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HIPOXANTINA ALTERA PERFIL INFLAMATÓRIO EM ESTRIADO DE RATOS

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Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de Bacharela em Biomedicina.

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PORTO ALEGRE

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DEDICO ESTE TRABALHO

À minha família, pela oportunidade de

tornar o sonho de Biomedicina em realidade.

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RESUMO

Lesch-Nyhan é uma doença metabólica hereditária ligada ao sexo, que acomete o metabolismo das purinas, sendo caracterizada pela deficiência da enzima hipoxantina-guanina fosforibosiltransferase (HPRT), resultando no acúmulo de oxipurinas, principalmente hipoxantina. O quadro clínico manifesta-se cedo na vida dos pacientes, o qual inclui alterações motoras e cognitivas, retardo mental, espasticidade e automutilação. Embora os mecanismos subjacentes da disfunção cerebral na doença de Lesch-Nyhan sejam pouco compreendidos, o acúmulo de hipoxantina parece contribuir para o dano neurológico. O objetivo do presente estudo foi investigar o efeito da hipoxantina sobre o perfil neuroinflamatório, avaliando alguns parâmetros inflamatórios como dosagem de citocinas IL-6 e TNF-α, imunoconteúdo de NF-κB e iNOS, níveis de nitritos, atividade e imunoconteúdo da acetilcolinesterase utilizando um modelo de cirurgia estereotáxica em ratos infantos e adultos jovens. Ratos Wistar de 21 e 60 dias de vida foram submetidos a cirurgia estereotáxica e receberam uma administração intraestriatal sendo divididos em dois grupos: (1) controle (infusão de soro fisiológico 0,9%), (2) Hipoxantina (infusão de 20 pmol/2 µL). Os animais foram decapitados 30 minutos após a administração de hipoxantina, os cérebros foram dissecados, sendo as análises feitas no estriado. Resultados mostraram que a administração intraestriatal de hipoxantina foi capaz de aumentar os níveis de IL-6 em estriado de ratos de ambas as idades analisadas. TNF-α aumentou somente em ratos de 21 dias submetidos ao modelo. Houve um aumento do imunoconteúdo da subunidade p65 de NF-κB nuclear, iNOS e AChE em estriado de ratos infanto e adultos jovens. Os níveis de nitritos foram diminuídos em estriado de ratos de 21 dias de vida submetidos à administração intraestriatal de hipoxantina. De acordo com nossos resultados, a hipoxantina aumentou alguns parâmetros inflamatórios em estriado de ratos infanto e adultos jovens, sugerindo que esse processo pode estar envolvido, pelo menos em parte, com as disfunções neurológicas encontradas por pacientes com doença de Lesch-Nyhan.

Palavras-chave: doença de Lesch-Nyhan, inflamação, citocinas, acetilcolinesterase, NF-κB, iNOS.

LISTA DE ABREVIATURAS

ACh - Acetilcolina

AChE - Acetilcolinesterase

EIM - Erros inatos do metabolismo

HPRT - Hipoxantina-guanina fosforibosiltransferase

IL-6 - Interleucina 6

iNOS - Óxido nítrico sintase induzível

LT - Leucotrieno

NF-κB - Fator nuclear kappa B

NO - Óxido nítrico

PG - Prostaglandina

ROS - Espécies reativas de oxigênio

SNC - Sistema nervoso central

TNF-α - Fator de necrose tumoral alfa

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1. INTRODUÇÃO

1.1 Erros inatos do metbolismo

Erros inatos do metabolismo (EIM) são doenças hereditárias, multifatoriais, caracterizadas por defeitos genéticos que promovem a síntese anômala de uma proteína, geralmente uma enzima com atividade parcial ou totalmente reduzida, resultando na alteração da via metabólica com consequente acúmulo de substrato e diminuição da síntese de produto. Outras substâncias tóxicas podem ser originadas por esse bloqueio metabólico através de rotas metabólicas alternativas (Scriver, 2008).

Correspondendo a cerca de 10% de todas as doenças genéticas, os EIM somam aproximadamente 1000 doenças diferentes identificadas até o momento (Mak et al., 2013). As doenças decorrentes dos EIM são consideradas raras, quando analisadas individualmente. Porém, em conjunto, apresentam alta frequência, acometendo aproximadamente 1:1000 recém-nascidos vivos (Scriver, 2008).

A área do metabolismo afetada determina a classificação dos EIM, que podem ser: de aminoácidos, de ácidos orgânicos, de glicídios, de lipídios, de glicosaminoglicanos, de glicoproteínas, de enzimas eritrocitárias, de metais, de lipoproteínas, de hormônios, de proteínas plasmáticas e de purinas e pirimidinas. Dentre os erros inatos do metabolismo das purinas, destaca-se a doença de Lesch-Nyhan, caracterizada pela deficiência na enzima hipoxantina-guanina fosforibosilransferase (HPRT), resultando em acúmulo tecidual de hipoxantina, xantina e ácido úrico (Lesch and Nyhan, 1964).

1.2 Doença de Lesch-Nyhan

A doença de Lesch-Nyhan é um EIM associado à herança recessiva ligada ao sexo. Caracteriza-se pela deficiência da enzima HPRT, a qual catalisa a conversão de hipoxantina e guanina em seus respectivos nucleotídeos, inosina monofosfato e guanosina monofosfato (Jinnah and Friedmann, 2001; Jinnah et al., 2013; Lesch and Nyhan, 1964; Nyhan, 1978; Visser et al., 2000). Essa alteração acarreta o acúmulo tecidual de xantina, ácido úrico e, principalmente, de hipoxantina. O aumento no nível de hipoxantina parece causar efeitos tóxicos para o sistema nervoso central (SNC) (Bavaresco et al., 2004; Jinnah and Friedmann, 2001). Diversos estudos apontam para as características metabólicas (Puig et al., 1989), cognitivas (Matthews et al., 1995; Schretlen et al., 2001) e comportamentais (Cauwels and Martens, 2005) associadas à doença. Em geral, o tratamento se baseia na administração de alopurinol, o qual inibe enzima xantina oxidase que é responsável por converter a hipoxantina em xantina, e a mesma em ácido úrico. Contudo, sabe-se que o alopurinol não apresenta melhora dos sintomas neurológicos (Torres and Puig, 2008). Pacientes afetados por essa doença apresentam retardo mental, alterações motoras, cognitivas e comportamentais, aliado a hiperuricemia, gota e auto-mutilação, a qual é caracterizada por mordeduras de lábios, língua e dedos, com marcante perda tecidual (Cauwels and Martens, 2005; Jinnah et al., 2006; Schretlen et al., 2001).

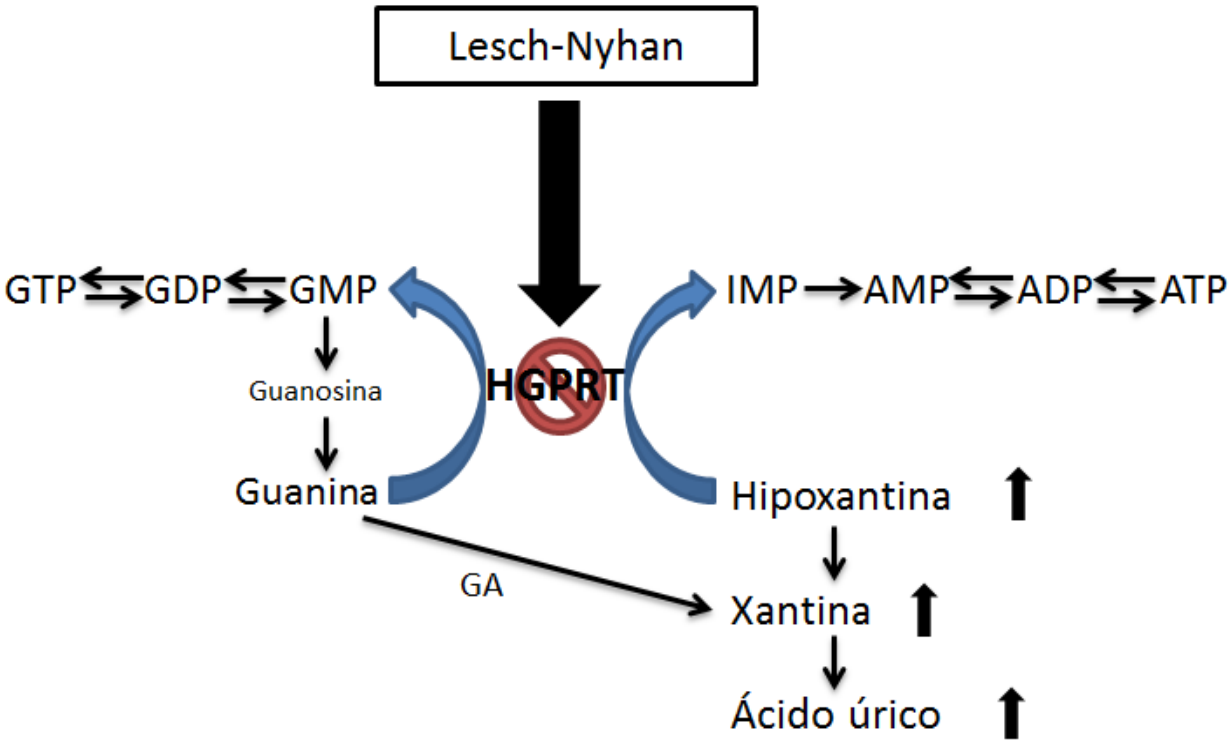

Figura 1. Bloqueio metabólico no metabolismo de purinas encontrado na doença de Lesch-Nyhan. Abreviações: HGPRT, hipoxantina-guanina fosforibosiltransferase; ADP, adenosina difosfato; AMP, adenosina monofosfato; ATP, adenosina trifosfato; GA, guanase; GDP, guanina difosfato; GMP, guanosina monofosfato; GTP, guanosina trifosfato; IMP, inosina monofosfato; PP-ribose-P, fosforibosilpirofosfato (Bavaresco et al., 2004).

1.3 Inflamação

A inflamação é, essencialmente, uma resposta protetora cujo propósito final é eliminar o agente indutor de injúria, podendo ser micro-organismo, estímulos físicos e agentes químicos, prevenindo a injuria ao tecido e/ou iniciando o processo de reparo. O

agente danoso é inicialmente reconhecido pelos componentes do sistema imune do organismo, seguido da liberação de mediadores químicos e ativação de diversos tipos de células que se acumulam no sítio inflamatório (Ali et al., 1997; Zhang, 2008). Alguns mediadores importantes de inflamação são: histamina, serotonina, prostaglandinas (PGs), leucotrienos (LTs), fator de ativação plaquetário, espécies reativas de oxigênio (ROS), óxido nítrico (NO), citocinas, quimiocinas, proteínas de fase aguda, fatore de transcrição nuclear kappa B (NF-κB) e o sistema complemento (Das, 2007). Embora a inflamação tenha uma função protetora no controle de infecções e promova a reparação tecidual, também pode causar danos nos tecidos e doenças (Abbas et al., 2012).

