATPase activity has been documented in the brain of AD patients (Mata et al. 2011). Further, we also showed an increase in Mg^{2+}-ATPase in the cerebral cortex suggesting that alterations in intracellular Mg^{2+} concentration also contribute to neurological and memory dysfunctions associated with AD. Of particular importance, Mg^{2+} is an important cofactor for Na^+, K^+-ATPase activity (Apell et al. 2017).

Inosine treatment was capable in regulating the ion pump activities in the brain structures of the rat AD model. Studies evaluating the effects of inosine in brain ATPase activities are scarce; however, Darlington and Gann (2005) related the potential of this compound in stimulating Na^+, K^+-ATPase activity to be independent of adenosine receptors. Considering that membrane-bound proteins are highly vulnerable to oxidative stress (Hitschke et al. 1994; Zaidi and Michaelis 1999), it is plausible to suggest that the antioxidant effect of inosine may be related to the modulation of ion pump activities as observed in our findings.

In fact, our results also demonstrated an increase in the markers of oxidative damage such as ROS, nitrite, and TBARS levels and a depletion in antioxidant enzyme activities such as catalase and GPX in both the hippocampus and cerebral cortex. It is well described in literature that intracerebroventricular administration of STZ induces oxidative stress in the brain (Huang et al. 2016; Gulyaeva et al. 2017; Pacheco et al. 2018). Abnormal production of reactive oxygen and nitrogen species might lead to oxidative modification of biomolecules and disruption of nerve terminal activity causing alterations and loss of synapses (Agostinho et al. 2010). These mechanisms may be correlated with the memory decline observed and the enzyme alterations in the present study.

Inosine treatment showed antioxidant action against STZ-induced oxidative stress. Inosine reduced the levels

of ROS and nitrite and increased the activity of CAT and GPx enzymes in the brain structures evaluated in the both doses used (50 and 100 mg/kg). Ruhal and Dhingra (2018) demonstrated that administration of inosine (100 and 200 mg/kg) for 15 days reduced lipid peroxidation and nitrite levels and increased the levels of glutathione reduction and superoxide dismutase activity in the hippocampus and frontal cortex of aged female rats. On the contrary of our results, Ruhal and Dhingra (2018) showed that the inosine (50 mg/kg) failed to prevent alterations in brain oxidative damage induced by age.

The antioxidant properties of inosine can be associated with its action as a scavenger of free radicals. Gudkov et al. (2006) reported that inosine could reduce the production of hydrogen peroxide and hydroxyl radicals and prevent radiation-induced oxidative DNA damage. Other possible mechanisms involved in the antioxidant effect of inosine may be associated with its metabolite, uric acid.

Uric acid is a natural antioxidant that may reduce oxidative stress through its actions as a scavenger of peroxynitrite and its derivatives (Squadrito et al. 2000; Glantzounis et al. 2005). The antioxidant effect of uric acid appears to be particularly important to prevent brain damage in neurodegenerative diseases (Rentzos et al. 2006; Andreadou et al. 2009; Koch and De Keyser 2006). Considering that inosine is a precursor of uric acid, we evaluated the levels of this molecule after inosine treatment. In fact, inosine at the dose of 100 mg/kg was effective in increasing the uric acid levels in the serum of rats suggesting that this mechanism might be involved in memory improvement. Corroborating with our hypothesis, Euser et al. (2009) showed that high levels of uric acid are associated with a decreased risk of dementia and better cognitive function later in life.

In conclusion, our findings showed that inosine ameliorates memory dysfunctions induced in an experimental model of AD. This effect can be attributed to the interaction of inosine with multiple molecular targets in brain regions involved with cognitive functions, such as cholinergic enzymes, ion pump activities, and redox status. However, more studies are required to evaluate inosine as a potential alternative for future AD therapeutic strategies against AD.

Acknowledgments This research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS). This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES)—Finance code 001. R.M.S. is recipient of CNPq fellowship (309299/2017-9).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Agostinho P, Cunha RA, Oliveira C (2010) Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. Curr Pharm Des 16:2766–2778

Aksenov MY, Markesbery WR (2001) Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci Lett 302:141–145

Ali SF, LeBel CP, Bondy SC (1992) Reactive oxygen species formation as a biomarker of methyl mercury and trimethyltin neurotoxicity. Neurotoxicol 13:637–648

Andreadou E, Nikolaou C, Gournaras F et al (2009) Serum uric acid levels in patients with Parkinson's disease: their relationship to treatment and disease duration. Clin Neurol Neurosurg 111:724–728

Apell HJ, Hitzler T, Schreiber G (2017) Modulation of the Na,K-ATPase by magnesium ions. Biochemistry 56:1005–1016

Aperia A (2007) New roles for an old enzyme: Na,K-ATPase emerges as an interesting drug target. J Intern Med 261:44–52

Assmann CE, Cadoná FC, Bonadiman BDSR, Dornelles EB, Trevisan G, Cruz IBMD (2018) Tea tree oil presents in vitro antitumor activity on breast cancer cells without cytotoxic effects on fibroblasts and on peripheral blood mononuclear cells. Biomed Pharmacother 103: 1253–1261

Benarroch E (2011) Na+, K+-ATPase: functions in the nervous system and involvement in neurologic disease. Neurology 76:287–293

Carvalho FB, Gutierres JM, Bohnert C, Zago AM, Abdalla FH, Vieira JM, Palma HE, Oliveira SM, Spanevello RM, Duarte MM, Lopes ST, Aiello G, Amaral MG, Pippi NL, Andrade CM (2015) Anthocyanins suppress the secretion of proinflammatory mediators and oxidative stress, and restore ion pump activities in demyelination. J Nutr Biochem 26:378–390

Chen YG (2018) Research progress in the pathogenesis of Alzheimer's disease. Chin Med J 131:1618–1624

Cinalli A, Guarracino J, Fernadez V et al (2013) Inosine induces presynaptic inhibition of acetylcholine release by activation of A3 adenosine receptors at the mouse neuromuscular junction. Br J Pharmacol 169:1810–1823

Dachir S, Shabashov D, Trembovler V, Alexandrovich AG, Benowitz LI, Shohami E (2014) Inosine improves functional recovery after experimental traumatic brain injury. Brain Res 1555:78–88

Darlington DN, Gann DS (2005) Purine nucleosides stimulate Na/K ATPase and prolong survival in hemorrhagic shock. J Trauma 58: 1055–1060

Deiana S, Platt B, Riedel G (2011) The cholinergic system and spatial learning. Behav Brain Res 10:389–411

Dekosky S, Scheff S (1990) Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurob 27:457–464

Dellu F, Fauchey V, LeMoal M, Simon H (1997) Extension of a new two-trial memory task in the rat: influence of environmental context on recognition processes. Neur Learn Memory 67:112–120

Deshmukh R, Kaudal M, Bansal V et al (2016) Caffeic acid attenuates oxidative stress, learning and memory deficit in intracerebroventricular streptozotocin induced experimental dementia in rats. Biomed Pharmacother 81:56–62

Dixon A, Gubitz A, Sirinathsinghji D et al (1996) Tissue distribution of adenosine receptor mRNAs in the rat. Br J Pharmacol 118:14–16

Doyle C, Cristofaro V, Sullivan MP, Adam RM (2018) Inosine-a multifunctional treatment for complications of neurologic injury. Cell Physiol Biochem 49:2293–2303

Ellman GL, Courtney KD, Andres V et al (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Esterbauer H, Cheeseman KH (1990) Determination of aldehydic lipid peroxidation. Methods Enzymol 186:407–421
Euser SM, Hofman A, Westendorp RG, Breteler MM (2009) Serum uric acid and cognitive function and dementia. Brain 132:377–382
Gamaro GD, Suyenaga E, Borsoi M et al (2011) Effect of rosmarinic and caffeic acids on inflammatory and nociception process in rats. Pharmacol 451682
Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA (2005) Uric acid and oxidative stress. Curr Pharm Des 11:4145–4151
Grieb P (2016) Intracerebroventricular streptozotocin injections as a model of Alzheimer's disease: in search of a relevant mechanism. Mol Neurobiol 53:1741–1752
Grünblatt E, Salkovic-Petrisic M, Osmanovic J, Riederer P, Hoyer S (2007) Brain insulin system dysfunction in streptozotocin intracerebroventricularly treated rats generates hyperphosphorylated tau protein. J Neurochem 101:757–770
Gudkov SV, Shtarkman IN, Smirnova VS, Chernikov AV, Bruskov VI (2006) Guanosine and inosine as natural antioxidants and radioprotectors for mice exposed to lethal doses of γ-radiation. Dokl Biochem Biophys 407:47–50
Gulyaeva NV, Bobkova NV, Kolosova NG et al (2017) Molecular and cellular mechanisms of sporadic Alzheimer's disease: studies on rodent models in vivo. Biochem 82:1088–1102
Gumusyayla S, Vural G, Bektas H et al (2016) A novel oxidative stress marker in patients with Alzheimer's disease: dynamic thiol-disulphide homeostasis. Acta Neuropsychiatr 28:315–320
Gutierres JM, Carvalho FB, Schetinger MRC, Marisco P, Agostinho P, Rodrigues M, Rubin MA, Schmatz R, da Silva CR, de P Cognato G, Farias JG, Signor C, Morsch VM, Mazzanti CM, Bogo M, Bonan CD, Spanevello R (2014) Anthocyanins restore behavioral and biochemical changes caused by streptozotocin-induced sporadic dementia of Alzheimer's type. Life Sci 96:7–17
Haskó G, Sitkovsky MV, Szabó C (2004) Immunomodulatory and neuroprotective effects of inosine. Trends Pharmacol Sci 25:152–157
Herman JP, Watson SJ (1987) The rat brain in stereotaxic coordinates (2nd edn) by George Paxinos and Charles Watson. Trends Neurosci 10:439–439
Hitschke K, Btihler R, Apell G (1994) Stark inactivation of the Na, K-ATPase by radiation-induced free. Evidence for a radical-chain mechanism. FEBS Lett 353:297–300
Huang WC, Lin YS, Wang CY et al (2009) Glycogen synthase kinase-3 negatively regulates anti-inflammatory interleukin-10 for lipopolysaccharide-induced iNOS/NO biosynthesis and RANTES production in microglial cells. Immunol 128:275–286
Huang WJ, Zhang X, Chen WW (2016) Role of oxidative stress in Alzheimer's disease (review). Biomed Rep 4:519–522
Ibanez V, Pietrini P, Alexander G et al (1998) Regional glucose metabolismo absnormalities are not the result of atrophy in Alzheimer's disease. Neurology 50:1585–1593
Jeong S (2017) Molecular and cellular basis of neurodegeneration in Alzheimer's. Dis Mol Cells 40:613–620
Jin X, Shephered R, Duling B et al (1997) Inosine binds to A3 adenosine receptors and stimulates mast cell degranulation. J Clin Invest 100: 2849–2857
Koch M, De Keyser J (2006) Uric acid in multiple sclerosis. Neurol Res 28:316–319
Kuricova M, Ledecky V, Liptak T, Madari A, Grulova I, Slovinska L, Nagyova M, Cizkova D (2014) Oral administration of inosine promotes recovery after experimental spinal cord injury in rat. Neurol Sci 35:1785–1791
Lores Arnaiz GR, Ordieres MG (2014) Brain Na(+), K(+)-ATPase activity in aging and disease. Int J Biomed Sci 10:85–102
Lowry O, Rosebrough N, Farr A (1951) Protein measurement with the folin phenol reagent. Anal Biochem 217:220–230
Mabley JG, Pacher P, Murthy KGK, Williams W, Southan GJ, Salzman AL, Szabo C (2009) The novel inosine analogue INO-2002 exerts an anti-inflammatory effect in a murine model of acute lung injury. Shock 32:258–262
Markowitz CE, Spitsin S, Zimmerman V, Jacobs D, Udupa JK, Hooper DC, Koprowski H (2009) The treatment of multiple sclerosis with inosine. J Altern Complement Med 15:619–625
Martini F, Rosa SG, Klann IP, Fulco BCW, Carvalho FB, Rahmeier FL, Fernandes MC, Nogueira CW (2019) A multifunctional compound ebselen reverses memory impairment, apoptosis and oxidative stress in a mouse model of sporadic Alzheimer's disease. J Psychiatr Res 109:107–117
Mata A, Sepulveda R (2010) Plasma membrane Ca^{2+}-ATPases in the nervous system during development and ageing. World J Biol Chem 26:229–234
Mata A, Berrocal M, Sepulveda M (2011) Impairment of the activity of the plasma membrane Ca^{2+}-ATPase in Alzheimer's disease. Send to Biochem Soc Trans 39:819–822
Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175
Mosconi L (2005) Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. Eur J Nucl Med Mol Imaging 32:486–510
Muto J, Lee H, Lee H et al (2015) Oral administration of inosine produces antidepressant-like effects in mice. Sci Rep 4:4199
Pacheco SM, Soares MSP, Gutierres JM, Gerzson MFB, Carvalho FB, Azambuja JH, Schetinger MRC, Stefanello FM, Spanevello RM (2018) Anthocyanins as a potential pharmacological agent to manage memory deficit, oxidative stress and alterations in ion pump activity induced by experimental sporadic dementia of Alzheimer's type. J Nutr Biochem 56:193–204
Rentzos M, Nikolaou C, Anagnostouli M et al (2006) Serum uric acid and multiple sclerosis. Clin Neurol Neurosurg 108:527–531
Ruhal P, Dhingra D (2018) Inosine improves cognitive function and decreases aging-induced oxidative stress and neuroinflammation in aged female rats. Inflammopharmacol 26:1317–1329
Sonntag KC, Ryu WI, Amirault KM et al (2017) Late-onset Alzheimer's disease is associated with inherent changes in bioenergetics profiles. Sci Rep 7:1–13
Soreq H, Seidman S (2001) Acetylcholinesterase: new roles for an old actor. Nat Rev Neurosci 2:294–302
Sorial ME, Sayed NSED (2017) Protective effect of valproic acid in streptozotocin-induced sporadic Alzheimer's disease mouse model: possible involvement of the cholinergic system. Naunyn Schmiedeberg's Arch Pharmacol 390:581–593
Squadrito GL, Cueto R, Splenser AE, Valavanidis A, Zhang H, Uppu RM, Pryor WA (2000) Reaction of uric acid with peroxynitrite and implications for the mechanism of neuroprotection by uric acid. Arch Biochem Biophys 376:333–337
Welihinda AA, Kaur M, Greene K, Zhai Y, Amento EP (2016) The adenosine metabolite inosine is a functional agonist of the adenosine A2A receptor with a unique signaling bias. Cell Signal 28:552–560
Yao Z, Zhang Y, Lin L et al (2010) Abnormal cortical networks in mild cognitive impairment and Alzheimer's disease. PLoS Comput Biol 18:e1001006
Zaidi A, Michaelis ML (1999) Effects of reactive oxygen species on brain synaptic plasma membrane Ca(2+)-ATPase. Free Radic Biol Med 27:810–821
Zhang LN, Sun YJ, Pan S et al (2013) Na+-K+-ATPase, a potent neuroprotective modulator against Alzheimer disease. Fundam Clin Pharmacol 27:96–103

4.3 Manuscrito 2

Effects of inosine on purinergic receptors, and neurotrophic and neuroinflammatory parameters in an experimental model of Alzheimer's disease

Status: em fase de submissão

Effects of inosine on purinergic receptors, and neurotrophic and neuroinflammatory parameters in an experimental model of Alzheimer's disease

Fernanda Cardoso **Teixeira**[1], Bruna da Siveira de **Mattos**[1], Mayara Sandrielly Pereira **Soares**[1], Eduardo **Blodorn**[4], Karine Paula **Reichert**[5], Fabiano **Carvalho**[2], Marilda **Cruz**[2] Jessie M. **Gutierrez**[2], Relber Aguiar **Gonçales**[3], Vinicius Campos **Farias**[4], Maria Rosa **Chitolina Schetinger**[5], Francieli Moro **Stefanello**[1], Roselia Maria **Spanevello**[1*]

[1] Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Campus Universitário S/N, Pelotas, RS, Brazil.

[2] Programa de Pós-Graduação em Patologia - Laboratório de Pesquisa em Patologia, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil.

[3] Laboratório de Biologia Molecular, Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal de Goiás, Goiânia, GO, Brasil.

[4] Laboratório de Genômica Estrutural, Programa de Pós-Graduação em Bioquímica e Bioprospecção, Universidade Federal de Pelotas, Campus Universitário S/N, Pelotas, RS, Brazil.

[5]Laboratório de Enzimologia Toxicológica, Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, Universidade Federal de Santa Maria, RS, Brazil.

***** Corresponding author:

Roselia Maria Spanevello: Programa de Pós-Graduação em Bioquímica e Bioprospecção, Laboratório de Neuroquímica, Inflamação e Câncer, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Campus Universitário, Capão do Leão, 96010-900 Pelotas, RS, Brazil

Phone: +55 53 39217355 E-mail: rspanevello@gmail.com

Abstract

Alzheimer's disease (AD) is a neurodegenerative pathology characterized by progressive impairment of memory, associated with neurochemical alterations and limited therapy. The aim of this study was to evaluate the effects of inosine on memory, neuroinflammatory parameters, neurotrophic factors, expression of purinergic receptors, and morphological changes in the hippocampus and cerebral cortex of rat models of AD induced by streptozotocin (STZ). Male rats were divided into four groups: I - control, II - STZ, III - STZ plus inosine (50 mg/kg), and IV - STZ plus inosine (100 mg/kg). The animals received intracerebroventricular injections of STZ or saline. Three days after the surgical procedure, animals were treated with inosine (50 mg/kg or 100 mg/kg) for 25 days. Inosine was able to prevent memory deficits and decrease the immunoreactivity of the brain A2A adenosine receptor induced by STZ. Inosine also increased the levels of brain anti-inflammatory cytokines (IL-4 and IL-10), the expression of BDNF and its receptors, and prevented the increase in cholesterol levels and serum butyrilcholinesterase alterations induced by STZ. The morphological changes in the molecular layer of the hippocampus were attenuated by treatment with inosine 50 and 100 mg/kg in rats exposed to STZ. However, inosine was not capable of preventing the increase in GFAP in animals exposed to STZ. In conclusion, our findings suggest that inosine has therapeutic potential for AD treatment through the modulation of different brain mechanisms involved in neuroprotection.

Keywords: inosine, hippocampus, memory, purinergic receptor, BDNF

1. Introduction

Alzheimer's disease (AD) is characterized by progressive impairment of memory and other cognitive skills. It is a neurodegenerative disease that predominantly affects elderly people, with advanced age as the main risk factor; therefore, AD is a public health problem due to life expectancy [1–3]. Several mechanisms have been proposed to explain AD pathogenesis. Evidences have demonstrated that oxidative stress, dysfunction in cholesterol metabolism, neuroinflammation, low levels of brain-derived neurotrophic factor (BDNF), and alterations in cholinergic and purinergic signaling contribute to neurodegeneration and cognitive deficits [4–11].

BDNF and its high affinity to tyrosine kinase receptors (TrkB) play an important role in neuronal survival, cell differentiation, synaptic plasticity, and neuronal maintenance [11–13]. BDNF is a neurotrophin with the ability to increase long-term potentiation (LTP) in the hippocampal CA1 region, which is directly associated with memory [14–16]. In fact, hippocampal and cortical *post-mortem* samples of AD patients revealed a decrease in both BDNF and TrkB levels, indicating that deficits in BDNF signaling contribute to neuronal damage in this pathological condition [17–21].

AD is also associated with chronic inflammation in the central nervous system (CNS), characterized by an increase in the production of cytokines, chemokines, inflammatory factors as well as infiltration of immune cells followed by neurodegeneration [2, 22]. The activation of microglia and astrocytes, accompanied by an increase in the production of pro-inflammatory cytokines, demonstrates a causal relationship between pro-inflammatory cytokine production and cognitive dysfunction [22]. Thus, astrogliosis and neuroinflammation appear to be integral components of AD onset and progression, which together with cholinergic neuronal loss are common findings in brain analyses of post-mortem patients [2,7,23].

The participation of adenosine receptors in cognitive processes has been recognized over the years. Adenosine receptors such as A1 and A2A are located mainly at synapses and mediate the physiological actions of adenosine. The brain density of these receptors is affected by AD [24–27]. A1 loss appears to be among the factors related to cell death in the hippocampus, while an increase in A2A expression has been associated with neurodegenerative processes and memory deficits [28, 29]. On this note, the pharmacological modulation of adenosine receptors is an important target to be explored with the aim of preventing the action of signaling pathways involved in neurodegeneration in AD [27,28].

Inosine, an endogenous purine nucleoside, is formed by deamination of adenosine by the enzyme adenosine deaminase and has been shown to have neuroprotective, anti-inflammatory, and antioxidant properties [30,31]. Studies have suggested that the biological actions of inosine may be mediated through adenosine receptors or by the production of uric acid, an important peroxynitrite scavenger. Therefore, the aim of this study was to evaluate the effects of inosine on short-term memory, neuroinflammatory parameters, BDNF signaling, and expression of purinergic receptors in the hippocampus and cerebral cortex of
rat models of AD induced by streptozotocin (STZ).

2. Material and Methods

2.1 Chemicals

Inosine, STZ, Coomassie Brilliant Blue G, sodium citrate, butyrylcholine, hydrochloric acid, RIPA buffer, protease, and phosphatase inhibitors were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used in the detailed experiments were of analytical grade and the highest purity. TRIzol reagent and DNase I Amplification Grade were purchased from Invitrogen™ (Carlsbad, USA).

2.2 Animals

Adult male Wistar rats (60 days, 300–350 g) were provided by the Central Animal House of the Federal University of Pelotas. The animals were kept in cages under standard temperature (23 ± 1 °C), relative humidity (45–55%), and lighting conditions (12 h light/dark cycle), and free access to standard rodent pelleted diet and water *ad libitum*. The Committee of Ethics and Animal Experimentation of the Federal University of Pelotas, Brazil, under protocol number CEEA 4808–2017, approved all animal procedures. The use of animals was in accordance with the Brazilian Guidelines for the Care and Use of Animals in Scientific Research Activities (DBCA), which is in agreement with the National Council of Control of Animal Experimentation (CONCEA).

2.2 Intracerebroventricular (icv) injection of streptozotocin (STZ)

The animals were anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg) for all surgical procedures. The head was placed in position in the stereotaxic apparatus, and a midline sagittal incision was made in the scalp in each animal. The stereotaxic coordinates for the lateral ventricle were measured accurately as anterio-

posterior −0.8 mm, lateral 1.5 mm, and dorso-ventral −4.0 mm, relative to the bregma and ventral from the dura with the tooth bar set at 0 mm [9, 32]. Through a skull hole, the piston of a 28-gauge Hamilton® syringe of 10 µL attached to a stereotaxic apparatus was lowered manually into each lateral ventricle. The STZ groups received bilateral intracerebroventricular (ICV) injection of STZ (3 mg/kg, body weight) dissolved in citrate buffer (pH 4.4). The concentration was adjusted to deliver a 5 µL/injection at the site. Animals in the control group received ICV injection of the same volume of citrate buffer.

2.4 Inosine Treatment

The animals were divided into four experimental groups (n = 10 each): I – Control (C), II – STZ, III – STZ + inosine 50 mg/kg (STZ + Ino 50), and IV - STZ + inosine 100 mg/kg (STZ + Ino 100). The animals ingroups II, III, and IV received bilateral ICV injection of STZ while animals in group I received only citrate buffer. Three days after the surgical procedure, the animals in groups III and IV were treated with inosine (50 mg/kg or 100 mg/kg) and those in groups I and II received saline intraperitoneally (i.p.), as shown in figure 1. Inosine was dissolved in saline solution and administered for 25 days. The weight of the animals was evaluated weekly during the experimental period. Inosine dose was chosen based on previous studies indicating neuroprotection [33, 34].

2.5 Behavioral procedure

2.5.1 Open-field test

Locomotor behavior was evaluated using an open-field apparatus after 27 days of STZ injection. The open-field test was performed in an apparatus consisting of a box with the floor of the arena divided into 16 equal squares (18 × 18 cm), placed in a sound-free room. The number of quadrants crossed over a period of 5 min was the parameter used to evaluate locomotor activity. This test was carried out to identify motor disabilities, which might influence memory behavioral tests. The apparatus was cleaned with 40% ethanol and dried after each individual animal session [9].

2.5.2 Object Recognition

Twenty-four hours after the open-field test, which was also used as habituation to the apparatus, the animals underwent an object recognition test to evaluate short-term memory. The task was performed on the 28th day after STZ injection. The

animals were placed individually in a box with two identical objects (objects A and B) for 5 min for freely exploration (training). After 2 h, the animals were put back in the box for 5 min, and one of the previous objects (B) was replaced (object C). The time spent exploring the new and familiar objects was recorded. The results were calculated according to the recognition index = TC / (TA + TC) [9]. After this test, the animals were euthanized and each animal's brain and blood were collected for analysis. The brain structure samples were prepared and protein determination was performed according to each specific technique.

2.6 Western blot analysis of A1, A2A and P2X7 immunoreactivity

The A1R, A2AR, and P2X7R receptor immunoreactivity in the hippocampal and cortical membranes were evaluated by western blot analysis, as previously described by Rebola et al. (2005) [24]. Samples of the hippocampus and cerebral cortex were homogenized in ice-cold radioimmunoprecipitation assay buffer (RIPA buffer) with 1mM protease and phosphatase inhibitors and centrifuged at 12,000 rpm at 4°C for 10 min. The protein concentration was determined using a BCA Protein Assay Kit (Sigma-Aldrich, EUA). The diluted samples were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Amersham Biosciences, UK). After blocking, the membranes samples were incubated overnight at 4°C with primary antibodies; A1 (dilution 1:800, Santa Cruz Biotechnology, CA, USA), A2A (dilution 1:800, Santa Cruz Biotechnology, CA, USA), and P2X7 (dilution 1:800, Santa Cruz Biotechnology, CA, USA) membranes were incubated with anti-rabbit or anti-mouse secondary antibodies (dilution 1:10.000, Santa Cruz Biotechnology, CA, USA) for 90 min at room temperature. The membranes were incubated with an enhanced chemifluorescent substrate (Amersham Biosciences) and analyzed with Amersham Imager 600 (GE Healthcare Life Sciences, EUA). The membranes were reprobed and tested for β-actin immunoreactivity, as a control for protein concentration.

2.7 RNA extraction, cDNA synthesis, and quantitative real-time polymerase chain reaction of BDNF and tyrosine receptor kinase B (TrkB)

Total mRNA was extracted from 50 to 100 mg of the hippocampus and cerebral cortex tissue, using TRIzol reagent followed by DNase treatment with DNase I Amplification Grade, to ensure minimum DNA contamination of the samples. The total

RNA isolated was quantified, and its purity (260/280 and 260/230 ratios) was examined using a NanoVue spectrophotometer (GE, Fairfield, CT, USA).

cDNA synthesis was performed using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, UK) according to the manufacturer's protocol. For reverse transcription, 1 µg of total RNA was used in a reaction volume of 20 µL. The amplification was performed with GoTaq® qPCR Master Mix (Promega, Madison, WI) using CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories Inc., CA, USA). The sequences of the primers used are listed in Table 1. The qPCR conditions were as follows: 10 min at 95°C to activate the hot-start Taq polymerase, followed by 35 cycles of denaturation for 15 s at 95°C, primer annealing for 60 s at 60°C, and extension for 30 s at 72°C (fluorescence signals were detected at the end of every cycle). Baseline and threshold values were automatically set using the Bio-Rad CFX Manager software.

The number of PCR cycles required to reach the fluorescence threshold in each sample was defined as the Ct value, and each sample was analyzed in duplicate to obtain an average Ct. The 2-ΔΔCT method was used to normalize the fold change in gene expression, using β-actin as a housekeeping gene.

2.8 Quantification of cytokines in brain

Hippocampus and cerebral cortex tissues were homogenized in 10 mM Tris–HCl buffer (pH 7.4) on ice using a homogenizer. The homogenate was centrifuged at 14000×g for 30 min, and the supernatants were used for the analysis. The cytokines were detected by an enzyme-linked immune sorbent assay (ELISA) using OptEIA kit (Pharmingen, San Diego, CA, USA), according to the manufacturer's instructions. Standard curves allowed determination of cytokine concentrations in pg/mL. The absorbance was read at 450 nm using a Power Wave X microplate scanning spectrophotometer (BioTek Instruments, Inc.).

2.9 Butirilcholinesterase activity in serum (BuChE)

BuChE activity was determined using a modification of the method of Ellman and col (1961) and was expressed in µmol BuSCh/h/mg of protein [35]. The method is based on the formation of 5,5-dithiobis-acid nitrobenzoic measured at 412 nm, and the reaction was initiated by adding 0.8 mM butyrylthiocholine iodide (BuSCh).

2.10 Total cholesterol level

Serum cholesterol levels were determined using commercially available diagnostic kits supplied by Labtest® (Labtest, MG, Brazil).

2.11 Morphological parameters

2.11.1 Hematoxylin and eosin (HE)

The specimens were fixed in 10% buffered formalin, processed, and included in paraffin, and subjected to histological cut in a microtome set to a thickness of 4 µm. The cuts placed on matte sheets were heated in an oven at 80 °C for 1 h, deparaffinized in xylol, rehydrated in ethyl alcohol staggered, and washed in distilled water. The slides were placed in Harris hematoxylin dye for 5 min, washed in running water, differentiated in acid-alcohol for 1 min, washed again in running water, and dipped in 1% lithium carbonate. Subsequently, the slides were placed in eosin dye for 3 min, dehydrated in absolute alcohol, and placed in xylol for assembly with Entellan type resin.

2.11.2 Immunohistochemistry for Glial Fibrillary Acid Protein (GFAP)

The brains were fixed in 10% buffered formalin, processed, and included in paraffin, and subjected to histological cut in a microtome regulated to a thickness of 3 µm and placed on slides. The slides were heated in an oven at 75 °C for 2 h, deparaffinized in xylol, and rehydrated in ethyl alcohol and then in distilled water for 5 min in PBS. Antigenic recovery was performed in a water bath for 20 min at 94 °C in citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with a 5% solution of hydrogen peroxide in methanol for 20 min in the dark. Protein blocking was performed with skimmed-milk powder diluted to 5% in PBS for 20 min. The cuts were incubated overnight in a refrigerator at 2–8 °C, with the primary anti-GFAP antibody in 1:200 dilution. After incubation, the secondary IgGκ light chain HRP antibody conjugated at 1:200 dilution was applied and incubated for 1 h and 30 min at room temperature, and the reaction was visualized with Liquid Dab (Dako, K3468) according to the manufacturer's recommendations. After visualization, the slides were counterstained in Harris' hematoxylin for 20 seconds and differentiated in 2% ammoniacal water for 20 seconds. The cuts were dehydrated in absolute alcohol and placed in xylol for the assembly of the slides in Entellan-type resin.

The quantification of GFAP was performed through optical density (OD) analysis using an Olympus® BX 257 40 microscope coupled to a computer with the software

Image Pro Plus® 6.3 (Media 258 Cybernetics). Images of each region were captured per section.

2.12 Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for means comparison using GraphPad Prism version 5.0 Program (Intuitive Software for Science, San Diego, CA, USA). $P \leq 0.05$ was considered statistically significant in the analysis. All data are expressed as mean ± standard error (SEM).

3. Results

3.1 Effects in body weight of animals

As can be seen in Figure 1, the animals treated only with STZ showed a reduction in body weight in relation to the control group during all experimental periods ($P<0.001$). However, only inosine (100 mg/kg) was capable of increasing the body weight of the animals when compared to the STZ group.

3.2 Inosine prevents memory deficits induced by STZ

In the object recognition task, the percentage of exploratory preference of the new object of the STZ group was significantly lower than that of the control group ($P< 0.01$), indicating memory impairment. Treatment with inosine 100 mg/kg increased the percentage of exploratory preference of the new object when compared with the STZ group, demonstrating that inosine is capable of restoring memory deficits induced by this experimental model (Figure 3). As shown in Figure 3, neither STZ nor STZ plus inosine treatment altered locomotor activity in the open field test.

3.3 Inosine modulated the brain purinergic receptors

Concerning the density of receptors in the hippocampus, our results showed an increase in immunoreactivity of A1 and A2A in the STZ group ($P<0.05$). Inosine (100 mg/kg) increased the levels of A1, and both inosine concentrations were effective in decreasing the immunoreactivity of the A2A receptor when compared to the STZ group (Figure 4). In the cerebral cortex, only an increase in A2A density was observed in the STZ group ($P<0.05$), which was prevented by inosine (50 mg/kg). Inosine (100 mg/kg) also increased the levels of A1 in the cerebral cortex of the rats ($P<0.05$) (Figure 4).

3.4 Inosine modulates expression of BDNF and TrkB receptor

In the hippocampus of the STZ group, a significant reduction in TrkB levels was observed ($P<0.05$), while BDNF levels only demonstrated a tendency to decrease. Interestingly, inosine at both doses was capable of increasing the mRNA levels of TrkB and BDNF in the hippocampus (Figure 5). No changes were observed in the mRNA levels of receptor TrkB or BDNF in the cerebral cortex (Figure 5). Surprisingly, our results showed an increase in the mRNA levels of BDNF in the cerebral cortex when the animals were treated with inosine at a dose of 100 mg/kg (Figure 5).

3.5 Inosine prevent alterations in levels of anti-inflammatory citokines

In figure 6, the results show that STZ reduced the levels of IL-4 and IL-10 in both the hippocampus and cerebral cortex of rats ($P<0.05$). In the hippocampus, only the treatment with inosine 100 mg/kg was capable of preventing these alterations. On the other hand, in the cerebral cortex only the dose of inosine 50 mg/kg was effective in increasing the levels of anti-inflammatory cytokines in both the brain structures analyzed (Figure 6).

3.6 Inosine prevent alterations in BuChE activity and cholesterol levels

In serum, STZ caused an increase in BuChE activity and total cholesterol levels when compared to the control group ($P<0.05$) (Figure 7). Inosine treatment at both doses (50 and 100 mg/kg) prevented these serum alterations (Figure 7).

3.7 Morphological changes and immunoreactivity for GFAP in different regions of the hippocampus of rats treated with inosine and submitted in a model of AD induced by STZ

In figure 8, it can be seen that in the CA1 and DG regions, there is a change in the morphology of the cells that make up the granular layer (see black arrows). In the control group, the nuclei were rounded and large with a lighter chromatin with visible nucleoli (see white arrows). In the STZ group, the heterogeneity of the cells was smaller, the cytoplasm was not apparent, and nuclei had elongated or fusiform morphology. In addition, the presence of “dark bodies” along the molecular layer, which are characterized by cells with a more basophilic nucleus, greater dyeing by hematoxylin, and an elongated morphology. These morphological changes in the molecular layer were attenuated by treatment with inosine 50 and 100 mg/kg in rats exposed to STZ.

Figure 9 shows the immunoreactivity of GFAP in the CA1 (graph B), CA3 (graph C), and DG (graph D) regions. There was an increase in immunoreactivity of GFAP in CA1 (P>0.05, graph B), CA3 (P>0.05 graph C), and DG regions (P>0.05, graph D). Inosine 50 and 100 mg/kg were not able to protect against the increase in GFAP immunoreactivity.

4. Discussion

The STZ model is characterized by brain insulin resistance and represents a sporadic non-transgenic AD model. This model reproduced the molecular and behavioral characteristics of AD, such as memory deficits, neuroinflammation, oxidative stress, cholinergic alterations, and glia activation [36–39].

Using the behavioral task of object recognition, our results showed that the STZ group had less preference for the new object, confirming the memory deficits. Inosine 100 mg/kg was capable of preventing short-time memory dysfunctions induced by STZ. This finding corroborated with another group study and the effect of inosine in improving memory can be associated with the modulation of many neural pathways such as oxidative stress, ion pump activities, and cholinergic signaling [34]. In addition, in the present study, we also demonstrated that inosine is capable of modulating other mechanisms involved in neuroinflammation, purinergic system, brain-derived neurotrophic factor (BDNF) signaling, and cholesterol metabolism.

There are no known receptors specific for inosine; however, studies have suggested that this nucleoside is capable of interacting with adenosine receptors. Adenosine receptors have many roles in the brain, such as presynaptic and postsynaptic neuromodulatory activities [27, 40]. However, in neurodegenerative conditions, while A1 has been described to have a neuroprotective effect, A2A plays a crucial role in the neurodegeneration process [41]. In fact, Pagnussat et al. (2015) [42] showed that activation of A2A is necessary and sufficient to decrease short-term memory. On this note, an important finding of our study is the reduction of A2A expression in the hippocampus and cerebral cortex by inosine, which can be directly associated with memory improvement. Other studies have also demonstrated that inosine prevents the positive regulation of A2A in experimental models of autoimmune encephalomyelitis [43] and Parkinson’s disease [44]. Thus, our results corroborate with previous data and allow us to propose that this purine can be an innovative and useful tool for the prevention of inflammatory and neurodegenerative diseases.

Another important result of this study was the effect of inosine in increasing the expression of BDNF and its receptor, mainly in the hippocampus. BDNF through the activation of its receptor (TrkB) plays a crucial role in the nervous system by providing trophic support to neurons and by regulating synaptic transmission and plasticity, such as long-term potentiation (LTP), an important pathway associated with memory [45]. Alterations in BDNF signaling have been described in AD, and in line of this, the reestablishment of BDNF activity by inosine may be considered as a promising strategy of preventing neurodegeneration.

There is an association between inflammatory markers and cognitive decline in both animal models and patients with AD [46]. Previous studies have demonstrated a relationship between early cytokine production and cognitive dysfunction and glial activation, which, in turn, is accompanied by an increase in the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-12) [47, 50]. In this study, we showed a decrease in the levels of anti-inflammatory cytokines IL-10 and IL-4 in the STZ group. In the brain, IL-10 is capable of promoting neuronal survival by blocking the effects of proapoptotic cytokines, and limiting inflammation by reducing the synthesis of proinflammatory cytokines and suppressing cytokine receptor expression/activation [49]. In addition, IL-4 is very important to immunity and plays a critical role in brain functions, such as homeostasis, neurogenesis, memory, and learning [47, 48]. Thus, the increase caused by inosine in the levels of these brain anti-inflammatory cytokines is an important neuroprotective function that can explain, together with other results, the improvement of memory and reduction in damage in the hippocampus histology.