1.4 Neuroinflamação

Inflamação associada ao SNC, ou neuroinflamação, diferentemente dos outros tecidos é caracterizada pela ativação de microglia e astrócitos, principalmente a primeira (Rock et al., 2004). Essas células lançam uma série de fatores que modulam mediadores pró- e anti-inflamatórios (citocinas, quimiocinas, NO, PGs, fatores de crescimento e ROS), que, por sua vez, regulam positivamente moléculas de adesão, aumentam a permeabilidade da barreira hematoencefálica, facilitam a invasão de células imunitárias periféricas, induzem a liberação de moléculas potencialmente tóxicas, que podem comprometer células cerebrais (Lucas et al., 2006). Assim, o SNC pode ser influenciado não só por mediadores pró- e anti-inflamatórios produzidos dentro do cérebro, mas também através das ações de mediadores oriundos da periferia (Xanthos and Sandkuhler, 2014).

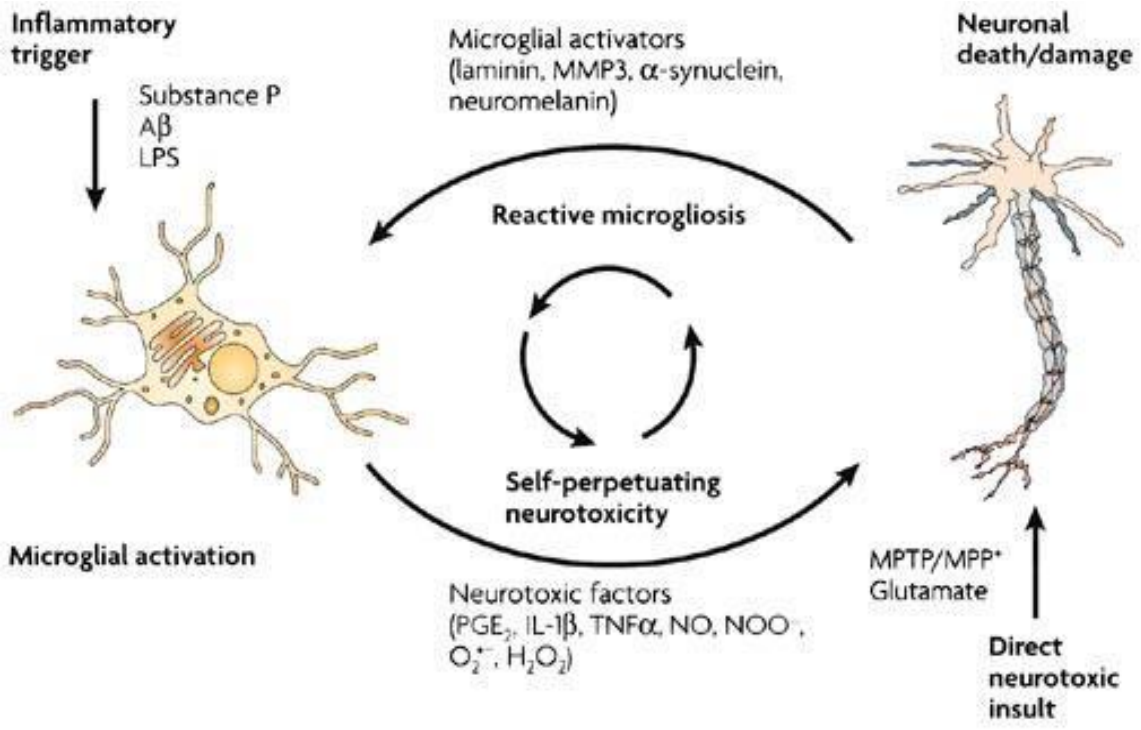

Figura 2. Participação da ativação da microglia na morte neuronal (Fonte Nature Reviews, 2007).

1.4.1 Citocinas

Produzidas em todos os tecidos e pela maioria das células dos sistemas imune inato e adaptativo, as citocinas são pequenas proteínas extremamente importantes para a resposta inflamatória. Estímulos inflamatórios ou antigênicos são capazes de desencadear sua produção. Em geral atuam localmente, de modo parácrino ou autócrino, mas algumas citocinas também podem ser produzidas em quantidades suficientes para exercer funções endócrinas (Abbas et al., 2012).

O fator de necrose tumoral alfa (TNF-α) é um adipocitocina envolvida na inflamação sistêmica, sendo um membro de um grupo de citocinas que estimulam a reação de fase aguda. Essa citocina é produzida principalmente por macrófagos, mas pode ser produzida também por linfócitos $CD4^+$, células *Natural killer*, neutrófilos, eosinófilos e neurônios (Hehlgans and Pfeffer, 2005).

A interleucina 6 (IL-6) é outra citocina com papel central na resposta inflamatória. Ela participa dos processos da imunidade inata e da adquirida, produzida em resposta ao TNF-α e interleucina 1 beta (IL-1β) e algumas células T ativadas. A IL-6 age estimulando a síntese de proteínas de fase aguda pelo fígado na resposta imune inata; já na imunidade adquirida, estimula o desenvolvimento dos plasmócitos (Hirota et al., 2005).

1.4.2 NF-κB

O NF-κB é um complexo proteico que controla a resposta celular a certos estímulos como estresse, citocinas, radicais livres, entre outros. Importante regulador na expressão de genes responsáveis pela síntese de citocinas pró-inflamatórias, quimiocinas, moléculas de adesão, além de mediadores envolvidos na inflamação (Tak and Firestein, 2001). Embora os genes alvos no SNC do NF-κB não são completamente elucidados (Kaltschmidt et al., 1993; Meberg et al., 1996), evidências atuais indicam que NF-κB está envolvido com regulação de neuroplasticidade (Albensi and Mattson, 2000), sobrevivência celular (Mattson et al., 2000), aprendizado e memória (Meffert et al., 2003). Hoje em dia, sabe-se que o NF-κB está presente no citoplasma na maioria das células de origem mesenquimal na sua forma inativada,

ligada a proteínas inibitórias específicas denominadas de IκB (Ghosh et al., 1998; Karin and Ben-Neriah, 2000). A partir de um estímulo pró-inflamatório, o NF-κB libera-se das proteínas inibitórias (IκB), desloca-se para o núcleo onde se liga a sequências promotoras de genes, produzindo a síntese de mediadores pró-inflamatórios (Handel et al., 2000), incluindo moléculas de adesão, enzimas, citocinas, entre outros (Christman et al., 2000). Regulação incorreta do NF-κB tem sido associada ao câncer, doenças inflamatórias e auto-imunes, choque séptico, infeções virais e desenvolvimento imunitário impróprio (Abraham, 2003).

1.4.3 Óxido Nítrico

Outro mediador bastante importante durante o processo inflamatório é o NO, o qual é um radical livre em estado gasoso que difunde-se livremente permeando membranas rapidamente. A produção de NO pode ser indicada por produtos da oxidação de NO, como nitritos e nitratos. O NO é produzido através da conversão do aminoácido, L-arginina à L-citrulina pela ação da enzima óxido nítrico sintase (NOS). Essa enzima possui diferentes isoformas, podendo ser constitutiva (cNOS) sendo encontrada nas células endoteliais (eNOS, ou tipo I), nos neurônios (nNOS, ou tipo III) e, também, nas células epiteliais, neutrófilos e plaquetas. Relacionado com a regulação da produção de grandes quantidades de NO, o terceiro tipo de isômero, denominado de induzível (iNOS, ou tipo II), expressa-se sob a ação de citocinas, endotoxinas, e outros mediadores inflamatórios, e pode ser encontrado, principalmente, em microglia, neutrófilos, macrófagos, fibroblastos, células endoteliais e musculatura lisa dos vasos (Moncada, 1993). Dentre as três isoenzimas, a iNOS é a única que quando expressada

sintetiza NO até finalizar o estoque intracelular de L-arginina (Forstermann and Sessa, 2012). O NO está envolvido no relaxamento do músculo liso, diminuição da agregação plaquetária, sinalização celular, aprendizado, além de respostas imunológicas citotóxicas e pode agir de forma autócrina ou parácrina (Moncada et al., 1991). O excesso de NO é problemático, uma vez que esta molécula é pró-inflamatória possibilitando o desenvolvimento de desordens neurodegenerativas (Calabrese et al., 2009).

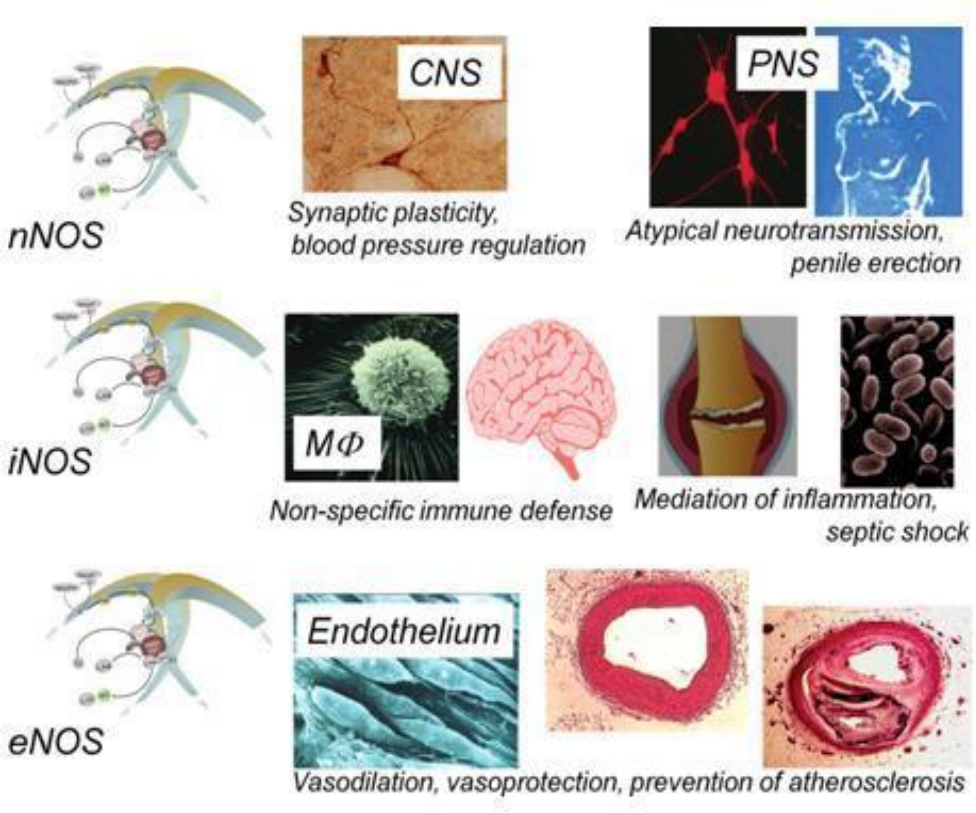

Figura 3. Principais funções das isoformas de NOS (Adaptado de Forstermann and Sessa, 2011).