Corroborating with the results described above, we also observed that STZ induced an increase in BuChE activity and inosine in both doses prevented these peripheral alterations. BuChE is a nonspecific cholinesterase able to hydrolyze acetylcholine, as well as other choline esters. Acetylcholine receptors are prominently expressed in immune cells and it is involved in the control of the production of many pro- and anti-inflammatory cytokines [51–53]. Studies have suggested that serum BuChE could be used as a possible marker of systemic inflammation [53] because an increase in this enzyme activity reduces the tissue acetylcholine levels, leading to disrupted cholinergic anti-inflammatory responses. Peripheral BuChE is an α-glycoprotein synthesized in the liver and its serum level can be correlated with several clinical conditions; for example, it is increased in patients with AD [54–56]. The STZ model mimics this pathological condition [54]. Thus, it is plausible that a reduction in

BuChE activity by inosine could increase the acetylcholine levels and contribute to the anti-inflammatory activity of this nucleoside. The effects of inosine on cholinergic signaling have also been reported in other studies [34].

In addition, an increase in GFAP was observed in both the hippocampus and cerebral cortex in the STZ group. In pathological conditions, astrocytes become reactive, leading to an upregulation of proinflammatory cytokines, which are associated with neuronal damage [53]. Astrocyte reactivity is characterized by morphological changes and overexpression of GFAP [57]. However, in our study, inosine was not able to prevent GFAP overexpression induced by STZ.

Lastly, elevated cholesterol levels are correlated with a higher incidence of memory impairment and dementia [58]. In fact, studies have investigated the potential therapeutic effects of lipid-lowering agents such as statins in experimental models of AD [59]. The relationship between lipid metabolism, especially cholesterol levels and AD, has been associated with apolipoprotein E4 (apoE4). ApoE4 is an important genetic risk factor for this neurodegenerative disease as it increases brain inflammation [60]. Interestingly, our results showed that inosine at both doses (50 and 100 mg/kg) was capable of preventing the increase in serum cholesterol levels induced by STZ. The exact mechanism associated with inosine and lipid metabolism has not been described in the literature, but recently Lima et al. (2020) [61] also demonstrated that inosine decreases cholesterol levels in a hypercholesterolemic animal model. In conclusion, our findings showed that inosine is capable of reestablishing memory deficits and modulating inflammatory and neurotrophic factors and their activities. Also, purinergic signaling is a promising alternative for AD therapeutics due to its multi-target action as shown in our results.

Acknowledgments

This research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico. R.M.S is a recipient of CNPq fellowship (309299/2017-9). This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brasil (CAPES) - finance code 001, and by Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) - grant number: 19/2551-0001712-0).

Conflicts of interest

The authors declare no conflicts of interest.

References

[1] Yiannopoulou KG, Papageorgiou SG (2020) Current and Future Treatments in Alzheimer Disease: An Update. Journal of Central Nervous System Disease, 12.

[2] Kempuraj D, Mentor S, Thangavel R, Ahmed ME, Selvakumar GP, Raikwar SP et al (2019) Mast cells in stress, pain, blood-brain barrier, neuroinflammation and Alzheimer's disease. Frontiers in cellular neuroscience, 13, 54.

[3] Gray SC, Kinghorn KJ, Woodling NS (2020) Shifting equilibriums in Alzheimer's disease: The complex roles of microglia in neuroinflammation, neuronal survival and neurogenesis. Neural Regeneration Research, 15(7), 1208.

[4] Whitehouse PJ, Price DL, Clark AW, Coyle JT, DeLong MR (1981) Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society, 10(2), 122-126.

[5] Moore AM, Mahoney E, Dumitrescu L, De Jager PL, Koran MEI, Petyuk VA (2020) APOE ε4-specific associations of VEGF gene family expression with cognitive aging and Alzheimer's disease. Neurobiology of aging, 87, 18-25.

[6] Butterfield DA, Mattson MP (2020) Apolipoprotein E and oxidative stress in brain with relevance to Alzheimer's disease. Neurobiology of Disease, 138, 104795.

[7] Hampel H, Caraci F, Cuello AC, Caruso G, Nisticò R, Corbo, M et al (2020) A Path Toward Precision Medicine for Neuroinflammatory Mechanisms in Alzheimer's Disease. Frontiers in Immunology, 11, 456.

[8] Stanciu GD, Luca A, Rusu RN, Bild V, Beschea Chiriac SI, Solcan C et al (2020) Alzheimer's disease pharmacotherapy in relation to cholinergic system involvement. Biomolecules, 10(1), 40.

[9] Pacheco SM, Soares MSP, Gutierres JM, Gerzson MFB, Carvalho FB, Azambuja JH et al (2018) Anthocyanins as a potential pharmacological agent to manag memory deficit, oxidative stress and alterations in ion pump activity induced by experimental sporadic dementia of Alzheimer's type. The Journal of nutritional biochemistry, 56, 193-204.

[10] Woods LT, Ajit D, Camden JM, Erb L, Weisman GA (2016) Purinergic receptors as potential therapeutic targets in Alzheimer's disease. Neuropharmacology, 104, 169-179.

[11] Tapia-Arancibia L, Aliaga E, Silhol M, Arancibia S (2008) New insights into brain BDNF function in normal aging and Alzheimer disease. Brain research reviews, 59(1), 201-220.

[12] Shen T, You Y, Joseph C, Mirzaei M, Klistorner A, Graham SL, Gupta V (2018) BDNF polymorphism: a review of its diagnostic and clinical relevance in neurodegenerative disorders. Aging and disease, 9(3), 523.

[13] Tebano MT, Martire A, Chiodi V, Ferrante A, Popoli P (2010) Role of adenosine A2A receptors in modulating synaptic functions and brain levels of BDNF: a possible key mechanism in the pathophysiology of Huntington's disease. The Scientific World journal, 10.

[14] Jerónimo-Santos A, Batalha VL, Mueller CE, Baqi Y, Sebastião AM, Lopes LV, Diógenes MJ (2014) Impact of in vivo chronic blockade of adenosine A2A receptors on the BDNF-mediated facilitation of LTP. Neuropharmacology, 83, 99-106.

[15] Lu Y, Christian K, Lu B (2008). BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? Neurobiology of learning and memory, 89(3), 312-323.

[16] Korte M, Kang H, Bonhoeffer T, Schuman E (1998) A role for BDNF in the late-phase of hippocampal long-term potentiation. Neuropharmacology, *37*(4-5), 553-559.

[17] Phillips HS, Hains JM, Armanini M, Laramee GR, Johnson SA, Winslow JW (1991) BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. Neuron, *7*(5), 695-702.

[18] Connor B, Young D, Yan Q, Faull RLM, Synek B, Dragunow M (1997) Brain-derived neurotrophic factor is reduced in Alzheimer's disease. Molecular brain research, 49(1-2), 71-81.

[19] Allen SJ, Wilcock GK, Dawbarn D (1999) Profound and selective loss of catalytic TrkB immunoreactivity in Alzheimer's disease. Biochemical and biophysical research communications, 264(3), 648-651.

[20] Ferrer I, Marín C, Rey MJ, Ribalta T, Goutan E, Blanco R et al (1999) BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. Journal of neuropathology and experimental neurology, 58(7), 729-739.

[21] Holsinger RD, Schnarr J, Henry P, Castelo VT et al (2000) Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction: decreased levels in Alzheimer's disease. Molecular Brain Research, 76(2), 347-354.

[22] Counil H and Krantic S (2020) Synaptic Activity and (Neuro) Inflammation in Alzheimer's Disease: Could Exosomes be an Additional Link? Journal of Alzheimer's Disease.

[23] Kamphuis W, Middeldorp J, Kooijman L, Sluijs JA, Kooi EJ, Moeton M, et al (2014) Glial fibrillary acidic protein isoform expression in plaque related astrogliosis in Alzheimer's disease. Neurobiology of aging, 35(3), 492-510.

[24] Rebola N, Pinheiro PC, Oliveira CR, Malva JO, Cunha RA (2003) Subcellular localization of adenosine A1 receptors in nerve terminals and synapses of the rat hippocampus. Brain research, 987(1), 49-58.

[25] Cunha RA (2005) Neuroprotection by adenosine in the brain: from A 1 receptor activation to A 2A receptor blockade. Purinergic signalling, 1(2), 111-134.

[26] Canas PM, Porciúncula LO, Simões AP, Augusto E, Silva HB, Machado NJ (2018) Neuronal adenosine A2A receptors are critical mediators of neurodegeneration triggered by convulsions. Eneuro, *5*(6).

[27] Blum D, Sandau U, Laurent C, Batalha V, Leboucher A, Hamdane M et al (2013) Adenosine receptors and Alzheimer's disease. In Adenosine, 385-407.

[28] Rahman A (2009) The role of adenosine in Alzheimer's disease. Current neuropharmacology, *7*(3), 207-216.

[29] Faivre E, Coelho JE, Zornbach K, Malik E, Baqi Y, Schneider M et al (2018) Beneficial effect of a selective adenosine A2A receptor antagonist in the APPswe/PS1dE9 mouse model of Alzheimer's disease. Frontiers in molecular neuroscience, 11, 235.

[30] da Rocha Lapa F, de Oliveira APL, Accetturi BG, de Oliveira Martins I, Domingos HV, de Almeida Cabrini D (2013) Anti-inflammatory effects of inosine in allergic lung inflammation in mice: evidence for the participation of adenosine A 2A and A 3 receptors. Purinergic signalling, *9*(3), 325-336.

[31] Ruhal P, Dhingra D (2018) Inosine improves cognitive function and decreases aging-induced oxidative stress and neuroinflammation in aged female rats. Inflammopharmacology, *26*(5), 1317-1329.

[32] Herman JP, Watson SJ (1987) The rat brain in stereotaxic coordinates (2nd edn) by George Paxinos and Charles Watson.Trends Neurosci, 10:439–439.

[33] Dachir S, Shabashov D, Trembovler V, Alexandrovich AG, Benowitz LI et al (2014) Inosine improves functional recovery after experimental traumatic brain injury. Brain research, 1555, 78-88.

[34] Teixeira FC, Gutierres JM, Soares MSP, de Mattos BDS, Spohr L, Do Couto CA (2020) Inosine protects against impairment of memory induced by experimental model of Alzheimer disease: a nucleoside with multitarget brain actions. Psychopharmacology, *237*(3), 811-823.

[35] Ellman G, Courtney K, Andres V, Feather-Stone R. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemistry. Pharmacology, 7, 88-95.

[36] Gulyaeva N V, Bobkova NV, Kolosova NG, Samokhin AN, Stepanichev MY (2017) Molecular and cellular mechanisms of sporadic Alzheimer's disease: Studies on rodent models in vivo. Biochemistry, 82(10), 1088-1102.

[37] Grieb P (2016) Intracerebroventricular streptozotocin injections as a model of Alzheimer's disease: in search of a relevant mechanism. Molecular neurobiology, 53(3), 1741-1752.

[38] Salkovic-Petrisic M., Hoyer S (2007) Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: an experimental approach. In Neuropsychiatric Disorders an Integrative Approach, 217-233.

[39] Knezovic A, Osmanovic-Barilar J, Curlin M, Hof PR, Simic G, Riederer P, Salkovic-Petrisic M (2015) Staging of cognitive deficits and neuropathological and ultrastructural changes in streptozotocin-induced rat model of Alzheimer's disease. Journal of neural transmission, 122(4), 577-592.

[40] Gomez G, Sitkovsky MV (2003) Differential requirement for A2a and A3 adenosine receptors for the protective effect of inosine in vivo Blood, 102 (13), 4472-4478.

[41] Stockwell J, Jakova E, Cayabyab FS (2017) Adenosine A1 and A2A receptors in the brain: current research and their role in neurodegeneration. Molecules, *22*(4), 676.

[42] Pagnussat N, Almeida AS, Marques DM, Nunes F, Chenet GC, Botton PHS (2015) Adenosine A2A receptors are necessary and sufficient to trigger memory impairment in adult mice. British journal of pharmacology, *172*(15), 3831-3845.

[43] Junqueira SC, dos Santos Coelho I, Lieberknecht V, Cunha MP, Calixto JB, Rodrigues ALS (2017) Inosine, an endogenous purine nucleoside, suppresses immune responses and protects mice from experimental autoimmune encephalomyelitis: a role for A2A adenosine receptor. Molecular neurobiology, 54(5), 3271-3285.

[44] El-Shamarka ME, Kozman MR, Messiha BA (2020) The protective effect of inosine against rotenone-induced Parkinson's disease in mice; role of oxido-nitrosative stress, ERK phosphorylation, and A2AR expression. Naunyn-Schmiedeberg's Archives of Pharmacology, 1-13.

[45] Lu B, Nagappan G, Lu Y (2014) BDNF and synaptic plasticity, cognitive function, and dysfunction. In Neurotrophic factors, 223-250.

[46] Schwab C, McGeer PL (2008) Inflammatory aspects of Alzheimer disease and other neurodegenerative disorders. Journal of Alzheimer's Disease, 13(4), 359-369.

[47] Gadani SP, Cronk JC, Norris GT, Kipnis J (2012) IL-4 in the brain: a cytokine to remember. The journal of immunology, 189(9), 4213-4219.

[48] Gambi F, Reale M, Iarlori C, Salone A, Toma L, Paladini C (2004) Alzheimer patients treated with an AchE inhibitor show higher IL-4 and lower IL-1β levels and expression in peripheral blood mononuclear cells. Journal of clinical psychopharmacology, 24(3), 314-321.

[59] Strle K, Zhou JH, Broussard SR, Venters HD, Johnson RW, Freund GG (2002) IL-10 promotes survival of microglia without activating Akt. Journal of neuroimmunology, 122(1-2), 9-19.

[50] Rossi C, Cusimano M, Zambito M, Finardi A, Capotondo A, Garcia-Manteiga JM, (2018) Interleukin 4 modulates microglia homeostasis and attenuates the early slowly progressive phase of amyotrophic lateral sclerosis. Cell death & disease, *9*(2), 1-16.

[51] De Jonge WJ, Ulloa L (2007) The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. British journal of pharmacology, 151(7), 915-929.

[52] Das UN (2007). Acetylcholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation. Medical Science Monitor, 13(12), RA214-RA221.

[53] Li C, Zhao R, Gao K, Wei Z et al (2011) Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer's disease. Current Alzheimer Research, 8(1), 67-80.

[54] Rao AA, Sridhar GR, Das UN (2007) Elevated butyrylcholinesterase and acetylcholinesterase may predict the development of type 2 diabetes mellitus and Alzheimer’s disease. Medical Hypotheses, 69, 1272–1276, 2007.

[55] Mushtaq G, H Greig N, A Khan J, A Kamal M (2014) Status of Acetylcholinesterase and Butyrylcholinesterase in Alzheimer’s Disease and Type 2 Diabetes Mellitus.CNS Neurol Disord Drug Targets,13(8):1432–1439.

[56] Barai P, Raval N, Acharya S, Acharya N (2018) Bergenia ciliata ameliorates streptozotocin-induced spatial memory deficits through dual cholinesterase inhibition and attenuation of oxidative stress in rats. Biomedicine & Pharmacotherapy,102:966-80.

[57] Ben Haim L, Carrillo-de Sauvage MA, Ceyzériat K & Escartin C (2015) Elusive roles for reactive astrocytes in neurodegenerative diseases. Frontiers in cellular neuroscience, 9, 278.

[58] Salkovic-Petrisic M, Knezovic A, Hoyer S & Riederer P (2013) What have we learned from the streptozotocin-induced animal model of sporadic Alzheimer’s disease, about the therapeutic strategies in Alzheimer’s research. Journal of neural transmission, 120(1), 233-252.

[59] Shepardson NE, Shankar GM & Selkoe DJ (2011) Cholesterol level and statin use in Alzheimer disease: I. Review of epidemiological and preclinical studies. Archives of neurology, 68(10), 1239-1244.

[60] Ophir G, Amariglio N, Jacob-Hirsch J, Elkon R, Rechavi G & Michaelson DM (2005) Apolipoprotein E4 enhances brain inflammation by modulation of the NF-κB signaling cascade. Neurobiology of disease, 20(3), 709-718.

[61] Lima D, Hacke ACM, Inaba J, Pessôa CA & Kerman K (2020) Electrochemical detection of specific interactions between apolipoprotein E isoforms and DNA sequences related to Alzheimer's disease. Bioelectrochemistry, 133, 107447.

Table 1. Primers used for quantitative real-time polymerase chain reaction. Listed are the forward and reverse primer sequences used to amplify each target gene as well as the GAPDH endogenous control.

Primer Name	Sequence
BDNF Forward	5' CAATCGAAGCTCAACCGAAGAG 3'
BDNF Reverse	5' AACCCGGTCTCATCAAAGCC 3'
TrkB Forward	5' CCAAGTTTGGCATGAAAGGTTTTG 3'
TrkB Reverse	5' GCAACAGTAGTCCCAGGAGTT 3'
β-actin Forward	5' ACCCGCGAGTACAACCTTCT 3'
β-actin Reverse	5'ATACCCACCATCACACCCTGG 3'

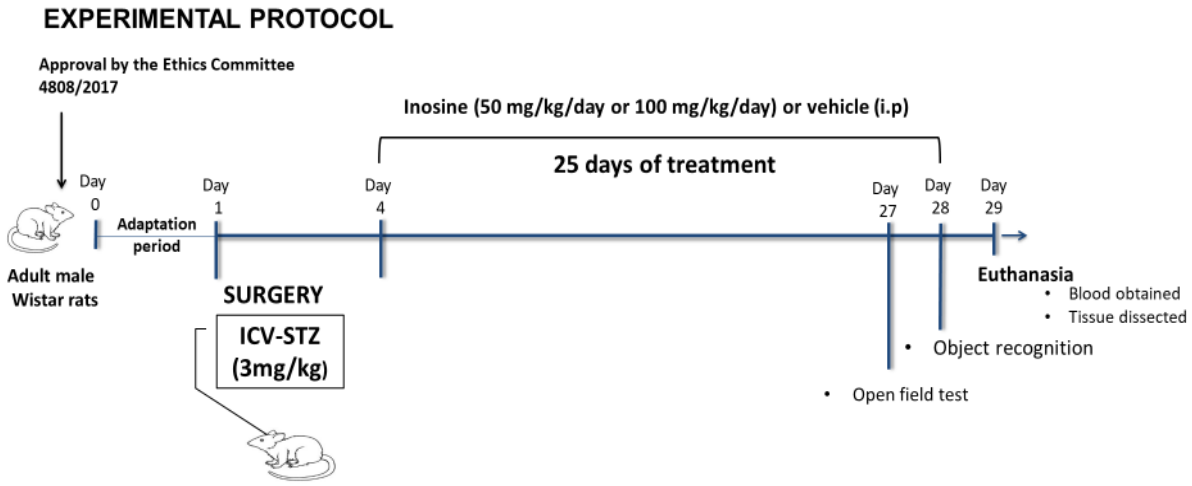


Figure 1 - Schematic for induction of a rat model of AD induced by STZ (3 mg/kg) and intraperitoneal (i.p.) administration of inosine (50 or 100 mg/kg) for 25 days.