1.4.4 Acetilcolinesterase

O sistema colinérgico é conhecido por modular diversas funções importantes, tais como a aprendizagem, memória, organização cortical do movimento, e controle do fluxo sanguíneo cerebral (Perry et al., 1999; Sarter and Bruno, 1997; Steriade, 1992). A acetilcolina (ACh) é um neurotransmissor clássico sintetizado pela enzima colina acetiltransferase a partir de acetato e colina, sendo armazenado em vesículas nos neurônios pré-sinápticos. Estudos recentes sugerem o envolvimento da ACh na inflamação através do "rota colinérgica anti-inflamatória", definida como sinais neurais transmitidos através do nervo vago para inibir a liberação de citocinas periféricas (Pavlov and Tracey, 2005; Rosas-Ballina and Tracey, 2009). A atividade colinérgica é controlada principalmente pela enzima acetilcolinesterase (AChE) que hidrolisa rapidamente a ACh nas sinapses colinérgicas e junção neuromuscular (Zimmerman and Soreq, 2006). Essa enzima tem sido considerada reguladora de inflamação, uma vez que ela controla a ação ACh (Das, 2007). Desse modo, a via colinérgica anti-inflamatória representa um mecanismo fisiológico pelo qual o sistema nervoso interage com o sistema imune inato a fim de controlar a resposta inflamatória (Gallowitsch-Puerta and Pavlov, 2007). Sabe-se que em diversas doenças como Alzheimer, hipertensão e diabetes, a atividade da AChE encontra-se elevada (Das, 2007; Pavlov et al., 2009).

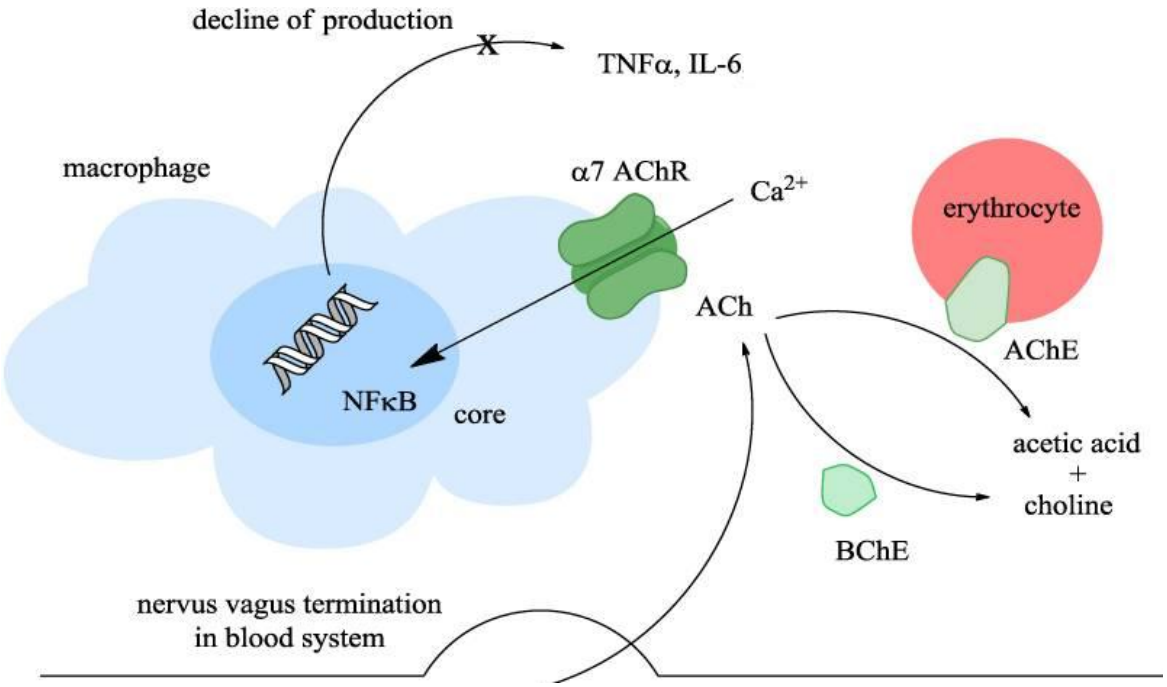

Figura 4. Princípio da rota anti-inflamatória colinérgica; abreviações: ACh- acetilcolina; AChE- acetilcolinesterase; BChE- butirilcolinesterase; IL-6- interleucina 6; TNF-α- fator de necrose tumoral alfa; NF-κB- fator nuclear kappa B (Adaptado de (Pohanka, 2014).

2. OBJETIVOS

2.1. Objetivo geral

Considerando que: a) há evidências na literatura mostrando que os níveis de oxipurinas, em concentrações semelhantes às encontradas na doença de Lesch-Nyhan, possuem efeitos neurotóxicos, b) os mecanismos pelos quais os pacientes com essa síndrome apresentam disfunções neurológicas não estão bem elucidados, c) e que a hipoxantina encontra-se elevada nessas condições, o presente trabalho tem como **objetivo geral** avaliar os efeitos da administração intraestriatal de hipoxantina sobre alguns parâmetros inflamatórios tais como, os níveis de citocinas IL-6 e TNF-α, imunoconteúdo de NF-κB e iNOS, níveis de nitritos, bem como a atividade e imunoconteúdo da AChE, buscando elucidar mecanismos neuroinflamatórios envolvidos na doença de Lesch-Nyhan.

2.2. Objetivos específicos

- Investigar os efeitos da administração intraestriatal de hipoxantina sobre níveis de citocinas (IL-6 e TNF-α) em estriado de ratos Wistar de 21 e 60 dias de vida;
- Analisar os efeitos da administração intraestriatal de hipoxantina sobre o imunoconteúdo da NF-κB em estriado de ratos Wistar de 21 e 60 dias de vida;

- Verificar os efeitos da administração intraestriatal de hipoxantina sobre os níveis de nitritos em estriado de ratos Wistar de 21 e 60 dias de vida;
- Investigar os efeitos da administração intraestriatal de hipoxantina sobre o imunoconteúdo da iNOS em estriado de ratos Wistar de 21 e 60 dias de vida;
- Observar os efeitos da administração intraestriatal de hipoxantina sobre a atividade e imunoconteúdo da AChE em estriado de ratos Wistar de 21 e 60 dias de vida.

3. ARTIGO CIENTÍFICO

O artigo intitulado “**Hypoxanthine intrastriatal administration alters inflammatory profile in striatum of Wistar rats**” foi formatado conforme normas para publicação junto ao periódico Molecular Neurobiology

Hypoxanthine intrastriatal administration alters inflammatory profile in striatum of Wistar rats

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Abstract

Lesch–Nyhan disease is an inherited metabolic disorder of purine metabolism characterized by deficiency of the enzyme hypoxanthine–guanine phosphoribosyl-transferase (HPRT), resulting in accumulation of hypoxanthine. Cognitive deficits, motor damage and self-mutilation behavior are the major symptoms of this disease. Although the underlying mechanisms of brain dysfunction in Lesch–Nyhan disease are poorly understood, hypoxanthine accumulation seems to contribute to the neurological damage. In the present study we analyzed the effect of hypoxanthine on inflammatory parameters such as cytokine levels (IL-6 and TNF-α), immunocontent of NF-κB/p65 subunit and iNOS, nitrite levels; we also evaluated acetylcholinesterase activity and immunocontent of infant and young adult rats subjected to a model of stereotaxic surgery. Wistar rats of 21 and 60 days of life underwent stereotactic surgery and were divided into two groups: control (infusion of saline 0.9%) and hypoxanthine (infusion of 20 pmol/2 μL). Animals were decapitated 30 minutes after the administration. Results showed that intrastriatal administration of hypoxanthine increased IL-6 levels in striatum of both ages of rats analyzed, while TNF-α was increased only in 21-day-old rats subjected to the model. Results show an augmented nuclear immunocontent of NF-κB/p65 subunit in striatum of both ages of rats with hypoxanthine administration. Hypoxanthine administration decreased nitrite levels in the striatum of 21-day-old rats, but the immunocontent of iNOS was increased in striatum of hypoxanthine groups. AChE immunocontent was increased in striatum of infant and young adult rats. According to our results, hypoxanthine increases intrastriatal administration of inflammatory parameters, suggesting that this process may be involved, at least in part, to neurological disorders found in patients with Lesch-Nyhan disease.

Keywords: Lesch-Nyhan disease, inflammation, cytokine, acetylcholinesterase, NF-κB, iNOS.

3.1. Introduction

A severe deficiency of Hypoxanthine-guanine phosphoribosyltranferase (HPRT, EC 2.4.2.8) activity leads to the inborn error of metabolism known as Lesch-Nyhan disease. This metabolic error of purines is an X-linked recessive trait and is characterized by tissue accumulation of oxypurines, mainly hypoxanthine. HPRT recycles hypoxanthine and guanine into inosine monophosphate and guanine monophosphate, respectively, playing an essential role on the purine salvage pathway by generating purine nucleotides [1-3]. The prevalence of Lesch-Nyhan disease is approximately 1:380.000 live births, occurring, with relatively equal frequency, in all populations that have been studied [4]. HPRT is present in all tissues, but with considerable higher levels in the basal ganglia, which explains the extrapyramidal effects, a characteristic of this disease [5]. Some of the symptoms presented by affected patients are motor dysfunction (spasticity, dystonia), cerebral palsy, cognitive and behavioral disturbances, and self-mutilation behavior [6, 7]. Lesch-Nyhan disease diagnosis is based on measurement of serum hypoxanthine, which in patients can reach concentrations of 10 μM, being eight times higher than normal levels [8].

Central nervous system (CNS) has several mechanisms responsible for maintaining immune homeostasis, for instance, the blood brain barrier and immunoregulatory cells that include endothelial cells, astrocytes, oligodendrocytes and microglia [9-11]. Microglia is the basic neuroinflammatory mediator, responding as the major brain's immune cells. Neuroinflammation is characterized as activation of microglia and the consequent release of many factors that modulate pro- and anti-

inflammatory mediators, such as cytokines, chemokines, nitric oxide (NO), PGEs, growth factors and superoxide species [12].

It is well described that the nuclear factor-kappaB (NF-κB) is activated in consequence of inflammation. Other member of the NF-κB family, NF-κB/p65, is situated in the cytoplasm bound to inhibitory proteins (IκB), hence inactivated. Activation induces nuclear translocation of NF-κB/p65, initiating gene transcription. These translocations are often frequently taken as indicators of NF-κB activation and are associated with cellular response to oxidants or to the inflammatory and acute immune response [13].

Another relevant inflammatory mediator is NO. Oxidation products, nitrite and nitrate are the main metabolites that can be used as an indicator of NO production [14]. NO is produced by converting the amino acid L-arginine to L-citrulline by the action of nitric oxide synthase (NOS). Inducible nitric oxide synthase (iNOS) is an isoform that is expressed via cytokines, endotoxins and other inflammation mediators being expressed mainly in microglial cells, neutrophils, macrophages, fibroblasts, endothelial cells and the vascular smooth muscle and mediates the production of large amounts of NO [15, 16].