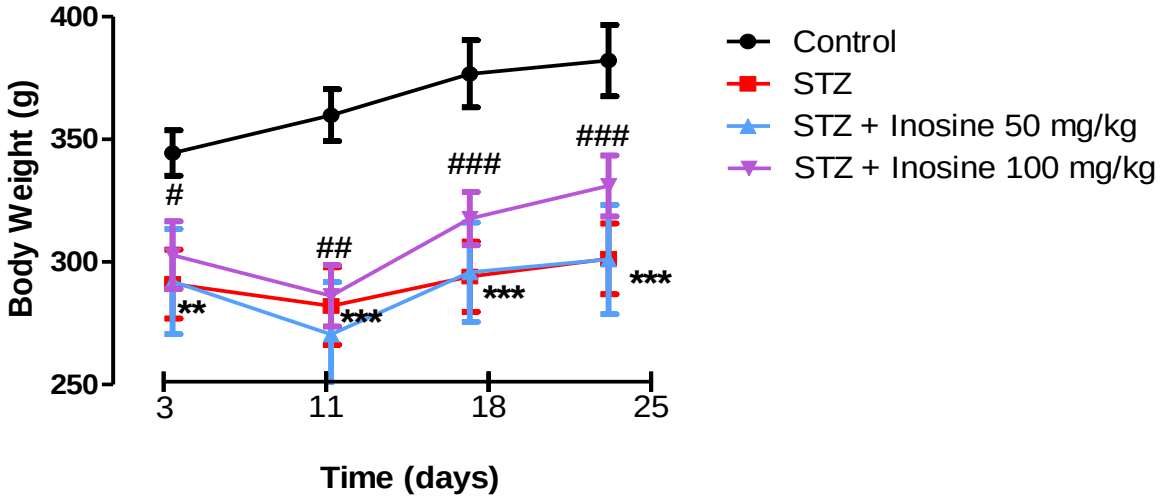


Figure 2 - Effects of treatment with inosine (50 or 100 mg/kg) on the body weight of animals submitted in a model of AD induced by STZ (3 mg/kg). ** P<0.01 and *** P<0.0001 when compared with the control group. # P<0.05 and ## P<0.01 when compared with the STZ group (n =10 per group).

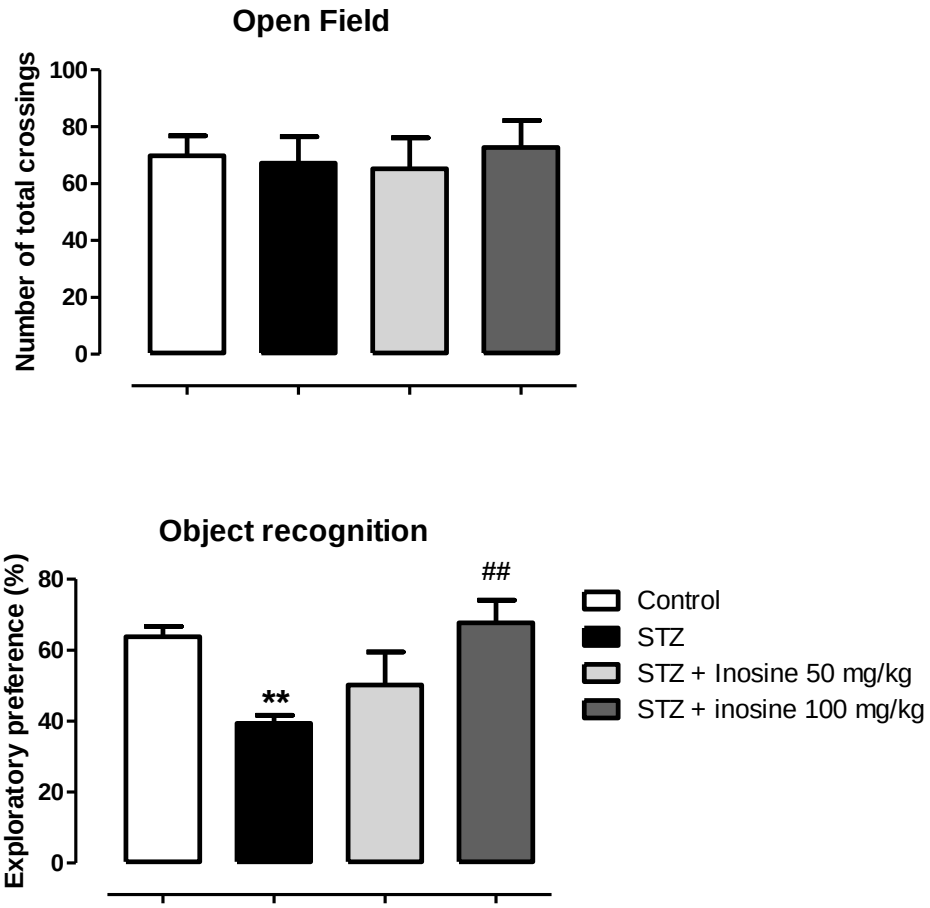


Figure 3 - Effects of treatment with inosine (50 or 100 mg/kg) in locomotor activity (using the open-field apparatus) and short time memory (using the object recognition test) of rats submitted in a model of AD induced by STZ (3 mg/kg). ** *P*<0.01 when compared with the control group. ## *P*<0.01 when compared with the STZ group (n=10 per group).

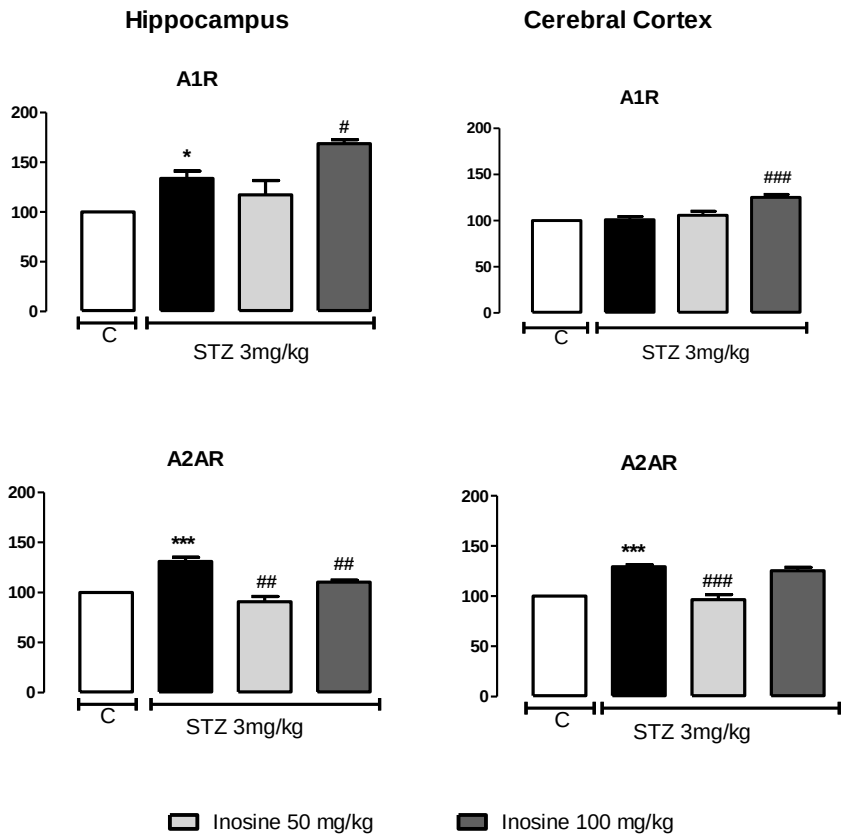


Figure 4 – Effect of inosine (50 and 100 mg/kg) on the immunoreactivity of purinergic receptors A1 and A2A in the hippocampal and cortical membranes of rats submitted in a model of AD induced by STZ (3 mg/kg). *P <0.05 and *** P<0.001 compared with the control group. ## *P*<0.01 and ### *P*<0.001 when compared with the STZ group (n=4-5 per group).

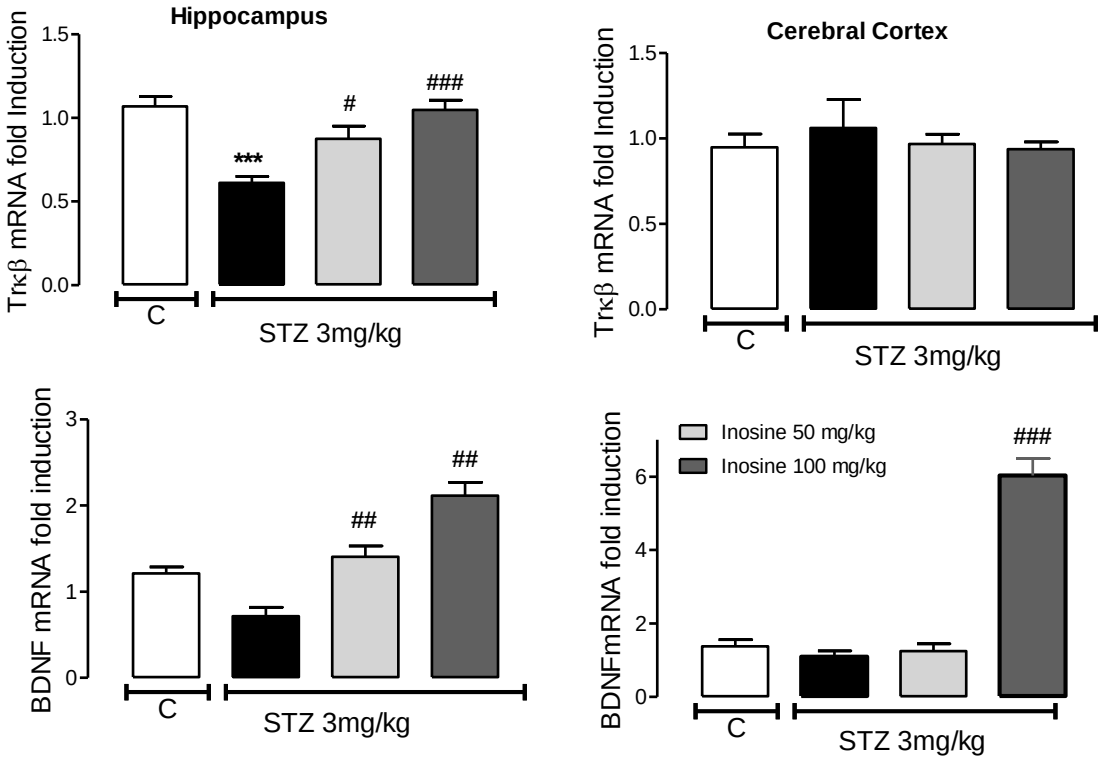


Figure 5 – Effects of treatment with inosine (50 or 100 mg/kg) on the rtPCR of expression of genes TrkB and BDNF in the hippocampus and cerebral cortex of rats submitted in a model of AD induced by STZ (3 mg/kg). ****P* <0.001 when compared with the control group. [#] *P*<0.05, [##] *P*<0.01 and [###] *P*<0.001 when compared with the STZ group (n=5-6 per group).

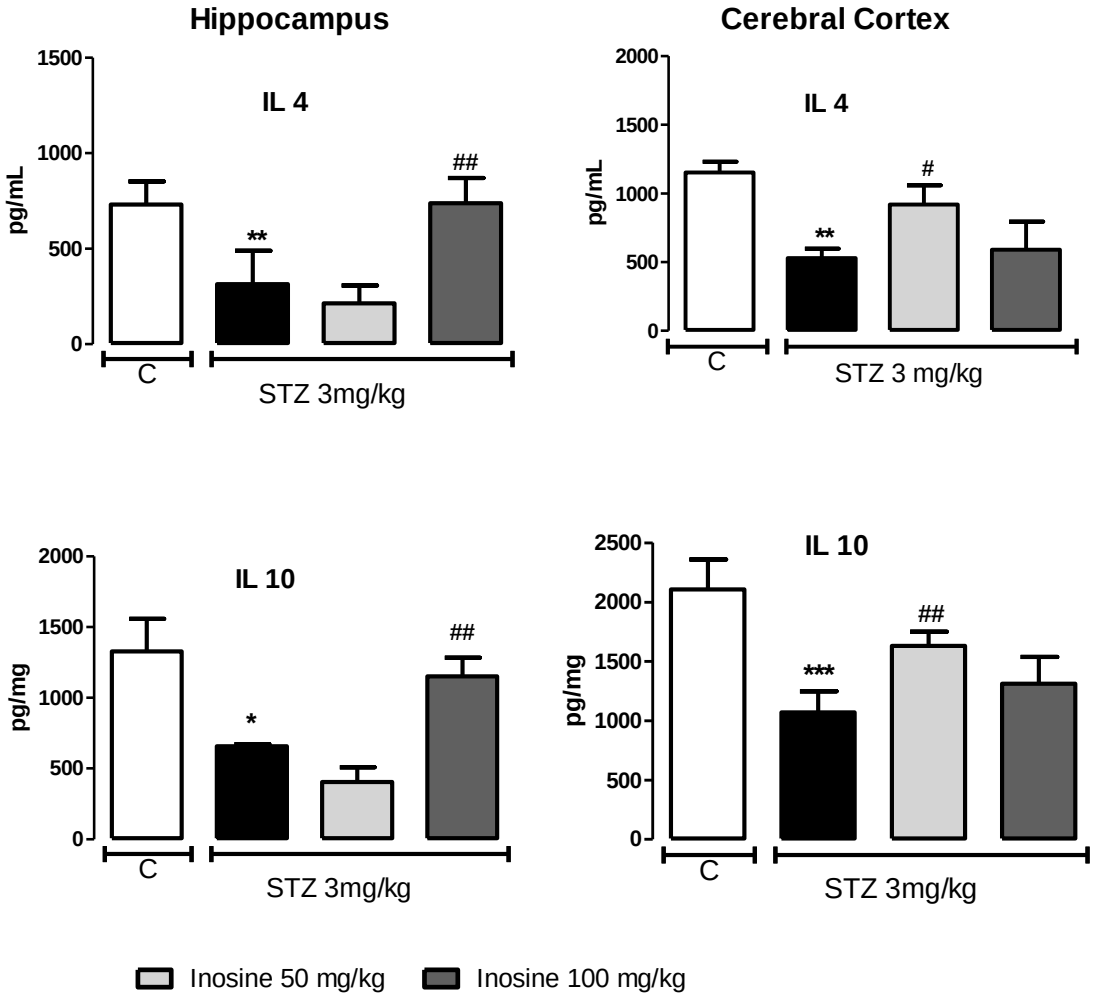


Figure 6 – Effects of treatment with inosine (50 or 100 mg/kg) on IL-4 and IL-10 levels in the hippocampus and cerebral of rats submitted in a model of AD induced by STZ (3 mg/kg). *P <0.05, ** *P*<0.01, and *** *P*<0.001 when compared with the control group. # *P*<0.05 and ## *P*<0.01 when compared with the STZ group (n=4-5 animals each group).

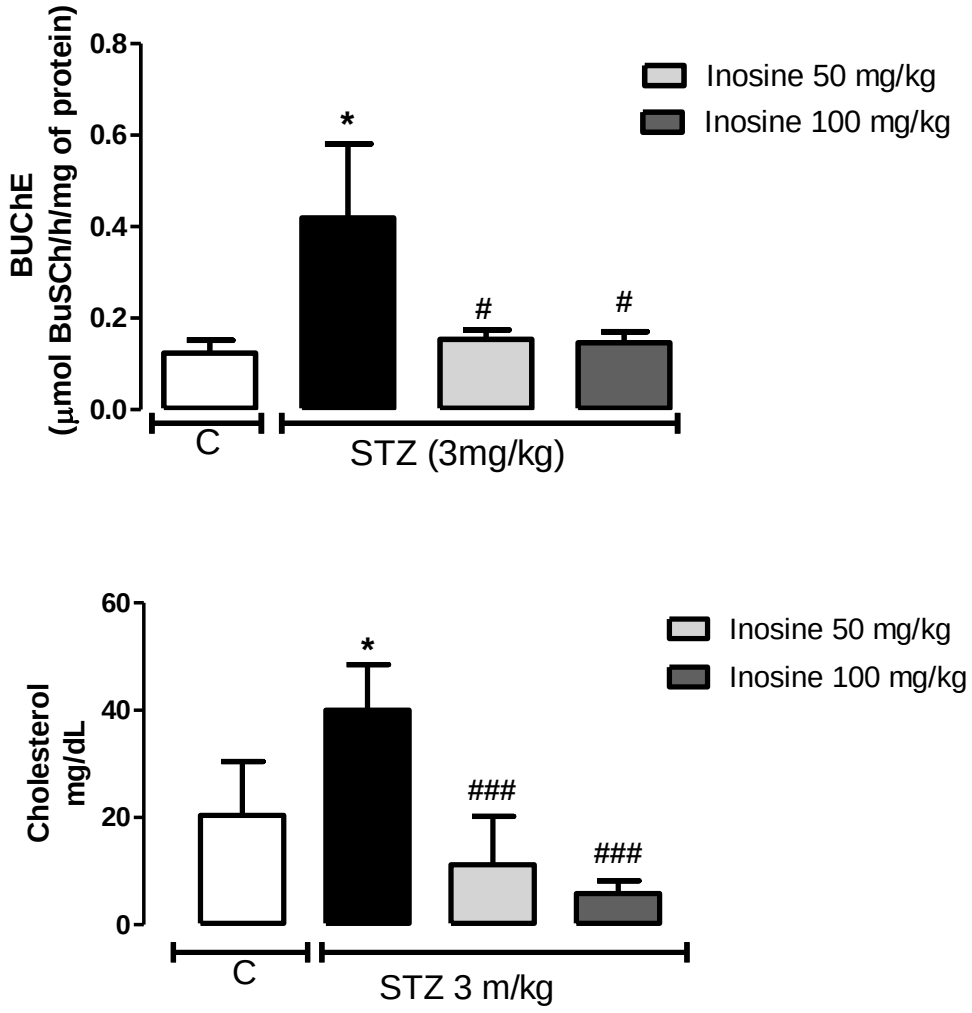


Figure 7 – Effects of treatment with inosine (50 or 100 mg/kg) on butyrylcholinesterase (BuChE) activity and cholesterol levels in serum of rats submitted in a model of AD induced by STZ (3 mg/kg). *P <0.05 when compared with the control group. # *P*<0.05 and ### *P*<0.001 when compared with the STZ group (n=4-5 per group).