AChE controls cholinergic activity, hydrolyzing ACh in cholinergic and neuromuscular junction [17]. Recent studies suggest the involvement of acetylcholine in inflammation process through the "cholinergic anti-inflammatory pathway", capable of down-modulate peripheral cytokines release via neural signals transmitted [18-20]. Hence, the cholinergic anti-inflammatory pathway is a physiological mechanism whereby the nervous system interacts with the innate immune system in order to control the inflammatory response [21].

In order to verify whether high hypoxanthine levels could alter inflammatory profile, in the present study, we evaluated the effect of intrastriatal hypoxanthine

administration on some inflammatory parameters such as cytokines (IL-6 and TNF-α), immunocontent of NF-κB as well as iNOS and nitrite levels. We also evaluated AChE activity and immunocontent in striatum of infant and young adult rats. The striatum was used as local drug administration due to the evident striatal lesions found in patients with Lesch–Nyhan disease [3, 22].

3.2. Materials and Methods

3.2.1. Animals and reagents

Female Wistar rats of 21 (infant) and 60-day-old (young adult) were obtained from the Central Animal House of the Department of Biochemistry of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. Animals were maintained under a standard dark–light cycle (lights on between 7:00 and 19:00 h) at a room temperature of 22±1°C, with free access to a 20% (w/w) commercial protein chow and water. Animal care followed the official governmental guidelines in compliance with the Federation of Brazilian Societies for Experimental Biology and was approved by the Ethical Committee of the Universidade Federal do Rio Grande do Sul, Brazil (#25717).

Acrylamide, bisacrylamide, SDS, and β-mercaptoethanol used in sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Polyclonal antibodies were purchased from Santa Cruz Biotechnology, BD Biosciences and Cell Signaling Technology. Anti-rabbit IgG peroxidase-conjugated and reagents to detect chemiluminescence (ECL) were

purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Hybond-C nitrocellulose membranes were from Hybond-ECL (Hybond-ECL-nitrocellulose membrane, Amersham Biosciences, Freiburg, Germany). All other chemical reagents used for analysis were obtained from Sigma Aldrich Co., St. Louis, MO, USA.

3.2.2. Experimental procedure

The model developed in this experimental study was based on the infusion of hypoxanthine directly into right striatum, using as a resource stereotactic surgery, which is widely described in the current scientific literature [23-25]. In this technique, the coordinates relative and specific to the right striatum for each age of rats were based on the stereotactic atlas coordinates developed by Paxinos and Watson [26], (coordinates relative from bregma for 21 and 60-day-old rats, respectively: AP -0.6 mm, ML -3.0 mm, V -4.0 mm from the dura; AP -0.5 mm, ML -2.5 mm, V -2.5 mm from the dura). In all experiments, the intrastriatal infusions of the drug occurred two days after surgery, to avoid any influence of anesthetic substances on the results, since studies show neuroprotective effect for xylazine and ketamine [27]. Later, a needle (0.9 mm) infusion was adjusted within the guide cannula placed in the animal and 2 µL of a solution of hypoxanthine (20 pmol/2 µL) or vehicle (saline 0.9%) were administered in the right striatum of the animal with a 2-minute break. Thus, the animals were divided into two groups: (1) control (saline infusion); (2) hypoxanthine (hypoxanthine infusion). Animals were euthanized by decapitation without anesthesia 30 minutes after the saline or hypoxanthine infusion, brain was dissected and the structure analyzed was striatum. Hypoxanthine dose used in our study was chosen according to Puig and colleagues [8],

being equivalent to that found in patients with Lesch–Nyhan disease. This model was used throughout all experiments described in this work.

3.2.3. Cytokines (IL-6 and TNF-α) assay

Striatum was homogenized in 1:5 (w/v) saline (0.9% NaCl). The homogenate was centrifuged at 800 x g for 10 minutes at 4ºC, being the supernatant used for the technic. IL-6 and TNF-α levels in striatum were quantified by a rat high-sensitivity enzyme-linked immunoabsorbent assays (ELISA) with commercially-available kits (invitrogenTM, Life Technologies, Carlsbad, CA, USA).

3.2.4. Cellular fractionation for cytosolic and nuclear NF-κB/p65 subunit

Striatum was homogenized in 300 µL hypotonic lysis buffer containing 10 mM HEPES (pH 7.9), 1.5 mM MgCl2, 10 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol (DTT), 5 mM NaF, 1 mM sodium orthovanadate plus protease inhibitor cocktail. Samples homogenate were then lysed with 18 µL 10% IGEPAL. The homogenate was centrifuged (14000 × g, 30 s, 4 °C), and supernatants containing the cytosolic fraction were stored at −80 °C. The nuclear pellet was resuspended in 200 µL ice-cold hypertonic extraction buffer (10 mM HEPES (pH 7.9), 0.40 M NaCl, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM PMSF, 1 mM DTT, 5 mM NaF, 1 mM sodium orthovanadate, 0.25 mM EDTA, 25% glycerol plus protease inhibitor cocktail). After 40 min of intermittent mixing, extracts were centrifuged (14000 × g, 10 min, 4 °C),

and supernatants containing nuclear protein were secured [28]. Aliquots were taken for protein determination and, for electrophoresis analysis, were dissolved in 25% (v/v) of a solution containing 40% glycerol, 5% mercaptoethanol, 50 mM Tris–HCl, pH 6.8.

3.2.5. Western blotting analysis of NF-κB/p65 subunit

Protein samples were separated by 10% SDS-PAGE (30 μg/lane of total protein) and transferred (Trans-blot SD semidry transfer cell, BioRad) to nitrocellulose membranes for 1 h at 15 V in transfer buffer (48 mM Trizma, 39 mM glycine, 20% methanol, and 0.25% SDS). The blot was then washed for 10 min in Tris-buffered saline (TBS) (0.5 M NaCl, 20 mM Trizma, pH 7.5), followed by a 2-hour incubation in blocking solution (TBS plus 5% bovine serum albumin (BSA). After incubation, the blot was washed twice for 5 min with blocking solution plus 0.05% Tween-20 (T-TBS) and then incubated overnight at 4 °C in blocking solution containing anti-NF-κB p65 (1:1000; Santa Cruz Biotechnology) and anti-β-actin (1:1000, Cell Signaling Technology). The blot was then washed twice for 5 min with T-TBS and incubated for 2 hours in antibody solution containing peroxidase-conjugated anti-mouse IgG or peroxidase-conjugated anti-rabbit IgG diluted 1:2000. The blot was again washed twice for 5 min with T-TBS and twice for 5 min with TBS. The blot was developed using a chemiluminescence kit (Immobilon Western Chemiluminescent HRP Substrate, Millipore) and detected by ImageQuant LAS 4000 (GE Healthcare Life Sciences).

3.2.6. Nitrite assay

For nitrite levels measurement 100 µL of supernatant of striatum was mixed with 100 µL Griess reagent (1:1 mixture of 1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water) and incubated in 96-well plates for 10 min at room temperature. The absorbance was measured on a microplate reader at a wavelength of 543 nm. Nitrite concentration was calculated using sodium nitrite standards [29].

3.2.7. Western blotting analysis of AChE and iNOS

Striatum of rats were homogenized in a lysis solution containing 100 mM Tris-HCl, 10% sodium dodecyl sulphate (SDS), 1% IGEPAL, 150 mM NaCl, Laemmli buffer and protease inhibitor cocktail. The samples were boiled for 3 min. Total protein homogenate were separated by 10% SDS–PAGE (30 µg/lane of total protein) and transferred (Trans-Blot SD Semi-Dry Transfer Cell, Bio-Rad) to nitrocellulose membranes for 1 h at 15 V in transfer buffer (48 mM Trizma, 39 mM glycine, 20 % methanol, and 0.25 % SDS). The blot was then washed for 10 min in Tris-buffered saline (TBS) (0.5 M NaCl, 20 mM Trizma, pH 7.5), followed by a 2-hour incubation in blocking solution (TBS plus 5% albumin). After incubation, the blot was washed twice for 5 min with blocking solution plus 0.05% Tween 20 (T-TBS) and then incubated overnight at 4°C in blocking solution containing AChE antibody (rabbit polyclonal IgG, AChE (H-134), Santa Cruz Biotechnology), anti-iNOS diluted 1:5000 (Sigma-Aldrich) and anti-β-actin (1:1000, Cell Signaling Technology). The blot was then washed twice for 5 min with T-

TBS and incubated for 2 hours in antibody solution containing peroxidase-conjugated anti-rabbit IgG diluted 1:10000. The blot was washed twice again for 5 min with T-TBS and twice for 5 min with TBS. The blot was developed using a chemiluminescence kit (Immobilon Western Chemiluminescent HRP Substrate, Millipore) and detected by ImageQuant LAS 4000 (GE Healthcare Life Sciences).

3.2.8. AChE activity assay

AChE activity was determined according to the method of Ellman and colleagues [30], with some modifications [31]. For AChE assay, striatum was homogenized in ten volumes of 0.1 mM potassium phosphate buffer, pH 7.5, and centrifuged for 10 min at 1000 × g, being the supernatants used for the enzymatic AChE analyses. Hydrolysis rates were measured at ACh concentration of 0.8 mM in 300 µL assay solution with 30 mM phosphate buffer, pH 7.5, and 1.0 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) at 25 °C. About 15 µL of sample supernatant was added to the reaction mixture and pre-incubated for 3 min. The hydrolysis was monitored by formation of the thiolate dianion of DTNB at 412 nm for 2–3 min (intervals of 30 seconds). All samples were run in triplicate.

3.2.9. Protein determination

Protein was measured by the Coomassie Blue method according to Bradford [32] or Lowry and colleagues [33] using bovine serum albumin as the standard.

3.2.10. Statistical analysis

Student's t-test was used to evaluate the different parameters. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, in a PC-compatible computer. Differences were considered statistically significant if $p < 0.05$.

3.3. Results

Initially we evaluated the effect of hypoxanthine administration on cytokine levels (IL-6 and TNF-α) in striatum of 21 and 60-day-old rats. Figure 1A shows that hypoxanthine administration increased IL-6 in rat striatum of 21 day-old [$t(5)=3.92$; $p<0.005$] and of 60 day-old [$t(5)=3.77$; $p<0,005$] (Fig 1B). TNF-α was significant increase in striatum of 21-day-old [$t(5)=2.57$; $p<0.05$], but not in striatum of 60-day-old rats [$t(5)=0.84$; $p>0.05$].

Since NF-κB regulate the innate immune response, and it is activated rapidly in response to a wide range of stimuli, including pro-inflammatory cytokines, such as TNF-α and IL-6 [34], in the present study we investigated the effect of hypoxanthine administration on immunocontent of cytosolic and nuclear fraction of NF-κB/p65 subunit. Figure 2A and B show that hypoxanthine administration significantly increased the immunocontent of nuclear fraction of NF-κB/p65 subunit in striatum of 21 [$t(5)=3.20$; $p<0.05$] and 60-day-old rats [$t(5)=6.39$; $p<0.01$], but no alteration was observed on cytosolic fraction of NF-κB/p65 subunit in rat striatum of 21 [$t(5)=1.09$; $p>0.05$] and 60-day-old [$t(5)=1.42$; $p>0.05$], as compared to control.