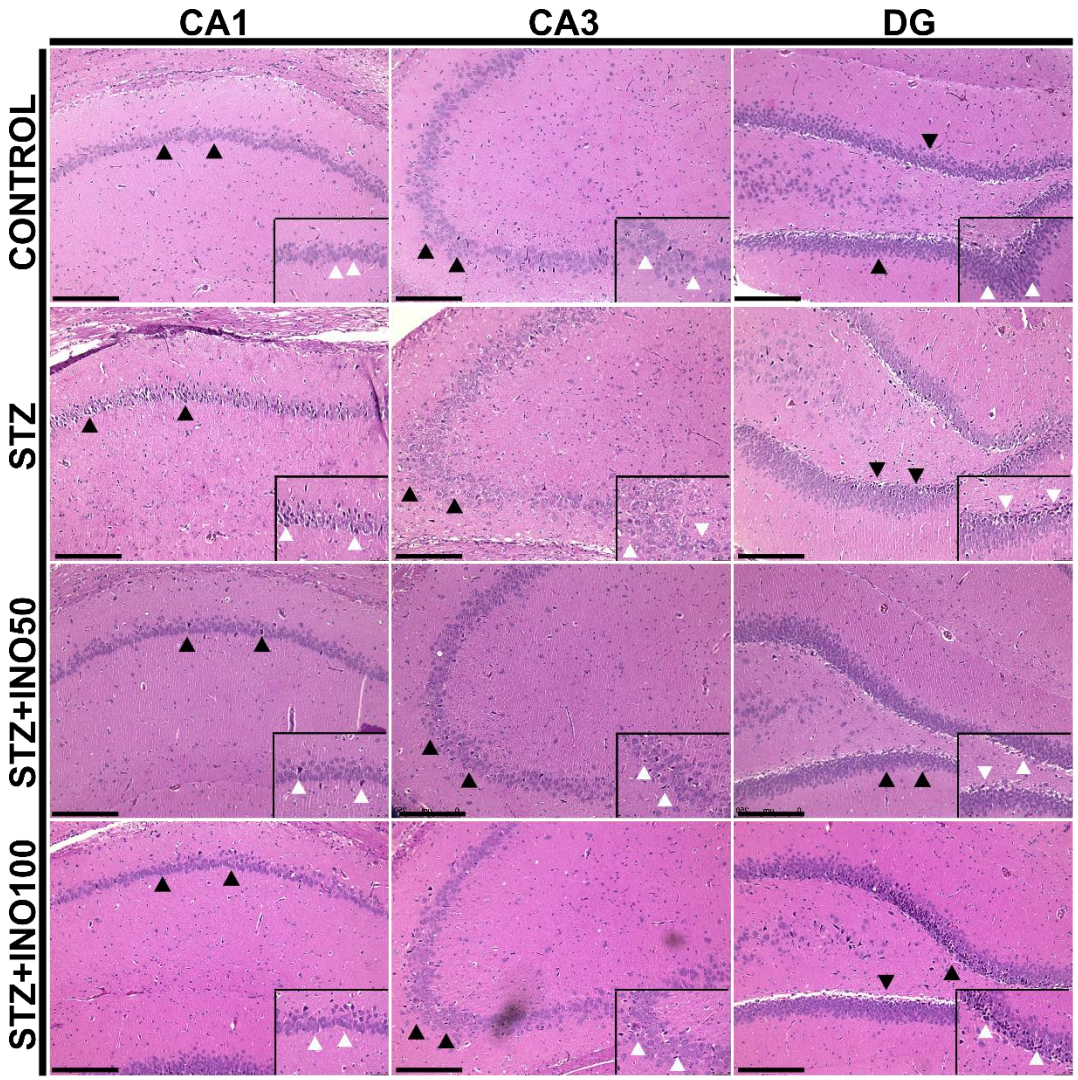


Figure 8- Histopathological changes by hematoxylin and eosin (H&E) staining in the CA1, CA3, and dentate gyrus (DG) regions of the hippocampus of rats treated with inosine (50 and 100 mg/kg, by i.p.) submitted in a model of AD induced by STZ (3 mg/kg). (100X magnification). The square in the lower right field represents an increase of 400X.

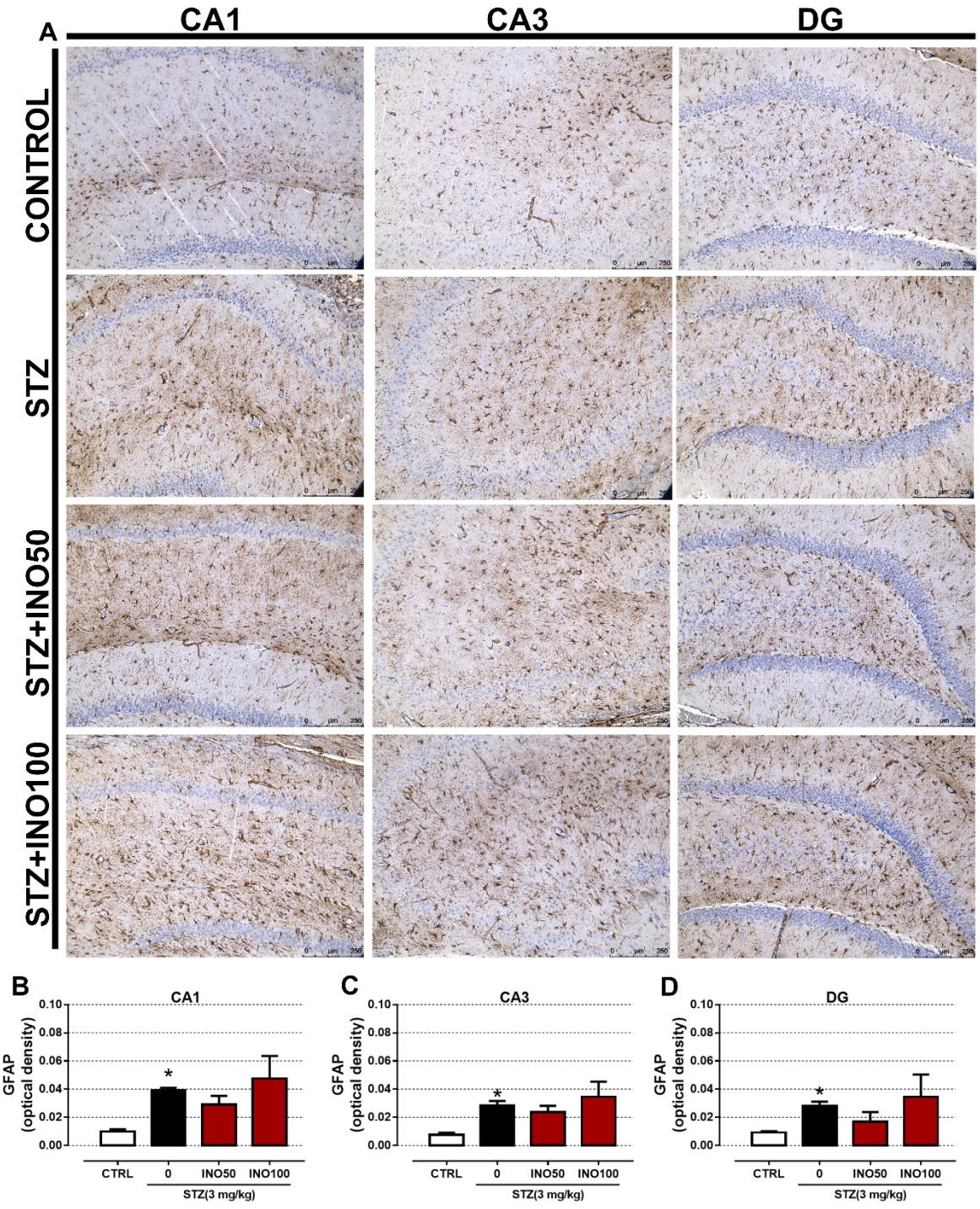


Figure 9 - Immunoreactivity of glial fibrillary acid protein (GFAP, astrocyte marker) in the CA1, CA3, and dentate gyrus (DG) (A) regions of the hippocampus of rats treated with inosine (50 and 100 mg/kg, by i.p.) submitted in a model of AD induced by STZ (3 mg/kg). Graphs represent the mean ± SEM of the cell density (optical density) of GFAP marked cells in the three analyzed regions: CA1 (graph B), CA3 (graph C), and DG (graph D). *P<0.05 when compared with the control group.

5 Discussão

Estudos prévios têm demonstrado que a inosina possui múltiplas ações terapêuticas, em diferentes modelos experimentais, tais como imunomodulação, neuroproteção e atividade antioxidante (HASKÓ; SITKOVSKY e SZABÓ, 2004; MABLEY et al., 2009; NASCIMENTO et al., 2010; da ROCHA-LAPA et al., 2013; MUTO et al., 2014; JUNQUEIRA et al., 2015; GONÇALVES et al., 2016). Entretanto, existem poucos trabalhos avaliando o papel desta purina na memória (DACHIR et al., 2014; RUHAL e DHINGRA et al., 2019) e cabe ressaltar ainda que até o presente momento, não existe nenhum estudo avaliando os efeitos deste nuclesídeo nos modelos experimentais de DA usados nesta pesquisa.

Concomitantemente com o protoloco realizado para o artigo 1 realizamos o estudo do manuscrito 1. O objetivo principal desse estudo (manuscrito 1) foi investigar os efeitos neuroprotetores da inosina em diferentes estágios da memória (aquisição e consolidação) utilizando a SCO com um indutor de ammésia dos animais.

Os resultados obtidos no estudo do manuscrito 1 demonstram que a SCO induziu um déficit tanto no protocolo de aquisição quanto no de consolidação da memória associado a um aumento de estresse oxidativo em hipocampo e córtex cerebral. Além disso, no protocolo de consolidação foi observado também alterações cerebrais nas enzimas AChE e Na^+,K^+-ATPase.

Embora a inosina tenha sido capaz de prevenir somente os déficits relacionados à consolidação de memória nos testes comportamentais, em relação aos parâmetros de estresse oxidativo pode-se perceber que ela foi efetiva em modular o status redox em ambos protocolos. O efeito antioxidante da inosina também foi relatado em estudo envolvendo envelhecimento (RUHAl e DHINGRA, 2018). Conforme mencionando anteriormente esse efeito antioxidante pode ter associação com a produção de urato, a forma aniônica do ácido úrico, no soro e no líquido cefalorraquidiano (SCHWARZSCHILD et al., 2014). Os resultados encontrados nesse manuscrito reforçam a atividade antioxidante da inosina ao reduzir niveis de EROs e reestabeler o equilibrio das enzimas antioxidantes CAT e SOD no hipocampo e no córtex cerebral, duas regiões importantes relacionadas ao processamento da memória.

Observou-se ainda que a inosina, em ambas as doses, foi capaz de reduzir a atividade da AChE, em hipocampo e cortex cerebral, somente no protocolo de consolidação, modulando um importante componente do sistema colinérgico. Tendo

em vista que o tratamento com a inosina atuou na fase de consolidação da memória, a atividade da Na^+,K^+-ATPase foi analisada devido a sua importante correlação com a processos cognitivos. A SCO reduziu a atividade desta enzima conforme dados prévios da literatura (ZHANG et al., 2013, GUTIERRES et al., 2014) e a inosina na dose de 100 mg/kg foi capaz de reverter essa alteração. Os dados apresentados nesse manuscrito estão de acordo com os resultados apresentados no artigo 1 dessa tese, demonstrando o efeito neuroprotetor da inosina em um modelo de amnésia através da associação da ação antioxidante, da modulação de enzimas colinérgicas e de bombas iônicas.

Um dos objetivos principais deste trabalho foi investigar os efeitos neuroprotetores da inosina utilizando a STZ com um indutor de déficit de memória nos animais, caracterizando assim, um modelo de demência esporádica do tipo Alzheimer. Esse modelo tem sido bastante utilizado segundo dados da literatura, pois mimetiza várias alterações neuroquímicas encontradas na DA (GRIEB, 2016). Os resultados relativos a esse protocolo estão dispostos no artigo 1 e no manuscrito 2. Utilizando testes comportamentais como reconhecimento de objeto, Y maze e esquiva inibitória foi possível constatar que a injeção ICV de STZ causou prejuízo de memória nos animais, corroborando com outros estudos prévios do grupo (GUTIERRES et al., 2014; PACHECO et al., 2018). O déficit de memória aversivo e comprometimento da memória espacial podem estar associados com alterações neuroquímicas observadas no hipocampo e córtex cerebral. De fato, alterações nessas estruturas cerebrais foram bem documentados em pacientes com DA e modelos experimentais que mimetizam essa patologia (DEKOSKY e SCHEFF 1990; YAO et al., 2010; GUTIERRES et al., 2014; PACHECO et al., 2018).

O tratamento com inosina nas duas doses utilizadas (50 e 100 mg/kg) foi capaz de prevenir os déficits de memória induzidos por STZ. O potencial da inosina na melhora da memória foi anteriormente descrito em outras condições experimentais como o modelo de traumatismo craniano (DACHIR et al., 2014) e em ratas idosas (RUHAL e DHINGRA, 2018), porém, seu efeito ainda não era relatado em modelos de DA. Com o objetivo de entender quais mecanismos poderiam estar envolvidos na melhora da memória, o artigo 1 analisou os efeitos do tratamento com inosina em parâmetros de estresse oxidativo, atividade de colinesterases e de bombas iônicas em hipocampo e córtex cerebral de ratos.

Alterações na sinalização colinérgica, especificamente ChAT e AChE foram correlacionadas com declínio cognitivo em pacientes e em modelos experimentais de DA (GUTIERRES et al., 2014; DESHMUKH et al., 2016; SORIAL e SAYED, 2017; PACHECO et al., 2018). Neste contexto, nossos resultados demonstraram que o STZ induziu um aumento da atividade da AChE associada a uma diminuição na expressão de enzima de síntese de ACh, a ChAT. Essas modicações nas enzimas colinérgicas poderia levar a uma diminuição dos níveis de ACh culminando com déficit cognitivo. Cabe ressaltar, que ACh além de ser um importante neurotransmissor envolvido na memória também possui importantes ações relacionadas a neurogênese, a sinaptogênese e a atividade anti-inflamatória (SOREQ e SEIDMAN, 2001; WANG et al., 2003; SHYTLE et al., 2004). Sendo assim, nossos resultados demonstraram que a inosina é capaz de proteger contra danos envolvidos na sinalização colinérgica em um modelo de DA e esse pode ser um dos mecanismos envolvidos com a melhora da memória observada nos resultados comportamentais.

Além disso, a inosina também foi capaz de reverter o aumento da Na^+, K^+-ATPase induzida pelo STZ. Considerando que alguns estudos associam a alterações da Na^+, K^+-ATPase com estresse oxidativo (HITSCHKE et al., 1994) é plausível sugerir que o efeito antioxidante da inosina, observado também neste mesmo trabalho, pode estar relacionado à modulação da atividade desta bomba de íons. Em relação a Ca^{2+}-ATPase os resultados obtidos demonstram que a administração de STZ causa a redução na atividade desta enzima. De fato, uma diminuição na atividade de Ca^{2+}-ATPase foi documentada no cérebro de pacientes com DA (MATA et al., 2011). No hipocampo, a inosina na dose de 100 mg/kg foi capaz de reverter essa alteração. Além disso, também mostramos um aumento na Mg^{2+}-ATPase no córtex cerebral, sendo que o tratamento com inosina em ambas as doses foi capaz de reverter essa alteração enzimática.

No artigo 1 também demonstramos que o STZ induziu estresse oxidativo em regiões cerebrais importantes para o processamento de memória, como hipocampo e córtex cerebral. É bem estabelecido que radicais livres podem causar danos a vários componentes celulares. A produção anormal de espécies reativas pode levar à modificação oxidativa da biomoléculas e interrupção da atividade terminal nervosa causando alterações e perda de sinapses contribuindo para o declínio de memória (CUNHA e AGOSTINHO, 2010). Observou-se que RUHAL e DHINGRA (2018) evidenciaram o efeito antioxidante da inosina em modelo animal de envelhecimento,

tanto em hipocampo quanto em córtex cerebral, principalmente na dose de 100mg/kg. Um possível mecanismo envolvido neste efeito benéfico da inosina pode estar associado ao aumento do urato, a forma aniônica do ácido úrico, no soro e no líquor (SCHWARZSCHILD et al., 2014). O urato é uma importante defesa antioxidante endógena contra danos oxidativos e nitrosativos, além de ser o produto final da via purinérgica (CROTTY, ASCHERIO, and SCHWARZSCHILD, 2017). Além disso, também foi demonstrado que o urato confere neuroproteção por meio da via Nrf2, um fator de transcrição que tem sido implicado como um regulador contra o estresse oxidativo ao controlar a transcrição de vários genes antioxidantes (ZHANG et al., 2014). Ademais, estudos associaram os níveis de ácido úrico a uma diminuição do risco de demência e melhor função cognitiva (EUSER et al., 2009). Dessa forma, nossos resultados corroboram com a literatura, uma vez que a inosina demonstrou atividade antioxidante em ambas as estruturas cerebrais analisadas nas doses de 50 e 100 mg/kg além de aumentar os niveis séricos de ácido úrico, no grupo tratado com inosina 100 mg/kg, indicando esses fatores como possíveis mecanismos para os efeitos neuroprotetores observados.

No manuscrito 2, usando o mesmo modelo de demência esporádica do tipo Alzheimer, o mesmo tempo de tratamento e as mesmas doses de inosina do artigo 1, novos alvos neuroprotetores para este nucleosídeo puderam ser evidenciados. Um dos resultados mais importantes deste manuscrito 2 é o fato da inosina ter modulado a expressão de receptores de adenosina A_1 e A_{2A}. A inosina ao ativar o receptor A_1, possivelmente pode inibir a liberação de glutamato, reduzir a ativação de receptores NMDA, reduzir o influxo de cálcio e também a excitotocicidade. Assim a inosina na dose de 100 mg/kg foi capaz de ativar a expressão do receptor A_1 tanto em hipocampo quanto em cortex, eluciando mais uma via responsável pelos efeitos biológicos do tratamento.

No entanto, em condições neurodegenerativas como a DP, enquanto os receptores A_1 foram descritos com efeitos neuroprotetores, os receptores A_{2A} tem um papel crucial no processo de neurodegeneração e neurotoxicidade (STOCKWELL, JAKOVA e CAYABYAB, 2017). Na verdade, Pagnussat et al. (2015) mostraram que a ativação dos receptores A_{2A} é necessária e suficiente para diminuir a memória de curto prazo. Nessa linha, um achado importante de nosso estudo é a redução da expressão dos receptores $A2_A$ no hipocampo e córtex cerebral pela inosina, efeito esse que pode estar diretamente associado à melhora da memória. Assim, nossos resultados

permitem concluir que a modulação da inosina nos receptores A_1 e A_{2A} permite que se alcance efeitos neuroprotetores e de prevenção de neurotoxicidade, respectivamente.

Outro mecanismo de ação importante da inosina foi sobre a expressão do BDNF e seu receptor. O BDNF e seu receptor específico TrKB são altamente expressos no hipocampo e esse fator tem um importante papel sobre a plasticidade sináptica nessa região. Essa plasticidade sináptica é a base da aprendizagem e da memória via LTP. SHIN e colaboradores (2014) apresentaram um peptídeo com atividade moduladora de BDNF, o tripéptido Neuropep-1. Nesse contexto, uma abordagem para aumentar os níveis cerebrais de BDNF poderia ser o uso de purinas como a inosina, visto que outras, guanosina e adenosina, já apresentam atividade neurotrófica descrita (DOYLE et al., 2018). Assim é plausível sugerir que esse efeito da inosina nos níveis de BDNF associado com aumentos de níveis de citocinas anti-inflamatórias cerebrais (IL-4 e IL10) também sejam vias envolvidas na melhora da memória observada no comportamento dos animais submetidos ao modelo de demência esporádica do tipo Alzheimer.

Os resultados desta tese enfatizam a inosina como agente neuroprotetor para modelos de DA através de modulação de vias cruciais para funções cognitivas. Cabe destacar que as doses de inosina utlizadas nesse trabalho foram iguais ou inferiores às demonstradas na literatura em que a inosina apresentou atividade antioxidante e antiinflamatória (DACHIR et al., 2014; RUHAL e DHINGRA, 2018).

Tendo em vista o conjunto de resultados apresentados nesta tese e mediante a necessidade de ferramentas terapêuticas inovadoras para o tratamento da DA, surge a inosina. Observa-se então, que esse nucleosídeo apresenta resultados promissores em diferentes modelos de deficit de memória em modelos animais pré-clinicos. A inosina tem se mostrado um composto efetivo em modular vias cruciais relacionadas com a memória, que se encontram alteradas na DA, apresentando um potencial multi-alvo (Figura 8). Destaca-se também a importância da continuidade da investigação da utilização da inosina como molécula neuroprotetora frente as alterações encontradas na DA, visto que é imprescindível a descoberta de agentes modificadores da doença na atualidade.