Nitrite levels were measured in striatum of 21 and 60-day-old rats subjected to hypoxanthine injection. Figure 3A and B show that hypoxanthine administration was able to diminished nitrite levels in striatum of infant rats [$t(6)=4.93$; $p<0.001$], but not in young adult rats [$t(6)=0.18$; $p>0.05$]. Since we observed a decrease in nitrite levels, we also evaluated the iNOS immunocontent in striatum of infant and young adult rats. Figure 4A and B show a significant increase on iNOS immunocontent in striatum of rats of both ages [$t(6)=2.64$; $p<0.05$ and $t(6)=5.04$; $p<0.001$].

In order to investigate if hypoxanthine would affect AChE, we analyzed the activity and immunocontent of this enzyme. Figure 5A shows that hypoxanthine did not alter AChE activity in rat striatum of 21-day-old [$t(6)=2.24$; $p>0.05$] and 60-day-old [$t(6)=1.18$; $p>0.05$]. We also evaluated the immunocontent of AChE in striatum of infant and young adult rats (figure 6) and results show an increase on AChE immunocontent in stratum of 21-day-old [$t(6)=2.66$; $p<0.05$] and 60-day-old rats [$t(6)=3.09$; $p<0.05$].

3.4. Figures

Figure 1

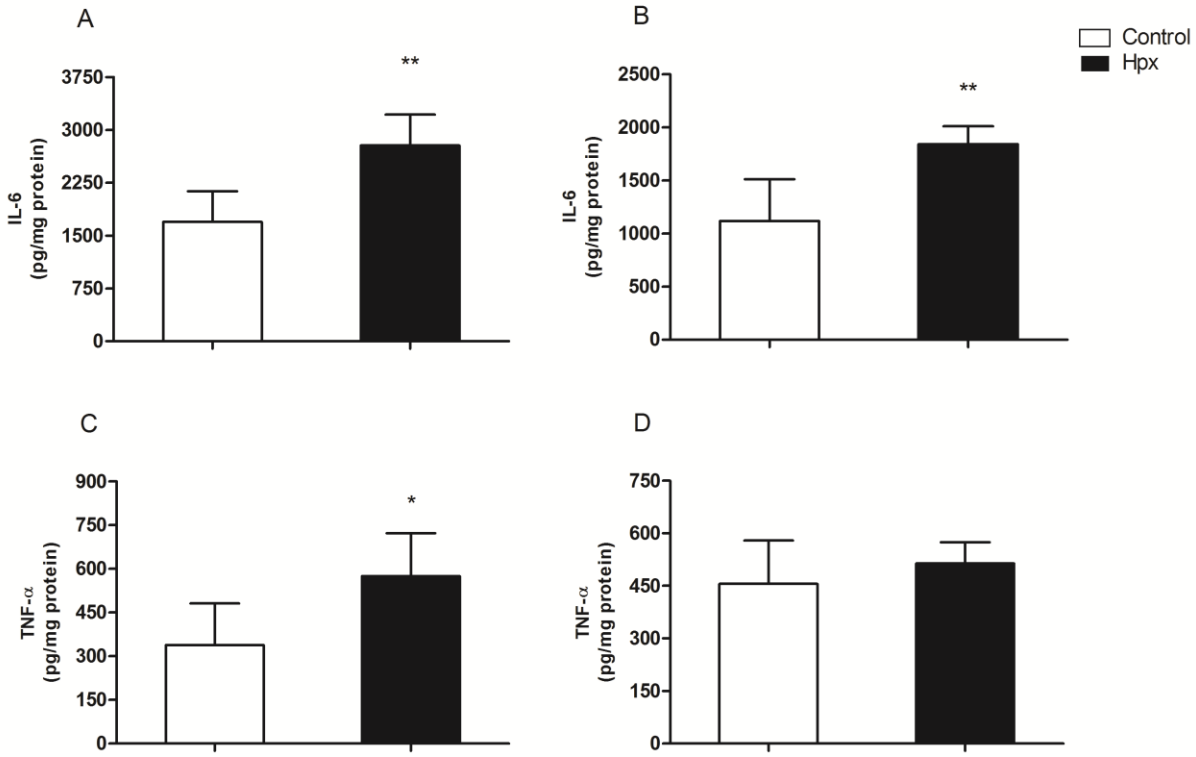

Figure 1. Effects of hypoxanthine intrastriatal administration on cytokines levels in striatum of infant and young adult rats. (A) IL-6 in 21-day-old rats, (B) IL-6 in 60-day-old rats, (C) TNF-α in 21-day-old rats and (D) TNF-α in 60-day-old rats. Results are expressed as picogram per milligram of protein. Data are mean ± SD for five to six animals in each group. Different from control, *p<0.05, **p<0.01 (Student's t test). Hpx, hypoxanthine; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha.

Figure 2

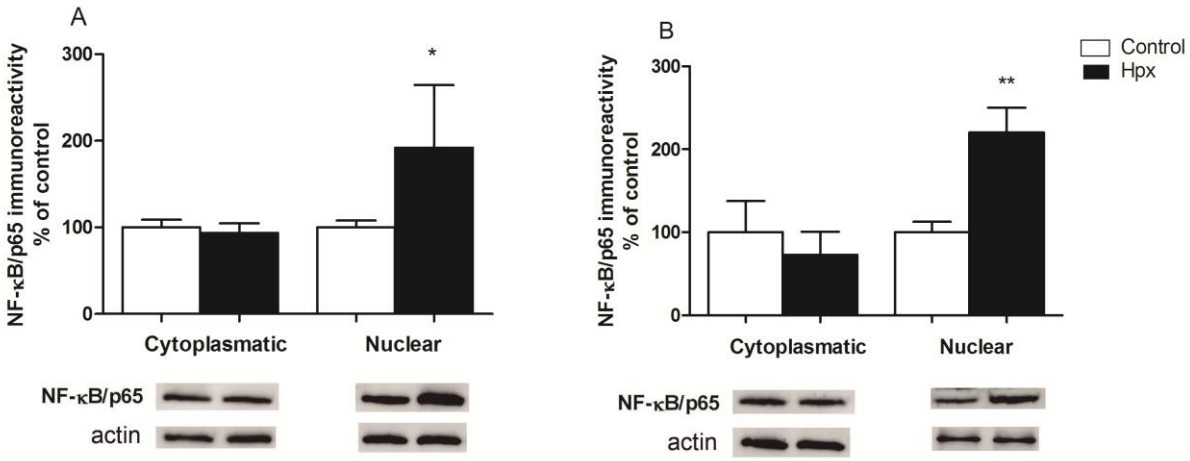

Figure 2. Effects of hypoxanthine intrastriatal administration on cytosolic and nuclear fraction of NF-κB/p65 subunit. (A) Striatum of 21-day-old rats and (B) striatum of 60-day-old rats. Results are expressed as percentage of control. Uniformity of gel loading was confirmed with β-actin as standard. Data are mean ± SD for six to seven animals in each group. Different from control, *p<0.05, **p<0.01 (Student's t test). Hpx, hypoxanthine.

Figure 3

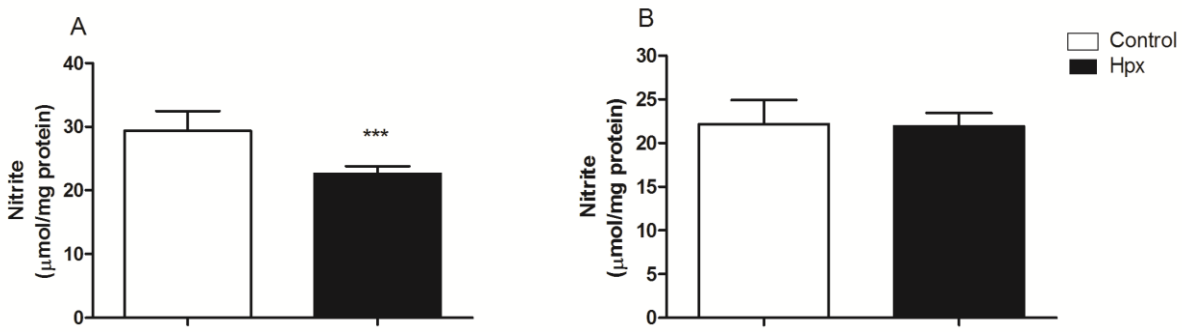

Figure 3. Effects of hypoxanthine intrastriatal administration on nitrite levels. (A) Striatum of 21-day-old rats and (B) striatum of 60-day-old rats. Results are expressed as micromole per milligram of protein. Data are mean ± SD for six to seven animals in each group. Different from control, ***p<0.001 (Student's t test). Hpx, hypoxanthine.

Figure 4

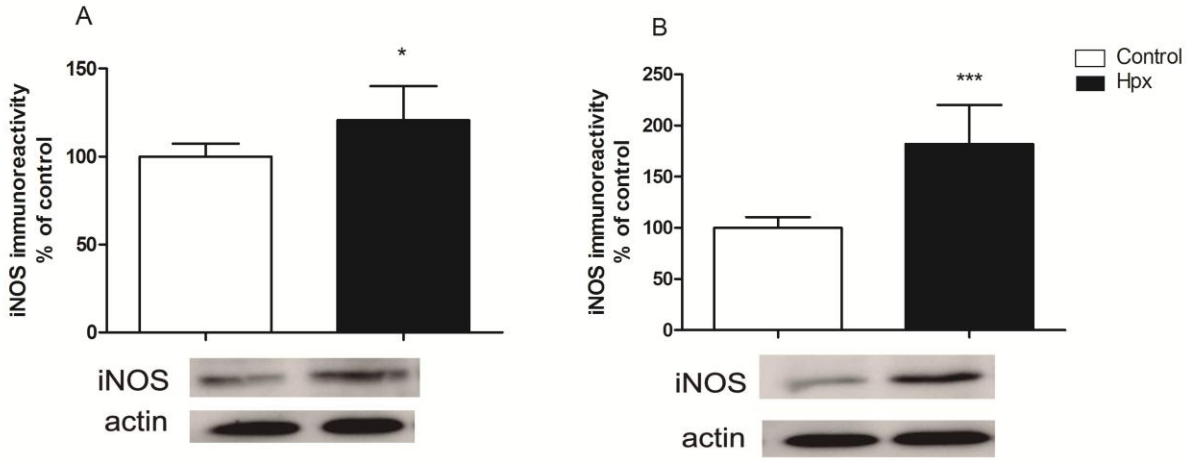

Figure 4. Effects of hypoxanthine intrastriatal administration on inducible nitric oxide synthase immunocontent. (A) Striatum of 21-day-old rats and (B) striatum of 60-day-old rats. Results are expressed as percentage of control. Uniformity of gel loading was confirmed with β-actin as standard. Data are mean ± SD for six to seven animals in each group. Different from control, *$p<0.05$, ***$p<0.001$ (Student's t test). Hpx, hypoxanthine; iNOS, inducible nitric oxide synthase.