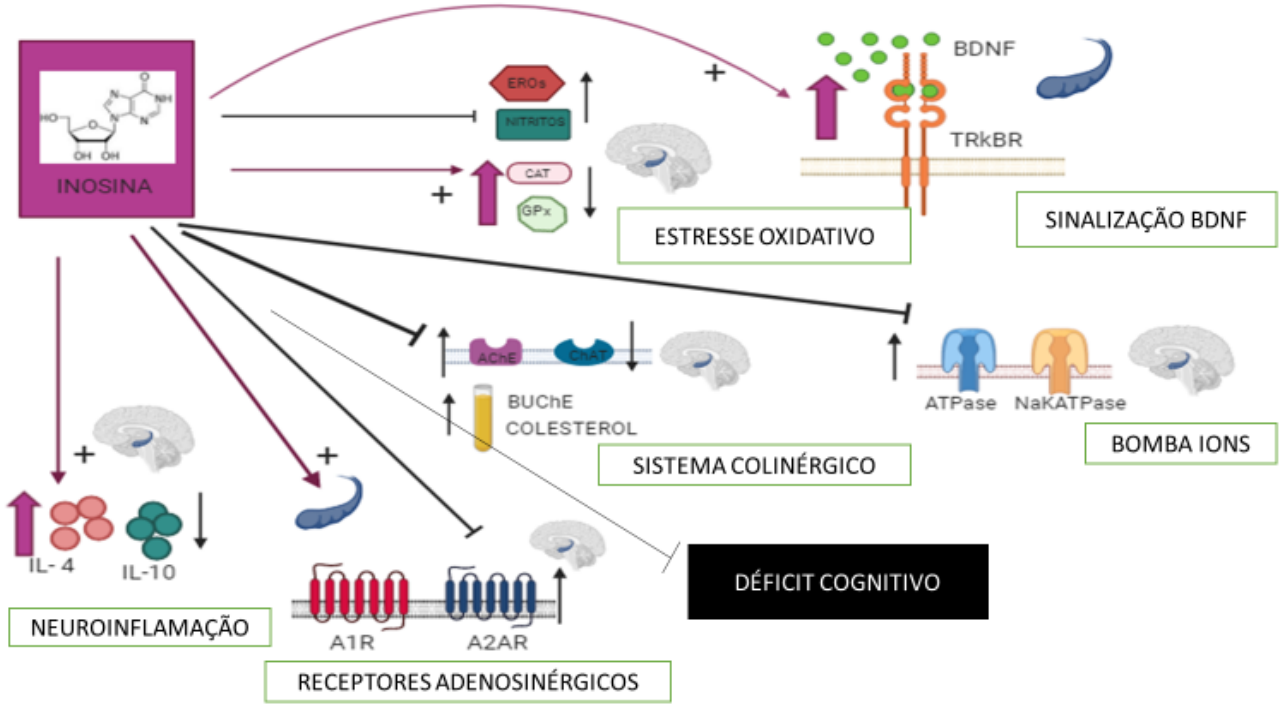


Figura 8. Esquema das ações multi-alvo da inosina. Fonte:autoria própria.

6 Conclusão

Esta tese demonstrou o efeitos da inosina em modelo de demência esporádica do tipo Alzheimer através da atividade antioxidante, anti-inflamatória bem como modulação de receptores de adenosina (A_1 e $A2_A$), bombas iônicas, enzimas colinérgicas e fatores neurotróficos (BDNF). Além disso, os dados histológicos demonstraram que este nucleosídeo reduziu danos hipocampais e foi responsável por melhorar a memória dos animais em testes comportamentais de esquiva inibitória, reconhecimento de objetos e labirinto em Y.

Além disso, em um modelo de déficit de memória induzido por SCO, os animais tratados com inosina apresentaram melhora da memória na fase específica da consolidação através da análise do teste comportamental de esquiva inibitória. Ademais, os achados encontrados nesse manuscrito 1 afirmam atividade antioxidante da inosina, bem como potencial de modulação de enzimas colinérgicas.

Essas descobertas podem ser promissoras na busca de novas alternativas terapêuticas coadjuvantes para o tratamento de patologias que apresentam déficit de memória, como a DA. Em suma, os resultados desta tese corroboram com as evidências pré-clínicas e clínicas que indicam que a inosina pode ser um agente terapêutico seguro, tolerável e eficaz para diminuir a intensidade e as consequências

de lesões celulares nervosas. Como relatado na discussão acima, a inosina atua através de uma variedade de mecanismos diferentes, o que potencializa seus efeitos biológicos. Considerando a natureza multifacetada, heterogênea, progressiva e interativa da DA é cada vez mais urgente a necessidade de tratamentos multi-alvos, assim como demonstra-se o potencial da inosina nesta tese.

7 Perspectivas

Considerando o envolvimento das alterações presentes nos componentes do sistema purinérgico na DA, vale averiguar a densidade /expressão por western blotting dos receptores P2X7, P2X4, P2Y1 e P2Y2 no modelo de demência do tipo Alzheimer e se o tratamento com inosina seria capaz de modular essas vias.

O peptídio beta amilóide é responsável pela ativação anormal das calpaínas, que por sua vez contribuem para hiperfosforilação da Tau, através de CDK5 e também para clivagem do TrKb que causa comprometimento neuromodulatório via BDNF. Assim, outro mecanismo de ação a ser analisado é se a inosina é capaz de inibir as calpaínas, proteases dependentes de cálcio, pois um vez inibidas previnem a neurodegeneração, restauram a função sináptica e a memória espacial.

Concluir as análises que não foram possíveis devido a pandemia COVID-19, como por exemplo: avaliar as concentrações dos componentes, nucleotídeos e nucleosídeos, do sistema purinérgico no líquor coletado dos animais no protocolo de demência esporádica do tipo Alzheimer, bem como verificar os possíveis mecanismos envolvidos nos efeitos da inosina frente ao desafio com lipopolissacarídeo em cultura primária de astrócitos.

Referências

ABEL, T. et al. Sleep, Plasticity and Memory from Molecules Review to Whole-Brain Networks. **Current Biology**, 23(17),774–788, 2013.

AGRAWAL, R. et al. A study of brain insulin receptors, AChE activity and oxidative stress in rat model of ICVSTZ induced dementia. **Neuropharmacology**, 56(4),779–787, 2009.

ALBASANZ, J. L. et al. Up-regulation of Adenosine Receptors in the Frontal Cortex in Alzheimer's Disease. **Brain Pathology**, 18(2),211–219, 2008.

ALBUQUERQUE, E. X. et al. Mammalian nicotinic acetylcholine receptors: from structure to function. **Physiological reviews**, 89(1),73-120, 2009.

ALLEN, J. S.; WATSON, J. J.; DAWBARN, D. The neurotrophins and their role in Alzheimer's disease. **Current neuropharmacology**, 9(4), p; 559-573, 2011.

ALLEN, N. J.; LYONS, D. A. Glia as Architects of Central Nervous System Formation and Function. **Science**, 362(6411)181–185, 2018.

ALZHEIMER'S ASSOCIATION, 2010. Disponível em: https://www.alz.org/ Acesso em: 25/6/19.

ALZHEIMER'S ASSOCIATION, 2017. Alzheimer's disease facts and figures. Alzheimers Dement. 13(4):325-373, 2017. Disponível em: https://www.alz.org/ Acesso em: 25/6/19.

AMATO, A.; TERZO, S.; MULÈ, F. Natural Compounds as Beneficial Antioxidant Agents in Neurodegenerative Disorders: A Focus on Alzheimer's Disease. **Antioxidants,** 8, 608, 2019.

AMERICAN PSYCHIATRIC ASSOCIATION. Disponível em: https://www.psychiatry.org/ Acesso em: 25/6/19.

AMIDFAR, M. et al. CREB and BDNF: Neurobiology and treatment of Alzheimer's disease. **Life sciences**, 118020, 2020.

ANAND, A. et al. The present and future of pharmacotherapy of Alzheimer's disease: a comprehensive review. **European Journal of Pharmacology**, 815:364-375, 2017.

ANDREASSON, K.I. et al. Targeting innate immunity for neurodegenerative disorders of the central nervous system. **Journal of Neurochemistry**, 138(5), 653–693, 2016.

ANGULO, E. et al. A1 Adenosine Receptors Accumulate in Neurodegenerative Structures in Alzheimer's Disease and Mediate Both Amyloid Precursor Protein Processing and Tau Phosphorylation and Translocation Brain Pathology. **Brain Patholology**, 13(4), 440-51, 2003.

ANSOLOAGA, B. et al. Deregulation of purine metabolism in Alzheimer's disease. **Neurobiology of Aging**, 36(1), 68–80, 2015.

ARENDASH, G.W. et al. Caffeine reverses cognitive impairment and decreases brain amyloid-beta levels in aged Alzheimer's disease mice. **Journal Alzheimers Disease**, 17(3), 661–80, 2009.

ARLUISON, M. et al. Distribution and anatomical localization of the glucose transporter 2 (GLUT2) in the adult rat brain--an immunohistochemical study. **Journal of Chemical Neuroanatomy**, 28(3), 117-136, 2004.

ARRANZ, A. M.; STROOPER B. The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications. **The Lancet Neurology,** 18(4), 406-414, 2019.

ASHFORD, J. W. Treatment of Alzheimer's Disease: The Legacy of the Cholinergic Hypothesis, Neuroplasticity, and Future Directions. **Journal of Alzheimer's Disease**, 47(1), 149-156, 2015.

AULD, D. S. et al. Alzheimer’s disease and the basal forebrain cholinergic system: relations to β -amyloid peptides, cognition and treatment strategies. **Progress in Neurobiology**, 68(3),209–245, 2002.

BAILEY, C. H.; KANDEL, E. R.; HARRIS, K. M. Structural Components of Synaptic Plasticity and Memory Consolidation. **Cold Spring Harbor Perspectives in Biology**, 7(7), a021758, 2015.

BAJRAMOVIC J.J. Regulation of innate immune responses in the central nervous system. **CNS & Neurological Disorders-Drug Targets,** 10(1),4-24, 2011.

BARANKIEWICZ, J.; COHEN, A. Purine nucleotide metabolism in resident and activated rat macrophages in vitro. **European Journal of Immunology**, 15(6), 627-31,1985.

BEKINSCHTEIN, P. et al. BDNF is essential to promote persistence of long-term memory storage. **Proceedings of the National Academy of Sciences**, 105(7), 2711-2716, 2008.

BENARROCH, E. E. Na+, K+-ATPase: Functions in the nervous system and involvement in neurologic disease. **Neurology**, 76(3), 287-293, 2011.

BENOWITZ, L. I. et al. Axon outgrowth is regulated by na intracellular purine-sensitive mechanism in retinal ganglion cells. **Journal of Biological Chemistry**, 273(45), 29626– 29634, 1998.

BERAN, J.; Salapová, E.; Spajdel, M. Inosine pranobex is safe and effective for the treatment of subjects with confirmed acute respiratory viral infections: analysis and subgroup analysis from a Phase 4, randomised, placebo-controlled, double-blind study. **The Journal of Infectious Diseases**,16(1), 648, 2016.

BERCHTOLD, N. C.; COTMAN, C. W. Evolution in the conceptualization of dementia and Alzheimer’s disease: Greco-Roman period to the 1960s. **Neurobiology of Aging**, 19(3),173-89,1998.

BLENNOW, K.; LEON, M. J.; ZETTERBERG, H. Alzheimer's disease. **Lancet**, 368(9533), 387–403, 2006.

BLOKLAND, A.; JOLLES, J. Behavioral and biochemical effects of an ICV injection of streptozotocin in old Lewis rats. **Pharmacology Biochemistry and Behavior**, 47(4),833–837, 1994.

BLUM, F. E.; ZUO, Z. Volatile anesthetics-induced neuroinflammatory and anti-inflammatory responses. **Medical Gas Research**, 3(1), 16, 2013.

BOISON, D.; CHEN, J. F.; FREDHOLM, B. B. Adenosine signaling and function in glial cells. **Cell Death Differention**, 17(7), 1071-82, 2010.

BUDNI, J. et al. The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. **Aging and Disease**, 6(5) 331, 2015.

BURNSTOCK, G. et al. Purinergic signalling: From normal behaviour to pathological brain function. **Progress in Neurobiology**, 95(2), 229–274, 2011.

BURNSTOCK, G. Purinergic signalling: Past, present and future**. Brazilian Journal of Medical and Biological Research,** 42(1), 3–8, 2009.

BURNSTOCK, G. Purinergic Signalling: Therapeutic Developments. **Frontiers in Pharmacology**, 8,661, 2017.

BUTTERFIELD, A.; HALLIWELL, B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. **Nature Reviews Neuroscience**, 20(3), 148–160, 2019.

BUTTERFIELD, D. A; MATTSON, M. P. Apolipoprotein E and oxidative stress in brain with relevance to Alzheimer's disease. **Neurobiology of Disease,** 138, 104795, 2020.

CALDEIRA, M. V. et al. BDNF regulates the expression and traffic of NMDA receptors in cultured hippocampal neurons. **Molecular and Cellular Neuroscience**, 35(2), 208–219, 2007.

CALSOLARO, V.; EDISON, P. Neuroinflammation in Alzheimer's disease: Current evidence and future directions. **Alzheimer's & Dementia,** 12(6),719-32, 2016.

CAO, C., et al. Caffeine suppresses amyloid-beta levels in plasma and brain of Alzheimer's disease transgenic mice. **Journal Alzheimers Disease,**17(3), 681–97. Erratum in: **Journal Alzheimers Disease**, 18(3),727, 2009.

CARVALHO, F. B. et al. Anthocyanins control neuroinflammation and consequent memory dysfunction in mice exposed to lipopolysaccharide. **Molecular Neurobiology,** 54(5), 3350-3367, 2017.

CARVALHO, F. B. et al. Anthocyanins suppress the secretion of proinflammatory mediators and oxidative stress and restore ion pump activities in demyelination. **The Journal of Nutritional Biochemistry**, 26(4), 378-390, 2015.

CELLAI, L. et al. The Adenosinergic Signaling: A Complex but Promising Therapeutic Target for Alzheimer's Disease. **Frontiers in Neuroscience**, 12, 520, 2018.

CHAKRABORTY, A.; DIWAN, A. "Alzheimer and It's Possible Therapy: A Review." **Journal of Experimental Neurology**, 1(4), 115-122, 2020.

CHEN, P. et al. Inosine induces axonal rewiring and improves behavioral outcome after stroke. **Proceedings of the National Academy of Sciences**, 99(13), 9031–9036, 2002.

CHEN, X. et al. Caffeine blocks disruption of blood brain barrier in a rabbit model of Alzheimer's disease. **Journal Neuroinflammation**, 5(1), 12-14, 2008.

CHEN, Y. G. Research Progress in the Pathogenesis of Alzheimer's Disease. **Chinese Medical Journal**, 131(13),1618–1624, 2018.

CHENG, Y.; JIANG, D. H. Therapeutic effect of inosine in Tourette syndrome and its possible mechanism of action. **Chinese Journal of Neurology and Psychiatry**, 23(2), 90–3, 1990.

CHU, Y.F. et al. Crude caffeine reduces memory impairment and amyloid β (1–42) levels in an Alzheimer's mouse model. **Food Chemestry**, 135(3),2095–2102, 2012.

CIEŚLAK, M.; WOJTCZAK, A. Role of purinergic receptors in the Alzheimer's disease. **Purinergic Signalling**, 14(4), 331–344, 2018.

ClinicalTrials.gov: NCT02279511Disponível em: https://clinicaltrials.gov/ct2/show/NCT02279511 Acesso em: 27/6/19

COHEN, J.; TORRES, C. Astrocyte senescence: evidence and significance. **Aging Cell**, 18(3), 12937, 2019.

CONNOR, B. et al. "Brain-derived neurotrophic factor is reduced in Alzheimer's disease." **Molecular brain research**, 49(1-2), 71-81, 1997.

COSTA, M.S. et al. Caffeine prevents age-associated recognition memory decline and changes brain-derived neurotrophic factor and tyrosine kinase receptor (TrkB) content in mice. **Neuroscience**, 153(4), 1071-1078, 2008.

COUTURIER, J. et al. Inhibition of double-stranded RNA-dependent protein kinase strongly decreases cytokine production and release in peripheral blood mononuclear cells from patients with Alzheimer's disease. **Journal of Alzheimer's Disease,** 21(4):1217-1231, 2010.

CRYSTAL, J.; GLANZMAN, D. A biological perspective on memory. **Current Biology**, 23(17), 728-31, 2020.

CROTTY, G.; ASCHERIO, A.; SCHWARZSCHILD, M. Targeting urate to reduce oxidative stress in Parkinson disease. **Experimental neurology**, 298, 210-224, 2017.

CUMMINGS, J. et al., Alzheimer's disease drug development pipeline, **Alzheimers Dementia**, 5, 272-293, 2019.

CUNHA, C.; BRAMBILLA, R.; THOMAS, K. L. A simple role for BDNF in learning and memory? **Frontiers in molecular neuroscience**, 3, 1, 2010.

CUNHA, R.; AGOSTINHO, P. Chronic Caffeine Consumption Prevents Memory Disturbance in Different Animal Models of Memory Decline. **Journal of Alzheimer's Disease**, 20(1), 95–116, 2010.

CUNHA, R. Neuroprotection by adenosine in the brain: From A1 receptor activation to A2A receptor blockade. **Purinergic Signalling**, 1(2), 111–134, 2005.

CUPINO, T.L.; ZABEL, M.K. Alzheimer's silent partner: cerebral amyloid angiopathy. **Translational Stroke Research**, 5(3), 330-337, 2013.

DA ROCHA-LAPA, et al. Anti-inflammatory effects of inosine in allergic lung inflammation in mice: evidence for the participation of adenosine A 2A and A 3 receptors. **Purinergic Signalling**, 3(9), 325-336, 2013.

DACHIR, S. et al. Inosine improves functional recovery after experimental traumatic brain injury. **Brain research**, 1555, 78-88, 2014.

DALL'LGNA, O. P. et al. Neuroprotection by caffeine and adenosine A 2A receptor blockade of β-amyloid neurotoxicity. **British Journal of Pharmacology**, 138(7), 1207– 1209, 2003.

DE OLIVEIRA, J. S. et al. Neuroprotective effects of berberine on recognition memory impairment, oxidative stress, and damage to the purinergic system in rats submitted to intracerebroventricular injection of streptozotocin. **Psychopharmacology**, 236(2), 641-655, 2018.

DEBOM, G. et al. Preventive effects of blueberry extract on behavioral and biochemical dysfunctions in rats submitted to a model of manic behavior induced by ketamine. **Brain Research Bulletin**, 127, 260-269, 2016.

DEKOSKY, S. T.; SCHEFF, S. W. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. **Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society**, 27(5), 457-464,1990.

DERECKI, N.C. et al. "Regulation of learning and memory by meningeal immunity: a key role for IL-4." **Journal of Experimental Medicine,** 207(5), 1067-1080, 2010.

DESFRERE, L. et al. Na, K-ATPase signal transduction triggers CREB activation and dendritic growth. **Proceedings of the National Academy of Sciences**, 106(7), 2212–2217, 2009.

DESHMUKH, R. et al. Caffeic acid attenuates oxidative stress, learning and memory deficit in intra-cerebroventricular streptozotocin induced experimental dementia in rats. **Biomedicine & Pharmacotherapy**, 81, 56-62, 2016.

DHULL, D. K.; KUMAR, A. Tramadol ameliorates behavioural, biochemical, mitochondrial and histological alterations in ICV-STZ-induced sporadic dementia of Alzheimer's type in rats. **Inflammopharmacology**, 26(4), 925-938, 2018.

DICKEY, C. A. et al. Dysregulation of Na+/K+-ATPase by amyloid in APP + PS1 transgenic mice. **Neuroscience**, 6(1), 1-11, 2005.