Figure 5

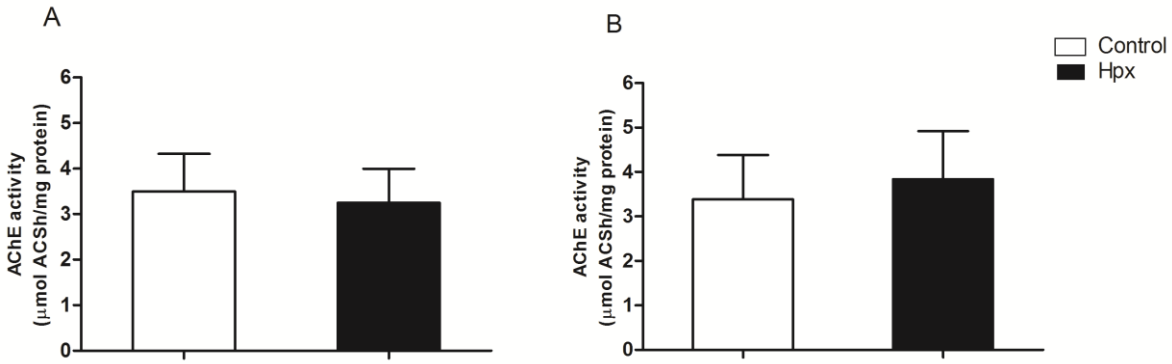

Figure 5. Effects of hypoxanthine intrastriatal administration on acetylcholinesterase activity. (A) Striatum of 21-day-old rats and (B) striatum of 60-day-old rats. Results are expressed as mean ± SD for six to seven animals in each group. Different from control, *p<0.05 (Student's t test). Hpx, hypoxanthine; AChE, acetylcholinesterase.

Figure 6

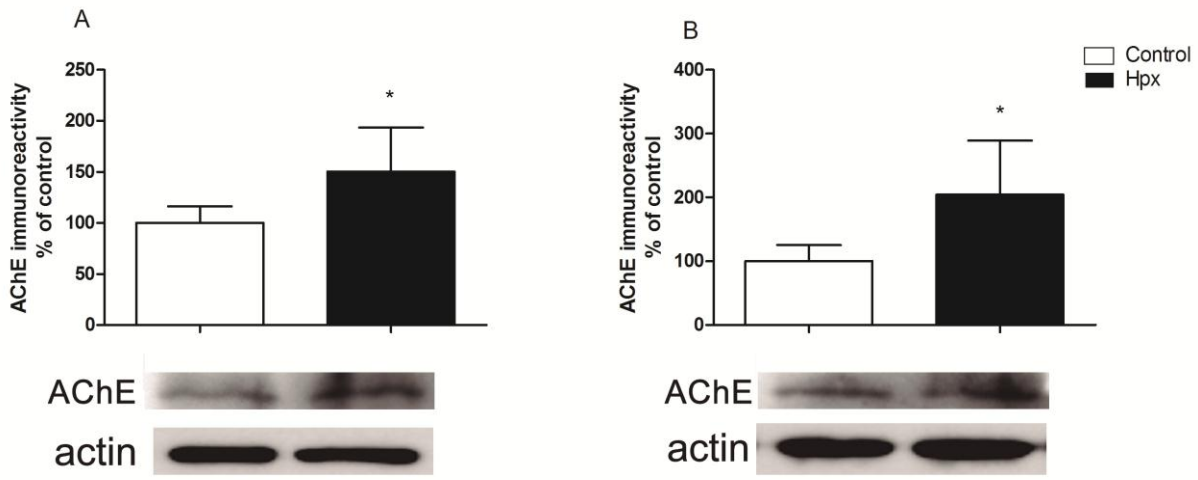

Figure 6. Effects of hypoxanthine intrastriatal administration on acetylcholinesterase immunocontent in striatum of infant and young adult rats. (A) 21-day-old rats and (B) 60-day-old rats. Results are expressed as percentage of control. Uniformity of gel loading was confirmed with β-actin as standard. Data are mean ± SD for six to seven animals in each group. Different from control, *$p<0.05$, (Student's t test). Hpx, hypoxanthine; AChE, acetylcholinesterase.

3.5. Discussion

Patients affected by Lesch-Nyhan disease present diverse signs and symptoms, such as mental retardation, self-mutilation behavior and dysfunction of the dopamine transmitter system of the basal ganglia [22, 35]. Increased levels of hypoxanthine in plasma, urine and cerebrospinal fluid are characteristic feature of Lesch-Nyhan disease [36, 37]. In this sense, tissue accumulation of hypoxanthine has been proposed to contribute to the neurological dysfunction characteristic of Lesch-Nyhan disease [3, 38], but the pathophysiology of this disease is still unclear.

For a long time CNS was considered to be “immune prerogative”, not being responsive or cooperate with inflammation. Nowadays, it is known that the CNS has a certain role in inflammation, and in response to injury, infection or disease, resident CNS cells, mainly microglia, that generates inflammatory mediators that modulate the pro- and anti-inflammatory mediators (cytokines, chemokine, NO, PGs, growth factors and the species of superoxide). These mediators up regulate adhesion molecules, increase permeability of the blood-brain barrier, facilitating the invasion of peripheral immune cells, inducing the release of potentially toxic molecules that can compromise brain cells [39]. The purpose of the present study was to evaluate pro-inflammatory cytokines levels, NF-κB/p65 subunit immunocontent, nitrite levels, iNOS immunocontent, as well as the activity and immunocontent of AChE in striatum of 21 and 60 day-old rats subjected to intrastriatal administration of hypoxanthine.

In the present study, we firstly analyzed cytokine levels in striatum of infant and young adult rats subjected to hypoxanthine. Cytokines are molecules involved in signal transmission between cells during the initiation of immune responses. Our results

showed that the hypoxanthine intrastriatal administration provoked an increase in pro-inflammatory cytokines, IL-6 and TNF-α, in striatum of 21-day-old and 60-day-old rats. In agreement with our results, Gudbjornsson and colleagues showed that hypoxanthine is related to inflammation of rheumatoid arthritis, which presents enhanced purine metabolism and diffusion of oxypurines [40]. Although the mechanism(s) by which hypoxanthine acts on inflammation are not fully understood, our findings suggest that the increase in cytokines levels could be closely related to oxidative stress caused by high levels of hypoxanthine [41].

NF-κB is a protein complex that controls the cellular response to certain stimuli such as stress, cytokines, free radicals, among others, by regulating gene expression responsible for the synthesis of pro-inflammatory cytokines, chemokines, adhesion molecules, and mediators involved in inflammation. Translocation of NF-κB is a pivotal stage in the coupling of extracellular stimuli to the transcriptional activation of specific target genes [42]. Once translocated into the nucleus NF-κB stimulates transcription by binding to cognate B sites in the promoter regions of target genes including cytokines, chemokines, and cell adhesion molecules [34]. This enzyme has been associated with a number of inflammatory mediators such as IL-6 [43-45], TNF-α [46, 47] and iNOS [48-50]. In our study, there was an increase on immunocontent of nuclear NF-κB/p65 subunit in striatum of infant and young adult rats subjected to intrastriatal injection of hypoxanthine. NF-κB could be activated by pro-inflammatory cytokines, such as IL-6 and TNF-α, as well as reactive species; the last parameter is highly related to hypoxanthine administration as seen in previous study [41].

We also evaluated nitrite levels in rats subjected to hypoxanthine administration. Results showed that hypoxanthine decreases nitrite levels in striatum of 21 day-old rats.

It has been shown that there is a relation between the formation of NO and synthesis of reactive species, such as superoxide and hydrogen peroxide [51]. Previous studies of our group showed a redox imbalance promoted by hypoxanthine administration [41]. Hydrogen peroxide can also react with NO generating peroxynitrite, a powerful cytotoxic substance [52], suggesting a diminished bioavailability of NO. This possibly explains the decrease in nitrite levels in striatum of infant rats. Since iNOS is responsible for catalyzing the formation of NO through inflammatory stimuli we also analyzed iNOS immunocontent. Our results show that this enzyme immunocontent was increased in striatum of 21 and 60 day-old rats. Increased protein expression of iNOS is an indicative of neuroinflammation and neurodegeneration also being related to microglial activation [51, 53].

It has been shown that AChE is associated with inflammation by the involvement of acetylcholine (ACh) in the "cholinergic anti-inflammatory pathway" [19]. Based on this evidence, we also investigate the effect of hypoxanthine on AChE activity and immunocontent. Our results showed that hypoxanthine administration increased AChE immunocontent in striatum of 21 and 60-day-old rats promoting a neuroinflammatory profile. AChE hydrolyzes ACh in neural synapses and motor end plate forming choline and acetic acid, allowing cholinergic neuron to return to basal state after excitation. Whereas ACh has a suppressive role on inflammation it is acceptable that AChE functions as an intrinsic regulator of inflammation [54]. The cholinergic inflammatory pathway begins by the release of ACh from the vagus nerve termination acting on α7 nicotinic acetylcholine receptor (α7 nAChR), consequently there is an influx of Ca^{2+} into macrophages [55]. Augmented levels of Ca^{2+} suppress IL-6 and TNF-α [20]. Other studies showed that peritoneal or intravenous injection of AChE inhibitors reduced

serum pro-inflammatory cytokine levels [56] and IL-1β in blood and brain in mice [57]. AChE activity was not altered in any group analyzed. So far we do not know the exact mechanisms by which hypoxanthine acts on AChE activity. One possibility may be the time analyzed; the increase in this enzyme immunocontent is not reflected on the activity yet.

In summary, our findings demonstrated that intrastriatal administration of hypoxanthine presented an increase in cytokine levels (IL-6 and TNF-α), NF-κB, iNOS and AChE immunocontent, as well as decreased nitrite levels, suggesting that this substance may promote a pro-inflammatory status in striatum of infant and young adult rats. This study provides new basis elucidating toxicity mechanisms of hypoxanthine on inflammatory parameters, suggesting that this process may be involved, at least in part, with disorders found in patients with Lesch-Nyhan disease.

3.6. Acknowledgements

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3.7. Conflict of interest

The authors declare that they have no conflict of interest.