DUELLI, R. et al. Intracerebroventricular injection of streptozotocin induces discrete local changes in cerebral glucose utilization in rats. **International Journal of Developmental Neuroscience**, 12(8), 737-743, 1994.

DOYLE, C. et al. Inosine-a Multifunctional Treatment for Complications of Neurologic Injury. **Cellular Physiology and Biochemistry,** 49(6), 2293-2303, 2018.

ESPAÑA, J. et al. β-Amyloid interrupts the transcription of the gene dependent on the activity required for memory through the CREB CRTC1 coactivator." **Journal of Neuroscience**, 30(28), 9402-9410, 2010.

EUSER, S. et al. Serum uric acid and cognitive function and dementia. **Brain**,132(2), 377-382, 2009.

FAHNESTOCK, M. Brain -derived neurotrophic factor: the link between amyloid -β and memory loss. **Future Neurology**, 6(5) 627-639, 2011.

FARHY-TSELNICKER, I.; ALLEN, N.J. Astrocytes, neurons, synapses: a tripartite view on cortical circuit development. **Neural Development**, 13(1), 1-12, 2018.

FARLOW, M. R.; MILLER, M. L.; PEJOVIC, V. Treatment options in Alzheimer's disease: Maximizing benefit, managing expectations. **Dementia and Geriatric Cognitive Disorders**, 25(5), 408–422, 2008.

FARTHING, D. E.; FARTHING, C. A.; XI, L. Inosine and hypoxanthine as novel biomarkers for cardiac ischemia: from bench to point-of-care. **Experimental Biology and Medicine**, 240(6), 821-831, 2015.

FEMMINELLA, G.D. et al. "Microglial activation in early Alzheimer trajectory is associated with higher gray matter volume." **Neurology**, 92(12), e1331-e1343, 2019.

FERREIRA-VIEIRA, T. H. et al. Alzheimer's Disease: Targeting the Cholinergic System. **Current Neuropharmacology**, 14(1), 101-115, 2016.

FERRER, I. Defining Alzheimer as a common age-related neurodegenerative process not inevitably leading to dementia. **Progress in Neurobiology**, 97(1), 38–51, 2012.

FERRETTI, E. et al. Canonical and non-canonical adenosinergic pathways. **Immunology Letters**, 205, 25–30, 2019.

FITZPATRICK, J.M. et al. Inosine in ischaemic renal surgery: long-term follow-up. **British Journal of Urology**, 53(6), 524–7, 1981.

FOUDA, A. Y. et al. "Anti-inflammatory IL-10 is upregulated in both hemispheres after experimental ischemic stroke: Hypertension blunts the response." **Experimental & Translational Stroke Medicine**, 5(1), 1-12, 2013.

FREDHOLM, B. B. et al. Adenosine and brain fuctions. **International Review of Neurobiology**, 63(1), 191-270, 2005.

FREDHOLM, B.B. et al. Nomenclature and classification of purinoceptors. **Pharmacological Reviews**, 46(2), 143-156, 1994.

FURINI, C. R. G. et al. New frontiers in the study of memory mechanisms. **Brazilian Journal of Psychiatry**, 35(2),173–177, 2013.

GADANI, S. P. et al. IL-4 in the Brain: A Cytokine to Remember. **Journal of Immunology**,189(9), 4213-4219, 2012.

GAHR, M. et al. Cerebral amyloidal angiopathy— a disease with implications for neurology and psychiatry. **Brain research,**1519, 19-30, 2013.

GAMBI, F. et al. Alzheimer Patients Treated with an AchE Inhibitor Show Higher IL-4 and Lower IL-1b Levels and Expression in Peripheral Blood Mononuclear Cells. **Journal of Clinical Psychopharmacology**, 24(3), 314–321, 2004.

GHAI, R. et al. Current strategies and novel drug approaches for Alzheimer disease. **CNS & Neurological Disorders-Drug Targets** (Formerly Current Drug Targets-CNS & Neurological Disorders), 2020.

GOEDERT, M.; SPILLANTINI, M. G. A Century of Alzheimer's Disease. **Science**, 777-81, 2006.

GOMES, F. C. A.; TORTELLI, V. P.; DINIZ, L. Glia: dos velhos conceitos às novas funções de hoje e as que ainda virão. **Estudos avançados**, 27(77), 61-84, 2013.

GOMEZ, G.; SITKOVSKY, M. V. Differential requirement for A2A and A3 adenosine receptors for the protective effetc of inosine in vivo. **Blood**, 102(13), 4472-8, 2003.

GONÇALVES, F. M. et al. Signaling pathways underlying the antidepressant-like effects of inosine in mice. **Purinergic Signalling**, 13(2), 203–214, 2016.

GRAHAM, W. V.; BONITO-OLIVA, A.; SAKMAR, T. P. Update on Alzheimer's disease therapy and prevention strategies. **Annual Review of Medicine**, 68, 413-430, 2017.

GRAY, S. C.; KINGHORN, K. J.; WOODLING, N. S. "Shifting equilibriums in Alzheimer's disease: The complex roles of microglia in neuroinflammation, neuronal survival and neurogenesis." **Neural Regeneration Research**,15(7), 1208, 2020.

GREENHALGH, A. D., DAVID, S., BENNETT, F. C. Immune cell regulation of glia during CNS injury and disease. **Nature Reviews Neuroscience**, 21, 1-14, 2020.

GREENWALD, B. S.; DAVIS, K. Experimental pharmacology of Alzheimer disease. **Advances in neurology**, 38, 87–102, 1983.

GRIEB, P. Intracerebroventricular streptozotocin injections as a model of Alzheimer's disease: in search of a relevant mechanism. **Molecular neurobiology**, 53(3), 1741-1752, 2016.

GUILLOT-SESTIER, M. V.; TOWN, T. Innate immunity in Alzheimer's disease: a complex affair. **CNS & Neurological Disorders-Drug Targets** (Formerly Current Drug Targets-CNS & Neurological Disorders), 12(5), 593-607, 2013.

GUMUSYAYLA, S. et al. A novel oxidative stress marker in patients with Alzheimer's disease: dynamic thiol-disulphide homeostasys. **Acta Neuropsychiatrica**, 28(6), 315-320, 2016.

GUO, Y. et al. Design, Synthesis, and Evaluation of Acetylcholinesterase and Butyrylcholinesterase Dual-Target Inhibitors against Alzheimer's Diseases. **Molecules**, 25(3), 489, 2020.

GUTIERRES, J. M. et al. Protective effects of anthocyanins on the ectonucleotidase activity in the impairment of memory induced by scopolamine in adult rats. **Life Sciences**, 91(23-24), 1221–1228, 2012.

GUTIERRES, J.M. et al. Anthocyanins restore behavioral and biochemical changes caused by streptozotocin-induced sporadic dementia of Alzheimer's type. **Life Science**, 96(1-2), 7-17, 2014.

HA, Z. Y.; MATHEW, S.; YEONG, K. Y. Butyrylcholinesterase: A Multifaceted Pharmacological Target and Tool. **Current Protein and Peptide Science**, 21(1), 99-109, 2020.

HALLIDAY, M.R. et al. Accelerated pericyte degeneration and blood–brain barrier breakdown in apolipoprotein E4 carriers with Alzheimer's disease. **Journal of Cerebral Blood Flow & Metabolism,** 36(1), 216-227, 2015.

HALLIWELL, B.; GUTTERIDGE, J. M. C. The Definition and Measurement of Antioxidants in Biological Systems. **Free Radical Biology and Medicine**, 18, 125-126, 1995.

HALLIWELL, B. Free radicals and antioxidants: updating a personal view. **Nutrition Reviews**, 70(5), 257-65, 2012.

HALLIWELL, B. Oxidative stress and neurodegeneration: where are we now? **Journal of Neurochemistry**, 97(6), 1634-1658, 2006.

HAMPEL, H. et al. On behalf of the Alzheimer Precision Medicine Initiative (APMI) A Path Toward Precision Medicine for Neuroinflammatory Mechanisms in Alzheimer's Disease. **Frontiers in Immunology,** 11, 456, 2020.

HAMPEL, H. et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. **Brain**, 141(7),1917-33, 2018.

HASKÓ, G.; SITKOVSKY, M. V.; SZABÓ C. Immunomodulatory and neuroprotective effects of inosine. **Trends in Pharmacological Sciences**, 25(3), 152-7, 2004.

TANILA, H. The role of BDNF in Alzheimer's disease, 2017.

HENEKA, M. T. et al. Neuroinfl ammation in Alzheimer’s disease. **Lancet Neurology**, 388-405, 2015.

HIPPIUS, H.; NEUNDORFER, G. The discovery of Alzheimer's disease. **Dialogues in Clinical Neuroscience**, 5(1), 101–108, 2003.

HITSCHKE, K. et al. Inactivation of the na, k-atpase by radiation-induced free radicals’ evidence for a radical-chain mechanism**. FEBS LETTERS**, 353(3), 297-300,1994.

HOLSINGER, R. M. D. et al. Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction: decreased levels in Alzheimer's disease. **Molecular Brain Research**, 76 (2), 347-354, 2000.

HOSOKAWA, M.; DOLCI, W.; THORENS, B. Differential Sensitivity of GLUT1- and GLUT2-Expressingβ-Cells to Streptozotocin. **Biochemical and Biophysical Research Communications**, 289(5), 1114–17, 2001.

HOYER, S. Causes and consequences of disturbances of cerebral glucose metabolism in sporadic Alzheimer disease: therapeutic implications. **Frontiers in Clinical Neuroscience**, 135-152, 2004.

INSTITUTO ALZHEIMER BRASIL- IAB Disponível em: https://www.institutoalzheimerbrasil.org.br/ acesso em: outubro de 2019.

HUANG, L. K.; CHAO, S. P.; HU, C. J. Clinical trials of new drugs for Alzheimer disease. **Journal of Biomedical Science**, 27(1), 1-13, 2020.

HUANG, W. J; ZHANG, X. I. A.; CHEN, W. W. Role of oxidative stress in Alzheimer's disease. **Biomedical reports**, 4(5), 519-522, 2016.

IDZKO, M.; FERRARI, D.; ELTZSCHIG, H. K. Nucleotide signalling during inflammation. **Nature**, 509(7500), 310–317, 2014.

INNISS, M. C.; SILVER, P. A. Building synthetic memory. **Current Biology**, 23(17), 812-816, 2013.

IZQUIERDO, I. et al. Pharmacological Findings on the Biochemical Bases of Memory Processes. **Neural Plasticity,** 11, 159–190, 2004.

IZQUIERDO, I. et al. Separate mechanisms for short- and long-term memory. **Behavioural Brain Research**, 103(1), 1-11,1999.

IZQUIERDO, I.; MCGAUGH, J. L. Behavioural pharmacology and its contribution to the molecular basis of memory consolidation. **Behavioural pharmacology**, 11(7), 517-534, 2000.

IZQUIERDO, I.; FURINI, C. R. G.; MYSKIW, J. C. Fear Memory. **Physiological Reviews**, 96(2), 695-750, 2016.

JERONIMO-SANTOS, A. et al. "Impact of in vivo chronic blockade of adenosine A2A receptors on the BDNF-mediated facilitation of LTP." **Neuropharmacology**, 83, 99-106, 2014.

JIN, X. et al. Inosine binds to A3 adenosine receptors and stimulates mast cell degranulation. **Journal of Clinical Investigation**, 100(11), 2849–57, 1998.

JUNQUEIRA, S. C. et al. Inosine, an Endogenous Purine Nucleoside, Suppresses Immune Responses and Protects Mice from Experimental Autoimmune Encephalomyelitis: Role for A2A Adenosine Receptor. **Molecular Neurobiology**, 54(5), 3271-3285, 2016.

KALARIA, R. N. et al. Alzheimer’s disease and vascular dementia in developing countries: prevalence, management, and risk factors. **The Lancet Neurology**, 7(9), 812–826, 2008.

KAMAT, P. K. et al. Mechanism of synapse redox stress in Okadaic acid (ICV) induced memory impairment: Role of NMDA receptor. **Neurochemistry International**, 76, 32–41, 2014.

KELLY C. C. et al. Low-grade chronic inflammation in women with polycystic ovarian syndrome. **The Journal of Clinical Endocrinology and Metabolism**, 86(6), 2453–2455, 2001.

KIM, D. et al. Inosine Enhances Axon Sprouting and Motor Recovery after Spinal Cord Injury. **Plos one**, 8(12), e81948, 2013.

KORTE, M.; SCHMITZ, D. Cellular and System Biology of Memory: Timing, **Molecules, and Beyond Physiological Reviews**, 96(2), 647-693, 2016.

KOUHESTANI, S.; JAFARI, A.; BABAEI P. Kaempferol attenuates cognitive deficit via regulating oxidative stress and neuroinflammation in an ovariectomized rat model of sporadic dementia. **Neural Regeneration Research**, 13(10), 1827, 2018.

KRAFT, A. D.; HARRY, G. J. Features of microglia and neuro-inflammation relevant to environmental exposure and neurotoxicity. **International Journal of Environmental Research and Public Health**, 8(7), 2980–3018, 2011.

KRASKA, A. et al. In vivo cross-sectional characterization of cerebral alterations induced by intracerebroventricular administration of streptozotocin. **PLoS One**, 7(9), e46196, 2012.

KROLAK-SALMON, P. et al. France will no more reimburse available symptomatic drugs against Alzheimer's disease. **Journal Alzheimers Disease**, 66(2), 425–7, 2018.

KURICOVA, K. et al. Oral administration of inosine promotes recovery after experimental spinal cord injury in rat. **Neurological Sciences**, 35(11), 1785-91, 2014.

LANGE, K W. et al. Do natural antioxidants play a role in Alzheimer's disease? **Journal of Food Bioactives**, 11, 2020.

LANGMEAD, C. J.; WATSONA, J.; REAVILLA, C. Muscarinic acetylcholine receptors as CNS drug targets. **Pharmacology & Therapeutics**, 117(2), 232-43, 2008.

LANNERT, H.; HOYER, S. Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. **Behaviour Neuroscience**, 112(5), 1199-1208, 1998.

LATTA, C.; BROTHERS, H. M.; WILCOCK, D. M. Neuroinflammation in Alzheimer's disease; a source of heterogeneity and target for personalized therapy. **Neuroscience**, 302, 103-111, 2014.

LEAL, G.; BRAMHAM, C. R.; DUARTE, C. B. BDNF and hippocampal synaptic plasticity. **Vitamins and Hormones**, 104, 153-95, 2017.

LEE, H. G. et al. Microglial P2X7 receptor expression is accompanied by neuronal damage in the cerebral cortex of the APPswe/PS1dE9 mouse model of alzheimer's disease. **Experimental and Molecular Medicine**, 43(1), 7–14, 2011.

LEE, Y. S.; SILVA, A. J. The molecular and cellular biology of enhanced cognition. **Nature Reviews Neuroscience**, 10 (2),126, 2009.

LEES, G. J. et al. The neurotoxicity of ouabain, a sodium-potassium ATPase inhibitor, in the rat hippocampus. **Neuroscience Letters**, 120(2),159-62, 1990.

LI, C. et al. Astrocytes: implications forneuroinflammatory pathogenesis of Alzheimer's disease. **Current Alzheimer Research**, 8(1), 67-80, 2011.

LIPTON, A. S.; ROSENBERG, P. A. Excitatory amino acids as a final common pathway for neurologic disorders. **New England Journal of Medice**, 330(9), 613-22,1994.

LIU, Y. J. et al. Research progress on adenosine in central nervous system diseases. **CNS Neuroscience & therapeutics,** 25(9), 899-910, 2019.

LÖWE, L. C.; GASER, C.; Franke, K. Alzheimer's Disease Neuroimaging Initiative. The effect of the APOE genotype on individual Brain AGE in normal aging, mild cognitive impairment, and Alzheimer's disease. **PloS one**, 11(7), 2016.

LUCHENA, C. et al. Contribution of Neurons and Glial Cells to Complement-Mediated synapse Removal During Development, Aging and In Alzheimer's Disease. **Mediators of Inflammation**, 2018.

MATA, A. M.; BERROCAL, M. and SEPÚLVEDA, M. R. Impairment of the activity of the plasma membrane Ca2+-ATPase in Alzheimer's disease, 819-822, 2011.

MABLEY, J. G. et al. The novel inosine analogue INO-2002 exerts an anti-inflammatory effect in a murine model of acute lung injury. **Shock**, 32(3), 258, 2009.

MAJKUTEWICZ, I. et al., Age-dependent effects of dimethyl fumarate on cognitive and neuropathological features in the streptozotocin-induced rat model of Alzheimer's disease. **Brain Research**, 1686, 19-33, 2018.

MAO, P.; REDDY, P. H. Aging and amyloid beta-induced oxidative DNA damage and mitochondrial dysfunction in Alzheimer's disease: Implications for early intervention and therapeutics. **Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease**, 1812(11), 1359-70, 2011.

MARISCO, P.C. et al. Piracetam prevents scopolamine-induced memory impairment and decrease of NTPDase, 5′-nucleotidase and adenosine deaminase activities. **Neurochemical Research**, 38(8), 1704–14, 2013.

MARKESBERY, W. R.; CARNEY, J. M. Oxidative alterations in Alzheimer's disease. **Brain pathology**, 9(1), 133-46, 1999.

MARKOWITZ, C. E. et al. The treatment of multiple sclerosis with inosine. **Journal of Alternative and Complementary Medicine**, 15(6), 619-25, 2009.

MARSH, S. E. et al. The adaptive immune system restrains Alzheimer's disease pathogenesis by modulating microglial function. **Proceedings of the National Academy of Sciences of the United States of America**, 113(9), 1316–25, 2016.

MCGEER, P. L. et al. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. **Neurology**, 38(8), 1285, 1988.

MCKHANN, G, et al. Clinical diagnosis of Alzheimer's disease Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. **Neurology**, 34(7), 939,1984.

MCLARNON, J. G. et al. Upregulated expression of purinergic P2X(7) receptor in Alzheimer disease and amyloid-beta peptide-treated microglia and in peptide-injected rat hippocampus. **Journal of Neuropathology and Experimental Neurology**, 65(11), 1090–97, 2006.

MEDEIROS, R.; LAFERLA, F. M. Astrocytes: conductors of the Alzheimer disease neuroinflammatory symphony. **Experimental neurology**, 239, 133–8, 2013.

MINTER, M.; TAYLOR, J.; CRACK, P. The contribution of neuroinflammation to amyloid toxicity in Alzheimer's disease. **Journal of Neurochemistry**, 136(3), 457–74, 2016.

MOBLEY, W. C.; CHEN, X. Exploring the pathogenesis of Alzheimer disease in basal forebrain cholinergic neurons: converging insights from alternative hypotheses. **Frontiers in Neuroscience**, 13, 446, 2019.

MOORE, A. M. et al. ApoEε4-specific associations of vegf gene family expression with cognitive aging and alzheimer's disease. **Neurobiology Aging**, 87, 18-25, 2019.

MORE, S. V. et al. Toxin-induced experimental models of learning and memory impairment. **International Journal of Molecular Sciences,** 17(9), 1447, 2016.

MOSELEY, A. E. et al. Deficiency in Na, K-ATPase α isoform genes alters spatial learning, motor activity, and anxiety in mice. **The Journal of Neuroscience**, 27(3), 616- 626. 2007.

MUTO, J. et al. Oral administration of inosine produces antidepressantlike effects in mice. **Scientific Reports**, 4, 4199, 2014.