3.8. References

1 Lesch M. and Nyhan W. L. (1964) A Familial Disorder of Uric Acid Metabolism and Central Nervous System Function. Am J Med. 36, 561-570

2 Nyhan W. L. (1978) Ataxia and disorders of purine metabolism: defects in hypoxanthine guanine phosphoribosyl transferase and clinical ataxia. Adv Neurol. 21, 279-287

3 Visser J. E., Bar P. R. and Jinnah H. A. (2000) Lesch-Nyhan disease and the basal ganglia. Brain Res Brain Res Rev. 32, 449-475

4 Kelley R. E. and Andersson H. C. (2014) Disorders of purines and pyrimidines. Handb Clin Neurol. 120, 827-838

5 Chen B. C., Balasubramaniam S., McGown I. N., O'Neill J. P., Chng G. S., Keng W. T., Ngu L. H. and Duley J. A. (2013) Treatment of Lesch-Nyhan disease with S-adenosylmethionine: experience with five young Malaysians, including a girl. Brain Dev. 36, 593-600

6 Jinnah H. A., Sabina R. L. and Van Den Berghe G. (2013) Metabolic disorders of purine metabolism affecting the nervous system. Handb Clin Neurol. 113, 1827-1836

7 Torres R. J. and Puig J. G. (2008) The diagnosis of HPRT deficiency in the 21st century. Nucleosides Nucleotides Nucleic Acids. 27, 564-569

8 Puig J. G., Jimenez M. L., Mateos F. A. and Fox I. H. (1989) Adenine nucleotide turnover in hypoxanthine-guanine phosphoribosyl-transferase deficiency: evidence for an increased contribution of purine biosynthesis de novo. Metabolism. 38, 410-418

9 Das Sarma J. (2014) Microglia-mediated neuroinflammation is an amplifier of virus-induced neuropathology. J Neurovirol. 20, 122-136

10 Gendelman H. E. (2002) Neural immunity: Friend or foe? J Neurovirol. 8, 474-479

11 Latta C. H., Brothers H. M. and Wilcock D. M. (2014) Neuroinflammation in Alzheimer's disease; A source of heterogeneity and target for personalized therapy. Neuroscience

12 Ghirnikar R. S., Lee Y. L. and Eng L. F. (1998) Inflammation in traumatic brain injury: role of cytokines and chemokines. Neurochem Res. 23, 329-340

13 Dallot E., Mehats C., Oger S., Leroy M. J. and Breuiller-Fouche M. (2005) A role for PKCzeta in the LPS-induced translocation NF-kappaB p65 subunit in cultured myometrial cells. Biochimie. 87, 513-521

14 Knowles R. G. and Moncada S. (1994) Nitric oxide synthases in mammals. Biochem J. 298 (Pt 2), 249-258

15 Lechner M., Lirk P. and Rieder J. (2005) Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin. Semin Cancer Biol. 15, 277-289

16 Moncada S. (1993) The L-arginine: nitric oxide pathway, cellular transduction and immunological roles. Adv Second Messenger Phosphoprotein Res. 28, 97-99

17 Zimmerman G. and Soreq H. (2006) Termination and beyond: acetylcholinesterase as a modulator of synaptic transmission. Cell Tissue Res. 326, 655-669

18 Pavlov V. A., Parrish W. R., Rosas-Ballina M., Ochani M., Puerta M., Ochani K., Chavan S., Al-Abed Y. and Tracey K. J. (2009) Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. Brain Behav Immun. 23, 41-45

19 Rosas-Ballina M. and Tracey K. J. (2009) Cholinergic control of inflammation. J Intern Med. 265, 663-679

20 Sun P., Zhou K., Wang S., Li P., Chen S., Lin G., Zhao Y. and Wang T. (2013) Involvement of MAPK/NF-kappaB signaling in the activation of the cholinergic anti-inflammatory pathway in experimental colitis by chronic vagus nerve stimulation. PLoS One. 8, e69424

21 Gallowitsch-Puerta M. and Pavlov V. A. (2007) Neuro-immune interactions via the cholinergic anti-inflammatory pathway. Life Sci. 80, 2325-2329

22 Jinnah H. A. and Friedmann T. (2001) Lesch Nyhan disease and it variants. In The Metabolic and Molecular Bases of Inherited (Scriver CR, Beaudet, A., Sly, W. and Valle, D., eds.). pp. 2537 – 2569, McGraw-Hill, New York

23 Engin E. and Treit D. (2007) The role of hippocampus in anxiety: intracerebral infusion studies. Behav Pharmacol. 18, 365-374

24 Tsenov G., Mateffyova A., Mares P., Otahal J. and Kubova H. (2007) Intrahippocampal injection of endothelin-1: a new model of ischemia-induced seizures in immature rats. Epilepsia. 48 Suppl 5, 7-13

25 Zugno A. I., Scherer E. B., Mattos C., Ribeiro C. A., Wannmacher C. M., Wajner M. and Wyse A. T. (2007) Evidence that the inhibitory effects of guanidinoacetate on the activities of the respiratory chain, Na+,K+-ATPase and creatine kinase can be differentially prevented by taurine and vitamins E and C administration in rat striatum in vivo. Biochim Biophys Acta. 1772, 563-569

26 Paxinos G. and Watson C. (2006) The rat brain in stereotaxic coordinates: hard cover edition. Academic press

27 Ozden S. and Isenmann S. (2004) Neuroprotective properties of different anesthetics on axotomized rat retinal ganglion cells in vivo. J Neurotrauma. 21, 73-82

28 Zanotto-Filho A., Gelain D. P., Schroder R., Souza L. F., Pasquali M. A., Klamt F. and Moreira J. C. (2009) The NF kappa B-mediated control of RS and JNK signaling in vitamin A-treated cells: duration of JNK-AP-1 pathway activation may determine cell death or proliferation. Biochem Pharmacol. 77, 1291-1301

29 Green L. C., Wagner D. A., Glogowski J., Skipper P. L., Wishnok J. S. and Tannenbaum S. R. (1982) Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 126, 131-138

30 Ellman G. L., Courtney K. D., Andres V., Jr. and Feather-Stone R. M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7, 88-95

31 Scherer E. B., da Cunha M. J., Matte C., Schmitz F., Netto C. A. and Wyse A. T. (2010) Methylphenidate affects memory, brain-derived neurotrophic factor immunocontent and brain acetylcholinesterase activity in the rat. Neurobiol Learn Mem. 94, 247-253

32 Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72, 248-254

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

34 Li Q. and Verma I. M. (2002) NF-kappaB regulation in the immune system. Nat Rev Immunol. 2, 725-734

35 Jinnah H. A., Gage F. H. and Friedmann T. (1990) Animal models of Lesch-Nyhan syndrome. Brain Res Bull. 25, 467-475

36 Harkness R. A., McCreanor G. M. and Watts R. W. (1988) Lesch-Nyhan syndrome and its pathogenesis: purine concentrations in plasma and urine with metabolite profiles in CSF. J Inherit Metab Dis. 11, 239-252

37 Puig J. G. and Mateos F. A. (1993) The Biochemical Basis of HGPRT Deficiency. In Molecular Genetics, Biochemistry and Clinical Aspects of Inherited Disorders of Purine and Pyrimidine Metabolism. pp. 12-26, Springer Berlin Heidelberg

38 Ma M. H., Stacey N. C. and Connolly G. P. (2001) Hypoxanthine impairs morphogenesis and enhances proliferation of a neuroblastoma model of Lesch Nyhan syndrome. J Neurosci Res. 63, 500-508

39 Lucas S. M., Rothwell N. J. and Gibson R. M. (2006) The role of inflammation in CNS injury and disease. Br J Pharmacol. 147 Suppl 1, S232-240

40 Gudbjornsson B., Zak A., Niklasson F. and Hallgren R. (1991) Hypoxanthine, xanthine, and urate in synovial fluid from patients with inflammatory arthritides. Ann Rheum Dis. 50, 669-672

41 Bavaresco C. S., Chiarani F., Wannmacher C. M., Netto C. A. and Wyse A. T. (2007) Intrastriatal hypoxanthine reduces Na(+),K (+)-ATPase activity and induces oxidative stress in the rats. Metab Brain Dis. 22, 1-11

42 Tak P. P. and Firestein G. S. (2001) NF-kappaB: a key role in inflammatory diseases. J Clin Invest. 107, 7-11

43 Libermann T. A. and Baltimore D. (1990) Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. Mol Cell Biol. 10, 2327-2334

44 Shimizu Y., van Seventer G. A., Horgan K. J. and Shaw S. (1990) Costimulation of proliferative responses of resting CD4+ T cells by the interaction of VLA-4 and VLA-5 with fibronectin or VLA-6 with laminin. J Immunol. 145, 59-67

45 Son Y. H., Jeong Y. T., Lee K. A., Choi K. H., Kim S. M., Rhim B. Y. and Kim K. (2008) Roles of MAPK and NF-kappaB in interleukin-6 induction by lipopolysaccharide in vascular smooth muscle cells. J Cardiovasc Pharmacol. 51, 71-77

46 Shakhov A. N., Kuprash D. V., Azizov M. M., Jongeneel C. V. and Nedospasov S. A. (1990) Structural analysis of the rabbit TNF locus, containing the genes encoding TNF-beta (lymphotoxin) and TNF-alpha (tumor necrosis factor). Gene. 95, 215-221

47 Shakhov A. N., Collart M. A., Vassalli P., Nedospasov S. A. and Jongeneel C. V. (1990) Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor alpha gene in primary macrophages. J Exp Med. 171, 35-47

48 Hughes J. E., Srinivasan S., Lynch K. R., Proia R. L., Ferdek P. and Hedrick C. C. (2008) Sphingosine-1-phosphate induces an antiinflammatory phenotype in macrophages. Circ Res. 102, 950-958

49 Morris K. R., Lutz R. D., Choi H. S., Kamitani T., Chmura K. and Chan E. D. (2003) Role of the NF-kappaB signaling pathway and kappaB cis-regulatory elements on the IRF-1 and iNOS promoter regions in mycobacterial lipoarabinomannan induction of nitric oxide. Infect Immun. 71, 1442-1452

50 Guo G. and Bhat N. R. (2006) Hypoxia/reoxygenation differentially modulates NF-kappaB activation and iNOS expression in astrocytes and microglia. Antioxid Redox Signal. 8, 911-918

51 Brown G. C. and Neher J. J. (2010) Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. Mol Neurobiol. 41, 242-247

52 Borutaite V., Budriunaite A. and Brown G. C. (2000) Reversal of nitric oxide-, peroxynitrite- and S-nitrosothiol-induced inhibition of mitochondrial respiration or complex I activity by light and thiols. Biochim Biophys Acta. 1459, 405-412

53 Ribeiro B. M., do Carmo M. R., Freire R. S., Rocha N. F., Borella V. C., de Menezes A. T., Monte A. S., Gomes P. X., de Sousa F. C., Vale M. L., de Lucena D. F., Gama C. S. and Macedo D. (2013) Evidences for a progressive microglial activation and increase in iNOS expression in rats submitted to a neurodevelopmental model of schizophrenia: reversal by clozapine. Schizophr Res. 151, 12-19

54 Pohanka M. (2014) Inhibitors of acetylcholinesterase and butyrylcholinesterase meet immunity. Int J Mol Sci. 15, 9809-9825

55 Pohanka M. (2012) Alpha7 nicotinic acetylcholine receptor is a target in pharmacology and toxicology. Int J Mol Sci. 13, 2219-2238

56 Hofer S., Eisenbach C., Lukic I. K., Schneider L., Bode K., Brueckmann M., Mautner S., Wente M. N., Encke J. and Werner J. (2008) Pharmacologic cholinesterase inhibition improves survival in experimental sepsis*. Critical care medicine. 36, 404-408

57 Pollak Y., Gilboa A., Ben-Menachem O., Ben-Hur T., Soreq H. and Yirmiya R. (2005) Acetylcholinesterase inhibitors reduce brain and blood interleukin-1beta production. Ann Neurol. 57, 741-745

4. CONCLUSÃO

Ao longo da última década, tem-se percebido que a inflamação desempenha um papel chave nas doenças que acometem SNC. A fim de verificar se os níveis elevados de hipoxantina alteraram padrões fisiológicos, provocando inflamação, analisamos alguns parâmetros inflamatórios. Em resumo, nossos resultados demonstraram que a administração intraestriatal de hipoxantina apresentou um aumento nos níveis de citocinas e imunoconteúdo de NF-κB, iNOS e AChE. Níveis de nitritos foram também alterados pela hipoxantina sugerindo que esta substância pode promover um estado pró-inflamatório em estriado de ratos infantos e adultos jovens. Este estudo esclarece novos mecanismos de neurotoxicidade de hipoxantina associados aos parâmetros inflamatórios, sugerindo que esse processo pode estar envolvido, pelo menos em parte, com os distúrbios neurológicos observados em pacientes com a doença de Lesch-Nyhan.

5. PERSPECTIVAS

- Verificar o efeito da administração intraestriatal de hipoxantina sobre o imunoconteudo de COX-2 em estriado de ratos de 21 e 60 dias;
- Avaliar o efeito da administração intraestriatal de hipoxantina sobre imunoconteudo de IBA1 em estriado de ratos de 21 e 60 dias;
- Avaliar expressão gênica de AChE em estriado de ratos submetidos ao modelo de injeção intraestriatal de hipoxantina;
- Dosar os níveis de ACh em estriado de ratos de 21 e 60 dias submetidos ao modelo de injeção intraestriatal de hipoxantina.

6. REFERÊNCIAS

Abbas, A.K., Lichtman, A.H., Pillai, S., 2012. Cellular and Molecular Immunology, Vol., Elsevier, Rio de Janeiro.

Abraham, E., 2003. Nuclear factor-kappaB and its role in sepsis-associated organ failure. J Infect Dis. 187 Suppl 2**,** S364-9.

Albensi, B.C., Mattson, M.P., 2000. Evidence for the involvement of TNF and NF-kappaB in hippocampal synaptic plasticity. Synapse. 35**,** 151-9.

Ali, H., Haribabu, B., Richardson, R.M., Snyderman, R., 1997. Mechanisms of inflammation and leukocyte activation. Med Clin North Am. 81**,** 1-28.

Bavaresco, C.S., Zugno, A.I., Tagliari, B., Wannmacher, C.M., Wajner, M., Wyse, A.T., 2004. Inhibition of Na+, K+-ATPase activity in rat striatum by the metabolites accumulated in Lesch-Nyhan disease. Int J Dev Neurosci. 22**,** 11-7.

Calabrese, V., Cornelius, C., Rizzarelli, E., Owen, J.B., Dinkova-Kostova, A.T., Butterfield, D.A., 2009. Nitric oxide in cell survival: a janus molecule. Antioxid Redox Signal. 11**,** 2717-39.

Cauwels, R.G., Martens, L.C., 2005. Self-mutilation behaviour in Lesch-Nyhan syndrome. J Oral Pathol Med. 34**,** 573-5.

Christman, J.W., Sadikot, R.T., Blackwell, T.S., 2000. The role of nuclear factor-kappa B in pulmonary diseases. Chest. 117**,** 1482-7.

Das, U.N., 2007. Acetylcholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation. Med Sci Monit. 13**,** RA214-21.

Forstermann, U., Sessa, W.C., 2011. Nitric oxide synthases: regulation and function. Eur Heart J. 33**,** 829-37, 837a-837d.

Forstermann, U., Sessa, W.C., 2012. Nitric oxide synthases: regulation and function. Eur Heart J. 33**,** 829-37, 837a-837d.

Gallowitsch-Puerta, M., Pavlov, V.A., 2007. Neuro-immune interactions via the cholinergic anti-inflammatory pathway. Life Sci. 80**,** 2325-9.

Ghosh, S., May, M.J., Kopp, E.B., 1998. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol. 16**,** 225-60.

Handel, M.L., Nguyen, L.Q., Lehmann, T.P., 2000. Inhibition of transcription factors by anti-inflammatory and anti-rheumatic drugs: can variability in response be overcome? Clin Exp Pharmacol Physiol. 27**,** 139-44.

Hehlgans, T., Pfeffer, K., 2005. The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. Immunology. 115**,** 1-20.

Hirota, Y., Osuga, Y., Hirata, T., Harada, M., Morimoto, C., Yoshino, O., Koga, K., Yano, T., Tsutsumi, O., Taketani, Y., 2005. Activation of protease-activated receptor 2 stimulates proliferation and interleukin (IL)-6 and IL-8 secretion of endometriotic stromal cells. Hum Reprod. 20**,** 3547-53.

Jinnah, H.A., Friedmann, T., 2001. Lesch Nyhan disease and it variants. In: The Metabolic and Molecular Bases of Inherited. Vol., Scriver CR, A. Beaudet, W. Sly, D. Valle, ed.^eds. McGraw-Hill, New York, pp. 2537 – 2569.

Jinnah, H.A., Visser, J.E., Harris, J.C., Verdu, A., Larovere, L., Ceballos-Picot, I., Gonzalez-Alegre, P., Neychev, V., Torres, R.J., Dulac, O., Desguerre, I., Schretlen, D.J., Robey, K.L., Barabas, G., Bloem, B.R., Nyhan, W., De Kremer, R., Eddey, G.E., Puig, J.G., Reich, S.G., 2006. Delineation of the motor disorder of Lesch-Nyhan disease. Brain. 129**,** 1201-17.

Jinnah, H.A., Sabina, R.L., Van Den Berghe, G., 2013. Metabolic disorders of purine metabolism affecting the nervous system. Handb Clin Neurol. 113**,** 1827-36.

Kaltschmidt, C., Kaltschmidt, B., Baeuerle, P.A., 1993. Brain synapses contain inducible forms of the transcription factor NF-kappa B. Mech Dev. 43**,** 135-47.

Karin, M., Ben-Neriah, Y., 2000. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. Annu Rev Immunol. 18**,** 621-63.

Lesch, M., Nyhan, W.L., 1964. A Familial Disorder of Uric Acid Metabolism and Central Nervous System Function. Am J Med. 36**,** 561-70.

Lucas, S.M., Rothwell, N.J., Gibson, R.M., 2006. The role of inflammation in CNS injury and disease. Br J Pharmacol. 147 Suppl 1**,** S232-40.

Mak, C.M., Lee, H.C., Chan, A.Y., Lam, C.W., 2013. Inborn errors of metabolism and expanded newborn screening: review and update. Crit Rev Clin Lab Sci. 50**,** 142-62.

Matthews, W.S., Solan, A., Barabas, G., 1995. Cognitive functioning in Lesch-Nyhan syndrome. Dev Med Child Neurol. 37**,** 715-22.

Mattson, M.P., Culmsee, C., Yu, Z., Camandola, S., 2000. Roles of nuclear factor kappaB in neuronal survival and plasticity. J Neurochem. 74**,** 443-56.

Meberg, P.J., Kinney, W.R., Valcourt, E.G., Routtenberg, A., 1996. Gene expression of the transcription factor NF-kappa B in hippocampus: regulation by synaptic activity. Brain Res Mol Brain Res. 38**,** 179-90.

Meffert, M.K., Chang, J.M., Wiltgen, B.J., Fanselow, M.S., Baltimore, D., 2003. NF-kappa B functions in synaptic signaling and behavior. Nat Neurosci. 6**,** 1072-8.

Moncada, S., Palmer, R.M., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev. 43**,** 109-42.

Moncada, S., 1993. The L-arginine: nitric oxide pathway, cellular transduction and immunological roles. Adv Second Messenger Phosphoprotein Res. 28**,** 97-9.

Nyhan, W.L., 1978. Ataxia and disorders of purine metabolism: defects in hypoxanthine guanine phosphoribosyl transferase and clinical ataxia. Adv Neurol. 21**,** 279-87.

Pavlov, V.A., Tracey, K.J., 2005. The cholinergic anti-inflammatory pathway. Brain Behav Immun. 19**,** 493-9.

Pavlov, V.A., Parrish, W.R., Rosas-Ballina, M., Ochani, M., Puerta, M., Ochani, K., Chavan, S., Al-Abed, Y., Tracey, K.J., 2009. Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. Brain Behav Immun. 23**,** 41-5.

Perry, E., Walker, M., Grace, J., Perry, R., 1999. Acetylcholine in mind: a neurotransmitter correlate of consciousness? Trends Neurosci. 22**,** 273-80.

Pohanka, M., 2014. Inhibitors of acetylcholinesterase and butyrylcholinesterase meet immunity. Int J Mol Sci. 15**,** 9809-25.

Puig, J.G., Jimenez, M.L., Mateos, F.A., Fox, I.H., 1989. Adenine nucleotide turnover in hypoxanthine-guanine phosphoribosyl-transferase deficiency: evidence for an increased contribution of purine biosynthesis de novo. Metabolism. 38**,** 410-8.

Rock, R.B., Gekker, G., Hu, S., Sheng, W.S., Cheeran, M., Lokensgard, J.R., Peterson, P.K., 2004. Role of microglia in central nervous system infections. Clin Microbiol Rev. 17**,** 942-64, table of contents.

Rosas-Ballina, M., Tracey, K.J., 2009. Cholinergic control of inflammation. J Intern Med. 265**,** 663-79.

Sarter, M., Bruno, J.P., 1997. Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. Brain Res Brain Res Rev. 23**,** 28-46.

Schretlen, D.J., Harris, J.C., Park, K.S., Jinnah, H.A., del Pozo, N.O., 2001. Neurocognitive functioning in Lesch-Nyhan disease and partial hypoxanthine-guanine phosphoribosyltransferase deficiency. J Int Neuropsychol Soc. 7**,** 805-12.

Scriver, C.R., 2008. Garrod's Croonian Lectures (1908) and the charter 'Inborn Errors of Metabolism': albinism, alkaptonuria, cystinuria, and pentosuria at age 100 in 2008. J Inherit Metab Dis. 31**,** 580-98.

Steriade, M., 1992. Basic mechanisms of sleep generation. Neurology. 42**,** 9-17; discussion 18.

Tak, P.P., Firestein, G.S., 2001. NF-kappaB: a key role in inflammatory diseases. J Clin Invest. 107**,** 7-11.

Torres, R.J., Puig, J.G., 2008. The diagnosis of HPRT deficiency in the 21st century. Nucleosides Nucleotides Nucleic Acids. 27, 564-9.

Visser, J.E., Bar, P.R., Jinnah, H.A., 2000. Lesch-Nyhan disease and the basal ganglia. Brain Res Brain Res Rev. 32, 449-75.

Xanthos, D.N., Sandkuhler, J., 2014. Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. Nat Rev Neurosci. 15, 43-53.

Zhang, C., 2008. The role of inflammatory cytokines in endothelial dysfunction. Basic Res Cardiol. 103, 398-406.

Zimmerman, G., Soreq, H., 2006. Termination and beyond: acetylcholinesterase as a modulator of synaptic transmission. Cell Tissue Res. 326, 655-69.