NABBI-SCHROETER, D. et al. Effects of long-term caffeine consumption on the adenosine A 1 receptor in the rat brain: An in vivo PET study with [18 F] CPFPX. **Molecular Imaging and Biology,** 20(2), 284-291, 2018.

NASCIMENTO, F. P. et al. Inosine Reduces Pain-Related Behavior in Mice: Involvement of Adenosine A1 and A2A Receptor Subtypes and Protein Kinase C Pathways. **The Journal of Pharmacology and Experimental Therapeutics**, 334(2), 590-598, 2010.

NAZEM, A. et al. Rodent models of neuroinflammation for Alzheimer's disease. **Journal of Neuroinflammation,** 12(1), 74, 2015.

NESTOR, P. J.; SCHELTENS, P.; HODGES, J. R. Advances in the early detection of Alzheimer's disease. **Nature Reviews Neuroscience**, 10(7), 34-41, 2004.

NICHOLLS. D. G. Bioenergetics and transmitter release in the isolated nerve terminal. **Neurochemistry Research**, 28(10), 1433-41, 2003.

NIEUWENHUYS, R.; VOOGD, J.; CHRISTIAAN V. H. The human central nervous system: a synopsis and atlas. **Springer Science & Business Media**, 2007.

OLABARRIA, M. et al. Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. **Glia**, 58(7), 831-838, 2010.

OLIVEIRA, P. et al. Southern Brazilian native fruit shows neurochemical, metabolic and behavioral benefits in an animal model of metabolic syndrome. **Metabolic Brain Disease**, 33(5), 1551-62, 2018.

OPHIR, G. et al. Apolipoprotein E4 enhances brain inflammation by modulation of the NF-KB signaling cascade. **Neurobiology of Disease**, 20(3), 709-18, 2005.

ORTEGA, F. et al. P2X7, NMDA and BDNF receptors converge on GSK3 phosphorylation and cooperate to promote survival in cerebellar granule neurons. **Cellular and Molecular Life Sciences**, 67(10), 1723–33, 2010.

OSBORN, L. M. et al. Astrogliosis: an integral player in the pathogenesis of Alzheimer's disease. **Progress in neurobiology**, 144, 121-41, 2016.

PACHECO, S. et al. Anthocyanins as a potential pharmacological agent to manage memory deficit, oxidative stress and alterations in ion pump activity induced by experimental sporadic dementia of Alzheimer's type. **Journal of Nutritional Biochemistry**, 56, 193-204, 2018.

PAGANONI, S.; SCHWARZSCHILD, M. A. Urate as a Marker of Risk and Progression of Neurodegenerative Disease. **Neurotherapeutics**, 14(1), 148–53, 2017.

PAGNUSSAT, N. et al. Adenosine A2A receptors are necessary and sufficient to trigger memory impairment in adult mice. **British Journal of Pharmacology**, 172(15), 3831-45, 2015.

PARVATHENANI, L. K. et al. P2X7 mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer’s disease. **Journal of Biological Chemistry**, 278(15),13309–17, 2003.

PENG, S. et al. Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. **Journal of Neurochemistry**, 93(6), 1412–21, 2005.

PEREZ-ASENSIO, F. J. et al. "Interleukin-10 regulates progenitor differentiation and modulates neurogenesis in adult brain." **Journal of Cell Science**, 126(18),4208-4219, 2013.

PHILLIPS, E. C. et al. Astrocytes and neuroinflammation in Alzheimer's disease. **Biochemical Society Transactions**, 2014.

SANTOS, P. et al. Alzheimer's Disease: A Review from the Pathophysiology to Diagnosis, New Perspectives for Pharmacological Treatment. **Current Medicinal Chemistry**, 25(26), 3141-59, 2018.

PICCIOTTO, M. R.; HIGLEY, M. J.; MINEUR, Y. S. Acetylcholine as a Neuromodulator: Cholinergic Signaling Shapes System Nervous System Function and Behavior. **Neuron**, 76(1), 16–29, 2012.

PRADO, V. F. et al. Mice deficiente for the vesicular acetylcholine transporter are myasthenic and have deficits in object and social recognition. **Neuron**, 51(5), 601-12, 2006.

PRESTON, A. R.; EICHENBAUM, H. Interplay of hippocampus and prefrontal cortex in memory. **Current Biology**, 23(17), 764–773, 2013.

PRINCE, M. et al. The global prevalence of dementia: a systematic review and metaanalysis. **Alzheimers Dement**, 9(1), 63-75, 2013.

PUGAZHENTHI, S. et al. Downregulation of CREB expression in Alzheimer's brain and in Aβ -treated rat hippocampal neurons. **Molecular neurodegeneration**, 6(1), 60, 2011.

RAHMAN, A. The Role of Adenosine in Alzheimer's Disease Current. **Neuropharmacology**, 7(3), 207-16, 2009.

RALEVIC, V.; BURNSTOCK, G. Receptors for purines and pyrimidines. **Pharmacological Reviews**, 50(3), 413-92, 1998.

RAMSER, E. M. et al. Amyloid-β oligomers induce tau-independent disruption of BDNF axonal transport via calcineurin activation in cultured hippocampal neurons. **Molecular biology of the cell**, 24(16), 2494-2505, 2013.

RAVELLI, K. G. et al. NADPH oxidase contributes to streptozotocin-induced neurodegeneration. **Neuroscience**, 358, 227-237, 2017.

REISS, A. B. et al. Adenosine A2A receptor occupancy stimulates expression of proteins involved in reverse cholesterol transport and inhibits foam cell formation in macrophages. **Journal of Leukocyte Biology**, 76(3), 727-734, 2004.

RIVERA-OLIVER, M.; DÍAZ-RÍOS, M. Using caffeine and other adenosine receptor antagonists and agonists as therapeutic tools against neurodegenerative diseases: a review. **Life Sciences**,101(1-2),1-9, 2014.

ROSA, E.; FAHNESTOCK, M. CREB expression mediates amyloid β -induced basal BDNF downregulation. **Neurobiology of Aging**, 36(8), 2406 -13, 2015.

RUHAL, P.; DHINGRA, D. Inosine improves cognitive function and decreases aging induced oxidative stress and neuroinflammation in aged female rats. **Inflammopharmacology**, 26(5), 1317-1329, 2018.

RYU, J. K.; MCLARNON, J. G. Block of purinergic P2X7 receptor is neuroprotective in an animal model of Alzheimer's disease. **NeuroReport,**19(17), 1715–19, 2008.

SALKOVIC-PETRISIC, M.; HOYER, S. Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: an experimental approach. **Journal of Neural Transmission**, 72, 217-33, 2007.

SALKOVIC-PETRISIC, M. et al. What have we learned from the streptozotocin-induced animal model of sporadic Alzheimer's disease, about the therapeutic strategies in Alzheimer's research. **Journal of Neural Transmission**, 120(1), 233–52, 2013.

SASI, M. et al. "Neurobiology of local and interellular BDNF signaling." **Pflügers Archiv-European Journal of Physiology**, 469 (5-6), 593-610, 2017.

SAWIKR, Y. et al. Neuroinflammation in Alzheimer's disease: the preventive and therapeutic potential of polyphenolic nutraceuticals. In: Advances in protein chemistry and structural biology. **Academic Press**, 108, 33-57, 2017.

SCHELTENS, P. et al. Alzheimer's disease. **Lancet**, 388 (10043), 505-17, 2016.

SCHETTERS, S. T. T. et al. Neuroinflammation: microglia and T cells get ready to tango. **Frontiers Immunology**, 8 (1905), 1-8, 2017.

SCHLIEBS, R.; ARENDT, T. The cholinergic system in aging and neuronal degeneration. **Behavioural Brain Research**, 221(2), 555-63, 2011.

SCHLIEBS, R.; ARENDT, T. The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. **Journal of Neural Transmission**, 113(1), 1625-44, 2006.

SCHWARZSCHILD, M.A. et al. Inosine to increase serum and cerebrospinal fluid urate in Parkinson disease: a randomized clinical trial. **JAMA Neurology**, 71(2), 141-50, 2014.

SEBASTIAO, A. M.; RIBEIRO, J. A. Adenosine receptors and the central nervous system. **Handbook of Experimental Pharmacology**, 7, 471-534, 2009.

SELKOE, D. J.; HARDY, J. The amyloid hypothesis of Alzheimer's disease at 25 years. **EMBO Molecular Medicine**, 8(6), 595–608, 2016.

SHIN, M. K. et al. Neuropep-1 ameliorates learning and memory deficits in an Alzheimer's disease mouse model, increases brain-derived neurotrophic factor expression in the brain, and causes reduction of amyloid beta plaques. **Neurobiology of Aging,** 35(5), 990-1001, 2014.

SHYTLE, R. D. et al. Cholinergic modulation of microglial activation by α7 nicotinic receptors. **Journal Neurochemestry**, 89(2), 337–43, 2004.

SJOBERG, E. A. Logical fallacies in animal model research. **Behavioral and Brain Functions,** 13(1), 1-13, 2017.

SKOU, J. C.; ESMANN, M. The Na, K-ATPase. **Journal of Bioenergetics and Biomembranes**, 24, 249-261, 1992.

SNEZHKINA, A. V. et al. ROS generation and antioxidant defense systems in normal and malignant cells. **Oxidative medicine and cellular longevity**, 2019.

SONG, J. H.; YU, J. T.; TAN, L. Brain -derived neurotrophic factor in Alzheimer's disease: risk, mechanisms, and therapy. **Molecular neurobiology**, 52(3), 1477 - 1493, 2015.

SONNTAG, K.C. et al. Late-onset Alzheimer's disease is associated with inherent changes in bioenergetics profiles. **Scientific reports**, 7(1), 1-13, 2017.

SORIAL, M. E.; El SAYED, N. S. E. D. Protective effect of valproic acid in streptozotocin-induced sporadic Alzheimer's disease mouse model: possible involvement of the cholinergic system**. Naunyn-Schmiedeberg's archives of pharmacology**, 390(6), 581-593, 2017.

SOREQ, H., and SEIDMAN, S. Acetylcholinesterase—new roles for an old actor. **Nature Reviews Neuroscience**, 2(4), 294-302, 2001.

STANCIU, G. D. et al. Alzheimer's Disease Pharmacotherapy in Relation to Cholinergic System Involvement. **Biomolecules**, 10(1), 40, 2019.

STOCKWELL, J., JAKOVA, E.; CAYABYAB, F. S. Adenosine A1 and A2A Receptors in the Brain, **Current Research and Their Role in Neurodegeneration.** Molecules, 22(4), 676, 2017**.**

STOLK, R. P. et al. Insulin and cognitive function in an elderly population. The Rotterdam Study. **Diabetes Care**, 20(5), 792-795,1997.

STONE, T. W.; CERUTI, S.; ABBRACCHIO, M. P. Adenosine receptors and neurological disease: neuroprotection and neurodegeneration. **Handbook of Experimental Pharmacology**, 535-87, 2009.

STRECK, E. et al. Inhibition of Rat Brain Na1, K1-ATPase Activity Induced by Homocysteine Is Probably Mediated by Oxidative Stress. N**eurochemical Research**, 26(11), 1195–1200, 2001.

TALAAT, R. M. Interleukin 10 (− 1082 G/A) and (− 819 C/T) gene polymorphisms in Egyptian women with polycystic ovary syndrome (PCOS). **Meta Gene**, 9, 254–258, 2016.

TAPIA-ARANCIBIA, L. et al. New insights into brain BDNF function in normal aging and Alzheimer disease. **Brain research reviews**, 59(1), 201-220, 2008.

TEJERA, D.; HENEKA, M. T. Microglia in Alzheimer's disease: the good, the bad and the ugly. **Current Alzheimer Research**, 13(4), 370-80, 2016.

TOGO, T. et al. Occurrence of T cells in the brain of Alzheimer's disease and other neurological diseases. **Journal of Neuroimmunology**, 124(1-2), 83–92, 2002.

UDDIN, M. et al. Novel ANTI-Alzheimer's therapeutic molecules targeting amyloid precursor protein processing. **Oxidative Medicine and Cellular Longevity**, 2020.

UŁAS, J. et al. Reduced density of adenosine A1 receptors and preserved coupling of adenosine A1 receptors to G proteins in alzheimer hippocampus: A quantitative autoradiographic study. **Neuroscience**, 52(2), 843–854, 1993.

UNGER, M.S. et al. Double cort inexpression in CD8+ T-cells and microglia at sites of amyloid-beta plaques: A potential role in shaping plaque pathology? **Alzheimers Dementia**, 14(8), 1022-37, 2018.

UNZETA, M. et al. Multi-target directed donepezil-like ligands for Alzheimer's disease. **Frontiers in neuroscience**, 10, 205, 2016.

VARMA, R. et al. Amyloid-β Induces a Caspase-mediated Cleavage of P2X4 to Promote Purinotoxicity. **NeuroMolecular Medicine**, 11(2), 63–75, 2009.

VIEGAS, T. X. et al. Pharmacokinetics and pharmacodynamics of peldesine (BCX-34), a purine nucleoside phosphorylase inhibitor, following single and multiple oral doses in healthy volunteers. **The Journal of Clinical Pharmacology**, 40(4), 410–20, 2000.

VITOLO, O. V. et al. Amyloid β -peptide inhibition of the PKA/CREB pathway and long -term potentiation: reversibility by drugs that enhance cAMP signaling. **Proceedings of the National Academy of Sciences**, 99(20), 13217-13221, 2002.

WANG, H. et al. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. **Nature**, 421(6921),384–8, 2003.

WATSON, G. S.; CRAFT, S. Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease. **European Jounal Pharmacology**, 490(1-3), 97-113, 2004.

WEI, C. J.; LI, W.; CHEN, J. F. Normal and abnormal functions of adenosine receptors in the central nervous system revealed by genetic knockout studies. **Biochimica et biophysica acta (BBA)-biomembranes**, 1808(5),1358-1379, 2011.

WELIHINDA, A. A. et al. Enhancement of inosine-mediated A2AR signaling through positive allosteric modulation. **Cell Signal**, 42, 227–235, 2018.

WELIHINDA, A. A. et al. The adenosine metabolite inosine is a functional agonist of the adenosine A2A receptor with a unique signaling bias. **Cell Signal**, 28(6), 552–60, 2016.

WOODS, L. T. et al. Purinergic receptors as potential therapeutic targets in Alzheimer's disease. **Neuropharmacology**, 104, 169-179, 2016.

WORLD HEALTH ORGANIZATION, WHO, 2015. Word health statistics 2015. Disponível em: https://www.who.int/gho/publications/world_health_statistics/2015/en/ Acesso em: 26/6/19.

WU, J. et al. Desing, synthesis and biological evaluation of novel carbamates as potencial inhibitors of acetylcholinesterase and butyrylcholinesterase. **Bioorganic & medicinal chemistry**, 28(5), 115324, 2020.

WU, Z. et al. Reduced cell death by inosine pretreatment after photochemically induced cerebral ischemia in adult rats. **Progress in Natural Science**, 18(12), 1513–1518, 2008.

YAN, R., HU, Z. Y.; ZHOU, W. X. Roles of adenosine receptors in Alzheimer's disease. **Yao xue xue bao= Acta pharmaceutica Sinica**, 49(6), 751-756. 2014.

YAO, Z. et al. Alzheimer's Disease Neuroimaging Initiative. Abnormal cortical networks in mild cognitive impairment and Alzheimer's disease. **PLoS Comput Biol**, 6(11), e1001006, 2010.

YEGUTKIN, G. G. Nucleotide-and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade**. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research**, 1783(5), 673-694, 2008.

YIANNOPOULOU, K. G.; PAPAGEORGIOU, S. G. Current and future treatments for Alzheimer's disease. **Therapeutic Advances in Neurological Disorders,** 6(1), 19-33, 2013.

YIANNOPOULOU, K. G.; PAPAGEORGIOU, S. G. Current and Future Treatments in Alzheimer Disease: An Update. **Journal of Central Nervous Syste Disease**, 12, 2020.

ZAI, L. et al. Inosine alters gene expression and axonal projections in neurons contralateral to a cortical infarct and improves skilled use of the impaired limb. **Journal of Neuroscience**, 29(5), 8187–8197, 2009.

ZHANG, L. N. et al. Na+-K+-ATPase, a potent neuroprotective modulator against Alzheimer disease. **Fundamental and clinical pharmacology,** 27(1), 96-103, 2013.

ZHOU, Z. et al. IL-10 promotes neuronal survival following spinal cord injury. **Experimental neurology**, 220(1), 183-190, 2009.

ZHUO, J. et al. Evaluation of type 2 diabetic mellitus animal models via interactions between insulin and mitogen-activated protein kinase signaling pathways induced by a high fat and sugar diet and streptozotocin**. Molecular Medicine Reports**, 17(4), 5132-5142, 2018.

ZIMMERMANN, H. Ectonucleotidases: Some recent developments and a note on nomenclature. **Drug development Research**, 52(1-2), 44-56, 2001.

Anexo 1- Carta CEEA/UFPEL

UFPel
CEEA
Comissão de Ética em Experimentação Animal

Pelotas, 08 de agosto de 2017

Certificado

Certificamos que a proposta intitulada **"Efeitos do tratamento com inosina em parâmetros comportamentais e bioquímicos em modelos *experimentais in vitro e in vivo* para a doença de Alzheimer"**, registrada com o n° **23110.004808/2017-71**, sob a responsabilidade de **Rosélia Maria Spanevello** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de 10/07/2017.

Finalidade	(X) Pesquisa ( ) Ensino	
Vigência da autorização	Início: 08/2017 Término: 08/2021	
Espécie/linhagem/raça	Rattus norvegicus / Ratos Wistar	
N° de animais	192	50
Idade	60 dias	1-3 dias
Sexo	Masculino	Masculino
Origem	Biotério Central da UFPel	

Solicitamos, após tomar ciência do parecer, reenviar o processo à *CEEA*.

Salientamos também a necessidade deste projeto ser cadastrado junto ao *COBALTO* para posterior registro no *COCEPE* (código para cadastro nº **CEEA 4808-2017**).

M.V. Dra. Anelize de Oliveira Campello Felix
Presidente da CEEA

Ciente em: 16/08/2017
Assinatura do Professor Responsável: Rosélia Spanevello

Anexo 2- Licença da revista Psychopharmacology para ultização do artigo 1

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Oct 17, 2020

This Agreement between Miss. FERNANDA CARDOSO TEIXEIRA ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4931420402538
License date	Oct 17, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Psychopharmacology
Licensed Content Title	Inosine protects against impairment of memory induced by experimental model of Alzheimer disease: a nucleoside with multitarget brain actions
Licensed Content Author	Fernanda Cardoso Teixeira et al
Licensed Content Date	Dec 13, 2019
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	full article/chapter
Will you be translating?	no

Circulation/distribution	1 - 29
Author of this Springer Nature content	yes
Title	Inosine protects against impairment of memory induced by experimental model of Alzheimer disease: a nucleoside with multitarget brain actions
Institution name	UFPEL
Expected presentation date	Oct 2020
Order reference number	0
Requestor Location	Miss. FERNANDA CARDOSO TEIXEIRA ALMIRANTE BARROSO 2114 RS, Solteiro 96010280 Brazil Attn: Miss. FERNANDA CARDOSO TEIXEIRA
Total	0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any

other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND
WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. ***BOOKS ONLY:*** Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [**the Licensor**]: on behalf of Cancer Research UK: : [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The [**the Licensor**]: on behalf of Cancer Research UK: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [**the Licensor**]: [**Book Publisher** (e.g. Palgrave Macmillan, Springer etc) [**Book Title**] by [**Book author**(s)] [**COPYRIGHT**] (year of publication)

Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.